Research Article

Evaluation of Copper and Manganese Concentrations in Commercial Fruit Juices and Nectars Consumed in Brazil by GF AAS

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The present work proposes a simple method for direct determination of Cu and Mn in commercial fruit juices and nectars by graphite furnace atomic absorption spectrometry (GF AAS). We analyzed samples of different flavors (orange, mango, passion fruit, peach, and grape) and brands of Brazilian commercial fruit juices and nectars. We also carried out a study to define a suitable temperature program and to optimize the calibration conditions. It was possible to determine Cu and Mn in the samples just after a simple dilution of samples with a $0.70 \, mol \, L^{-1}$ HNO_3 solution, except in the case of grape juice. We compared the results obtained with the proposed method to those obtained after a traditional treatment based on acid digestion in a microwave oven, and no significant differences were observed (except for grape juice). The accuracy of the method was assessed through a recovery test, which provided recovery percentages in the range of 81–117%. Precision was always better than 8%, and the limits of quantification for Cu and Mn were $6 \, \mu g \, L^{-1}$ and $9 \, \mu g \, L^{-1}$, respectively. We analyzed twenty-two samples, and the concentrations of Cu and Mn were in the range of 24.1–321 $\mu g \, L^{-1}$ and 116–3296 $\mu g \, L^{-1}$, respectively. Statistical analysis using a two-way analysis of variance (ANOVA) at 95% confidence level showed that flavor and brand impacted on the concentration of the analytes in the samples. Among the samples analyzed, the grape juice presented the highest concentrations of both Cu and Mn.

1. Introduction

Fruit juices are widely consumed all over the world, especially in tropical countries. According to ABIR (Brazilian Association of Soft Drinks and Non-Alcoholic Beverage Industries), the average consumption of nectar per capita in Brazil increased from 3.9 L in 2010 to 5.3 L per year in 2017 [1]. As the demand for fruit juices increases, it is necessary to develop fast and accurate methodologies to ensure quality and safe products to consumers.

Several studies on fruit juices have been conducted worldwide, especially on the determination of sugar concentration and fruit content. However, it is also necessary to monitor metal concentrations because the ingestion of excessive concentrations of these species can represent a risk for human health [2, 3]. On the other hand, some of them, such as Cu and Mn, are considered essential for the human development. On this basis, the Brazilian Health Regulatory Agency (ANVISA) regulates the minimum amount of minerals and vitamins that must be daily ingested in the form of food, including commercial juices and nectars. The amounts of Cu and Mn recommended by ANVISA are $900 \, \mu g$ and 2.3 mg, respectively [4]. Additionally, in some cases, these elements may have a negative impact on the production process, facilitating redox and precipitation reactions, formation of gels, and even altering the organoleptic characteristics of the juices [5].

Plasma-based atomic spectrometric techniques, such as inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS), are the most popular analytical techniques used to determine trace metal concentrations in fruit

juices [6–13]. On the other hand, the use of atomic absorption techniques such as FAAS and GF AAS is still limited to some specific situations [14–20].

One of the greatest challenges in the determination of trace metals in fruit juices is to deal with possible nonspecific interferences due to the typical high organic carbon content of the samples, which occurs due to the presence of sugars, proteins, and additives. These substances alter the physical characteristics of the samples, leading to the occurrence of strong transport and nebulization interferences. In order to minimize such interferences, most of the published works proposes the application of wet acid digestion [5, 7, 14, 16, 20] or dry ashing [12, 13] approaches to mineralize the carbon present in the samples and simplify their matrices.

Methodologies that aim to reduce the laborious sample pretreatment process have also been reported. In order to eliminate solid interferents present in the medium, some studies centrifuged and/or filtered the juices prior to analyte determination [11, 21, 22]. Direct determination is less usual due to the high viscosity and the complex organic content of the fruit juices [19]. Therefore, the analysis of samples diluted with nitric acid solution has been described as a good alternative to avoid sample digestion. The reported dilutions used in most studies ranged from 2 to 200 times [6, 8, 19, 23].

Only few works were found in the literature on the direct determination of metals in fruit juices using GF AAS. In 1993, Arruda et al. [24] determined aluminum in tomato juice by flow injection analysis coupled with GF AAS. In 2005, Oliveira et al. [25] determined selenium in mango, tomato, and grape juices. Both groups reported the necessity of using chemical modifiers and dilution of samples with 0.2% and 1% (v/v) nitric acid solutions, respectively.

Methods that avoid the previous treatment of the sample are of great interest in the determination of metals in fruit juices, since their use requires a lower time to process the samples, avoids possible losses of the analytes, and reduces the risk of contamination [26]. GF AAS allows a controlled thermal treatment of the samples inside the instrument, making possible the elimination of interferents before the measurement of the analytical signal. Thus, direct determination of metals in fruit juices by GF AAS can be considered a fast and practical alternative to accomplish this task. In this context, the present work proposes a method for the determination of Cu and Mn in nectars and commercial fruit juices consumed in Brazil by GF AAS with a minimum treatment of the samples. Once optimized, the method was applied to the analysis of twenty-two samples of nectars and juices of different flavors and brands commercially available in Brazil, and the impact of these factors on the metal concentrations in the samples was evaluated.

2. Materials and Methods

2.1. Apparatus. The quantification of the analytes in the solutions was carried out using a Varian AA240Z graphite furnace atomic absorption spectrometer (Mulgrave, Australia), coupled to a GTA-120 atomization unit and a Varian PSD 120 autosampler. The instrument was equipped with a Zeeman-effect background correction system. The graphite tubes with L'vov platform used in this work were longitudinally heated and also obtained from Varian. Individual copper and manganese cathode lamps from Varian were used by applying currents of 3.0 mA and 5.0 mA, respectively. Copper was determined by setting the wavelength at 324.8 nm with a slit width of 0.5 nm, and manganese was measured at 297.5 nm with a 0.2 nm slit. Argon with 99.99% purity (Linde Gases, Macaé, Brazil) was employed as the protective gas. A microwave oven from Berghof (Eningen, Germany), model SpeedWave 4, was employed for sample digestion always using DAK-100 (100 mL) perfluoroalkoxy (PFA) flasks.

2.2. Reagents, Solutions, and Samples. All solutions were prepared with deionized water (18.2 MΩ cm^{-1}) produced in a Direct Q-3 water purification system (Millipore, Bedford, MA, USA). The concentrated nitric acid used to acidify solutions and samples was of trace metal grade and purchased from Tedia (Fairfield, OH, USA).

Stock solutions (1000 mg L^{-1}) of copper and manganese used for the preparation of the standard solutions were both obtained from Tedia (São Paulo, Brazil). The standard solutions of Cu and Mn were prepared daily from dilutions of their respective stock solutions with a 0.70 mol L^{-1} HNO$_3$ solution.

Twenty-two samples of commercial fruit juices and nectars, purchased in the city of Rio de Janeiro (Brazil), were analyzed. All beverages were packed in Tetra Pak® packaging. Four different brands of nectars (A, B, C, and D) in five different flavors (orange, mango, passion fruit, peach, and grape) and two brands of fruit juice (A and B) in two different flavors (orange and grape) were evaluated in this work.

2.3. Material Decontamination. All materials used in this work were decontaminated through immersion in a nitric acid solution. Polyethylene tubes and glassware were washed with detergent and rinsed with current water, followed by a second rinse with deionized water. Then, they were immersed for 24 h, at least, in a 1.4 mol L^{-1} HNO$_3$ solution. Before their use, the materials were rinsed with deionized water and dried at 60°C (except volumetric material).

2.4. Direct Determination of Cu and Mn by GF AAS. Direct determination of the analytes by GF AAS in the samples was performed after their appropriate dilution with a 0.70 mol L^{-1} HNO$_3$ solution. Two independent aliquots of each sample were analyzed. Integrated absorbance signals were measured by introducing 20 μL of the sample (or standard) solution, previously treated, into the graphite furnace, followed by the application of the temperature program for each metal (Table 1). The measurements were performed in duplicate and without using any chemical modifier in order to avoid the increase in blank signals.

TABLE 1: Temperature program of the graphite furnace established for the measurement for Cu and Mn.

Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow rate (mL min^{-1})
Drying I	95	5.0	—	300
Drying II	120	10.0	14.0	300
Pyrolysis	1200* (Cu), 1300* (Mn)	5.0	4.0	300
Atomization	2100* (Cu), 2200* (Mn)	1.0	3.0	0.0
Cleaning	2200* (Cu), 2300* (Mn)	2.0	—	300

*Values established after construction of pyrolysis and atomization curves.

2.5. Microwave-Assisted Acid Digestion of Samples.

The microwave-assisted acid digestion of the samples was performed in order to have a reference value for the concentrations of Cu and Mn in the samples. For this purpose, 2.0 mL of the samples under the test was mixed with 5.0 mL of concentrated nitric acid directly into the liners, which were then sealed. Afterward, the liners were adjusted to the carrousel of the microwave oven, and the heating program (Table 2) was run. After finishing the heating program, the liners were taken out of the microwave oven cavity and left on the bench until cooling to the laboratory ambient temperature. Then, the liners were opened, their contents were quantitatively transferred to 50 mL volumetric flasks, and the volume was completed to the mark with deionized water. Two independent aliquots of each sample were digested and analyzed.

3. Results and Discussion

3.1. Optimization of the Temperature Program of GF AAS.

The first study was carried out to define a suitable heating program to determine the analytes in the samples by GF AAS. In order to know the thermal behavior of the analytes in the samples, pyrolysis and atomization curves were built up in three different media (aqueous solution, nectar, and juice) for each metal. In this study, we used the samples S_2 (orange nectar, brand B) and S_{20} (orange juice, brand B) to construct the pyrolysis and atomization curves. The samples were diluted with a 0.70 mol L^{-1} HNO$_3$ solution prior to run the measurements.

Pyrolysis and atomization curves of Cu and Mn are displayed in Figure 1. The thermal behavior of the two analytes was very similar in the three media, indicating that no important nonspecific interferences affected the measurements. The pyrolysis temperatures for Cu and Mn were set at 1200°C and 1300°C, respectively, in order to ensure total elimination of organic matter without losing the analyte by volatilization. The atomization temperatures were set at 2100°C and 2200°C, for Cu and Mn, respectively, to ensure maximum sensitivity for the measurements in the case of Cu and due to peak shape in the case of Mn. Although we observed lower analytical signals at 2200°C (in comparison with those observed at 2000°C), using this atomization temperature, the Mn peaks were narrower and taller, which improved their differentiation in relation to the baseline.

The temperatures of the drying step were defined to allow total evaporation of the water from the sample matrix or the standard solutions before the pyrolysis step, whereas

TABLE 2: Heating program employed in the microwave-assisted acid digestion of the samples.

Step	Temperature (°C)	Pressure (bar)	Ramp time (min)	Hold time (min)
1	100	60	1	10
2	200	60	5	10
3	50	60	1	5

the cleaning temperature was set at 100°C above the atomization temperature to ensure that any persistent component present in the graphite tube was eliminated. The complete temperature program is given in Table 1.

3.2. Evaluation of the Calibration Strategy.

Since GF AAS is very sensitive, allowing the quantification of trace amounts of metals, it is possible to work with diluted sample solutions, which can significantly reduce interferences caused by the matrix. Even so, the measurement of the GF AAS analytical signals in juices and nectars are challenging due to the high concentration of sugars. In order to test whether the dilutions were sufficient to eliminate possible nonspecific interferences, analytical curves of each metal were compared to analyte addition curves prepared in different media (nectar or juice, different flavors). These curves were prepared with the samples of five flavors of nectars and the two flavors of juices of brand B.

The comparison between the curves was performed by calculating the slope ratios, which can be defined as the ratios between the slope of analytical curve (S_{ac}) and analyte addition curve (S_{adc}) for each metal in each different situation. As can be seen in Table 3, the slope ratios for all nectars and orange juice were between 0.82 and 1.18 for both analytes. In these cases, we considered that there was no significant nonspecific interference, since the sensitivity of the analyte addition curves were between 80% and 120% of the sensitivity of the analytical curve. Additionally, Student's t-test was applied for the comparison of the slopes of the analyte addition curves with the analytical curve. As can also be seen in Table 3, the values of t were always lower than the critical one ($t_{critical} = 4.30$) at 95% confidence level, indicating that there were no differences among the curves. These results demonstrated that the determination of the analytes in the nectars of all flavors and orange juice could be performed using an external calibration approach.

On the other hand, in the case of the samples of grape juice, a significant difference between the sensitivities of the analytical and analyte addition curves for both metals was observed (t_{values} of 24.051 for Cu and 15.924 for Mn), with
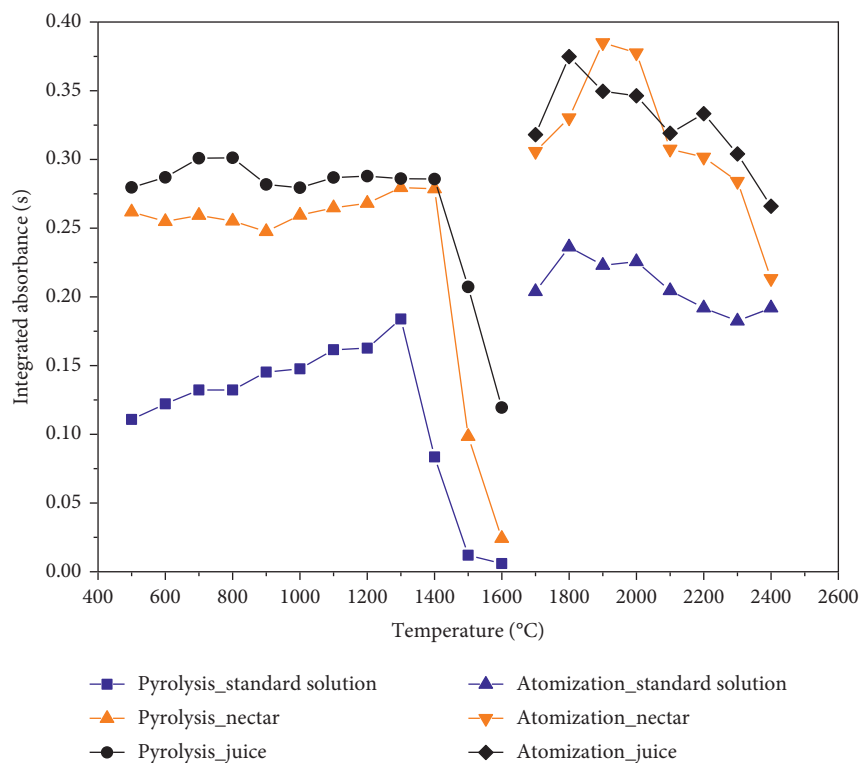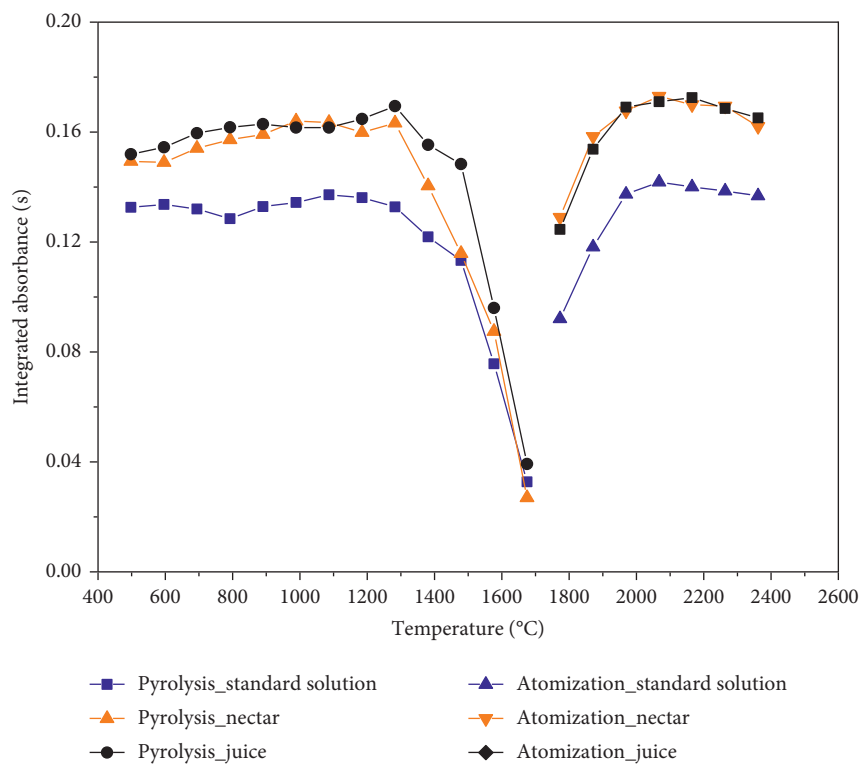
(a)

(b)

FIGURE 1: Pyrolysis and atomization curves of (a) Cu and (b) Mn in the two media under test (nectar and juice) and in aqueous standard solution (Cu concentration = 15 μg L^{-1} and Mn concentration = 5.0 μg L^{-1}). The curves for nectar and juice were constructed using the samples S_2 and S_{20} diluted (1 : 10 to 1 : 40) with 0.70 mol L^{-1} HNO3 solution.

Table 3: Results obtained in the evaluation of the calibration strategy using samples of different flavors.

Calibration strategy/sample	Type of sample/flavor	Cu[a]	Mn[a]
Analytical curve	Water	$Y = (0.0090 \pm 0.0002)\, x + (0.0048 \pm 0.0055)$, $r^2 = 0.999$	$Y = (0.0347\pm)\, x + (0.0026 \pm 0.0044)$, $r^2 = 0.998$
Analyte addition curve/S_2	Nectar/orange	$Y = (0.0094x \pm 0.0003)\, x + (0.1347 \pm 0.0039)$, $r^2 = 0.999$, SR = 1.04, t value = 2.495	$Y = (0.0316\pm)\, x + (0.3432 \pm 0.0184)$, $r^2 = 0.991$, SR = 0.91, t value = 2.784
Analyte addition curve/S_6	Nectar/mango	$Y = (0.0106x \pm 0.0009)\, x + (0.0830 \pm 0.0074)$, $r^2 = 0.999$, SR = 1.18, t value = 4.277	$Y = (0.0332\pm)\, x + (0.4046 \pm 0.0128)$, $r^2 = 0.997$, SR = 0.93, t value = 1.653
Analyte addition curve/S_9	Nectar/passion fruit	$Y = (0.0099x \pm 0.0005)\, x + (0.0428 \pm 0.0022)$, $r^2 = 0.999$, SR = 1.10, t value = 3.869	$Y = (0.0310\pm)\, x + (0.2016 \pm 0.0181)$, $r^2 = 0.998$, SR = 0.89, t value = 2.543
Analyte addition curve/S_{12}	Nectar/peach	$Y = (0.0100x \pm 0.0008)x + (0.113 \pm 0.0085)$, $r^2 = 0.999$, SR = 1.11, t value = 3.214	$Y = (0.0345\pm)\, x + (0.2512 \pm 0.0175)$, $r^2 = 0.995$, SR = 0.99, t value = 0.149
Analyte addition curve/S_{16}	Nectar/grape	$Y = (0.0099x \pm 0.0008)\, x + (0.0897 \pm 0.0076)$, $r^2 = 0.998$, SR = 1.10, t value = 2.665	$Y = (0.0293\pm)\, x + (0.2878 \pm 0.0297)$, $r^2 = 0.996$, SR = 0.84, t value = 3.521
Analyte addition curve/S_{20}	Juice/orange	$Y = (0.0087x \pm 0.0004)\, x + (0.1168 \pm 0.0055)$, $r^2 = 0.999$, SR = 0.96, t value = 1.466	$Y = (0.0284\pm)\, x + (0.2353 \pm 0.0281)$, $r^2 = 0.999$, SR = 0.82, t value = 3.816
Analyte addition curve/S_{22}	Juice/grape	$Y = (0.0144x \pm 0.0005)\, x + (0.2910 \pm 0.0096)$, $r^2 = 0.993$, SR = 1.60, t value = 24.051	$Y = (0.0628\pm)\, x + (0.1035 \pm 0.0062)$, $r^2 = 0.993$, SR = 1.81, t value = 15.924

[a]SR represents the slope ratios and were calculated as S_{adc}/S_{ac}, where S_{ac} = the sensitivity (slope) of the analytical curve and S_{adc} = sensitivity (slope) of the analyte addition curve. Y represents the integrated absorbance (s) and x is the concentration of the analyte ($\mu g\ L^{-1}$). t_{value} was compared to $t_{critical} = 4.30$ (degrees of freedom = 2 at 95% confidence level).

slope ratios of 1.60 and 1.81 for Cu and Mn, respectively. Therefore, for this sample, the direct determination of Cu and Mn by GF AAS could only be possible using an analyte addition approach.

In order to evaluate the results obtained from the slope ratio study, recovery tests were performed for each metal in all samples of brand B. For this purpose, the samples were spiked at four concentration levels (10, 20, 30, and 40 $\mu g\ L^{-1}$) with individual Cu and Mn standards. The recovery percentages were calculated at each level as the difference between the concentrations obtained for spiked and nonspiked samples. As expected, the recovery percentages were between 81% and 117% for all nectars and for orange juice, confirming the results obtained in the study of the calibration strategy. Again, grape juice showed a very different behavior. In this case, recovery percentages in the range of 170%–207% were observed, indicating that simple dilution of this type of sample with acid solution was not sufficient to eliminate possible nonspecific interferences. All results obtained in the recovery test are shown in Table 4.

3.3. Comparison of Sample Preparation Procedures. We also tested the accuracy of the proposed method through the comparison with a reference method. For this, all fruit juice and nectar samples of brand B were submitted to the traditional treatment based on the acid digestion in a microwave oven. The concentrations obtained from the samples treated by acid digestion were compared to the concentrations obtained by direct determination. Figure 2 shows the results for all samples of nectars (*n-*) and juices (*j-*) of the brand B analyzed by the two procedures. It is important to reinforce that the direct determination of Cu and Mn in the samples was performed using an external calibration strategy with aqueous standard solutions of the analytes, except in the

case of grape juice, in which analyte addition curves of Cu and Mn were used instead. In all cases, the samples were diluted with a 0.70 mol L^{-1} HNO_3 solution to fit in the linear portion of the calibration curves.

In order to confirm whether the results obtained by the two methods were statistically similar, we applied Student's t-test for each individual pair of results obtained for Cu and Mn. Before application of Student's t-test, each pair of results was evaluated in terms of their variances using the F-test. The results are shown in Table 5. As can be seen, all values of F were lower than the critical value ($F_{crit} = 39.00$), indicating that, in all cases, the data were homoscedastic. Also, the values of t were lower than the critical value ($t_{crit} = 4.30$) and, for this reason, we concluded that no significant difference was observed between the results, evidencing that the simple dilution of the sample with nitric acid solution followed by determination of Cu and Mn by GF AAS can be considered as an effective method for the quantification of metals in these samples.

On the other hand, the determination of both Cu and Mn in the sample of grape juice (also of brand B) presented a different behavior. In these cases, the statistical test showed that the results obtained by acid digestion and direct determination were significantly different at 95% confidence level ($t_{Cu} = 6.887$; $t_{Mn} = 6.612$; $t_{crit} = 4.30$), evidencing that nonspecific interferences cannot be corrected in the grape juice analysis even when using the analyte addition approach as the calibration strategy.

3.4. Determination of Analytical Features. After establishing the optimum experimental conditions for the determination of Cu and Mn in the samples, we estimated the analytical features of the method. The instrumental limits of detection (LOD) and quantification (LOQ) were estimated using a calibration curve (10–50 $\mu g\ L^{-1}$ for Cu and 2.0–10 $\mu g\ L^{-1}$ for

TABLE 4: Results obtained in the recovery tests using samples of brand B.

Sample	Type of sample/flavor	Concentration added (μg L^{-1})	Cu recovery (%)	Mn recovery (%)
S_2	Nectar/orange	10	114	106
		20	109	100
		30	104	99
		40	105	89
S_6	Nectar/mango	10	95	113
		20	102	98
		30	99	93
		40	102	95
S_9	Nectar/passion fruit	10	117	81
		20	106	94
		30	113	95
		40	100	89
S_{12}	Nectar/peach	10	106	117
		20	108	102
		30	114	105
		40	109	101
S_{16}	Nectar/grape	10	109	104
		20	113	91
		30	111	88
		40	111	85
S_{20}	Juice/orange	10	90	88
		20	93	93
		30	95	98
		40	95	93
S_{22}	Juice/grape	10	175	207
		20	171	201
		30	174	197
		40	170	182

Mn) with standard solutions prepared in 0.70 mol L^{-1} HNO$_3$ solution and performing ten measurements of the blank solution (0.70 mol L^{-1} HNO$_3$). These limits were calculated as LOD = 3.3σ/S and LOQ = 10σ/S [27], where σ represents the standard deviation of the ten measurements of the blank solution, and S represents the sensitivity (slope) of the calibration curve. On this basis, the LOD for Cu and Mn were 2 μg L^{-1} and 3 μg L^{-1}, respectively, whereas the LOQ were 6 μg L^{-1} and 9 μg L^{-1}, respectively. The typical equations of the calibration curves were $A = 0.0090$ (Cu (μg L^{-1})) + 0.0048 ($r^2 = 0.999$) and $A = 0.0347$ (Mn (μg L^{-1})) + 0.0026 ($r^2 = 0.998$). Precision was calculated from five independent determinations of the metals in the sample S_1, and it was always better than 8%.

3.5. *Application of the Developed Method in the Samples.* The concentrations of Cu and Mn in the nectars and orange juices were determined by the method of direct determination by GF AAS, except in the cases of grape juices, in which we determined the analytes after acid digestion of the sample followed by GF AAS determination. The results obtained for Cu and Mn determination are shown in Table 6.

The concentrations of the analytes in orange and grape juices were always higher than in their respective nectars, which can be attributed to the different concentrations of whole juice found in each type of beverage. Grape and orange nectars contain at least 50% of whole juice, whereas

the juice itself does not undergo any dilution. Such results may indicate that most metals come from the fruit itself and not from some type of contamination during the production process.

Considering the average concentrations found in the samples according to the flavor, Mn was always found in higher concentrations than Cu, in both nectars and juices. This trend is consistent with the values found in the Brazilian Table of Food Composition [28]. Possibly, the plants absorb more Mn from the soil than Cu, since Mn is vital for the process of energy production during photosynthesis [29, 30].

In order to evaluate whether flavor and brand impact the concentrations of the analytes in the beverages, a two-way ANOVA test was applied for each dataset (Cu and Mn). For both Cu and Mn, the influence of the two factors was considered statistically relevant at 95% confidence level. The test was performed considering the samples with all flavors of nectars, except passion fruit, since it was not possible to purchase passion fruit beverages of all brands.

Despite the ANOVA test indicated that brand impacted the concentration of the analytes, it is not possible to state that the difference observed among the brands is exclusively due to the production process. Several factors may contribute to the variability of metals concentrations in fruits such as soil, climate, seasonality, and others. To establish a safe link between the brand and the analyte concentration in the samples, the entire production process should be monitored and evaluated.
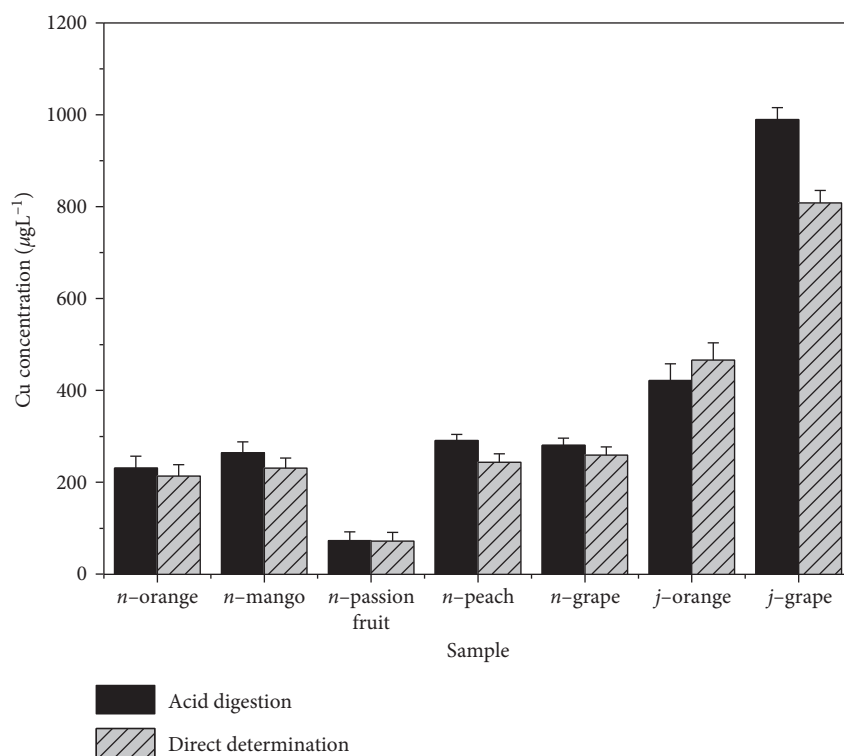
(a)
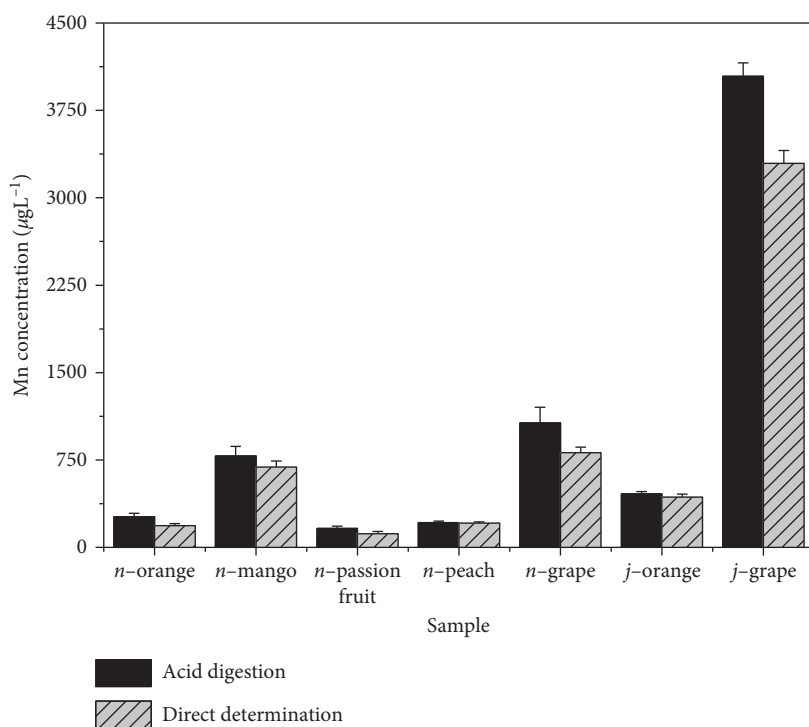
(b)

FIGURE 2: Comparison between the concentrations of (a) Cu and (b) Mn obtained by direct GF AAS determination and after acid digestion of samples. *n* and *j* indicates samples of nectar and juice, respectively.

The Cu concentrations determined in all samples were far below the maximum tolerance limit of 10 mg day^{-1} established by the World Health Organization (WHO) [31]. It is also important to highlight that the maximum tolerance limit of Cu in fruit juices is still 30 mg L^{-1}, established by an old Brazilian legislation of 1965 [32]. Due to the low toxicity of Mn, both the WHO and national legislation do not set tolerance limits for such metal in this type of beverage.

TABLE 5: Statistical evaluation (Student's t-test) of the results obtained in the determination of Cu and Mn by the direct GF AAS method and after acid digestion of samples.

Sample	F value (Cu)[a]	t value (Cu)[b]	F value (Mn)[a]	t value (Mn)[a]
n-orange	1.077	0.682	2.966	3.099
n-mango	1.162	1.484	2.578	1.378
n-passion fruit	1.011	0.075	1.021	2.411
n-peach	1.998	3.032	1.677	0.379
n-grape	1.358	1.279	7.735	2.764
j-orange	1.072	−1.209	1.635	1.166
j-grape	1.118	6.887	1.114	6.612

[a]$F_{2,2}$ (two-sided test) = 39.00, at 95% confidence level. [b]$t_{critical}$ = 4.30 (degrees of freedom = 2), at 95% confidence level. n- and j- indicates the samples of nectar and juice, respectively.

TABLE 6: Concentrations of Cu and Mn in the analyzed samples.

Sample	Type	Flavor	Brand	Cu (μg L^{-1})[b]	Mn (μg L^{-1})[b]
S_1	Nectar	Orange	A	179 ± 5 (20)	249 ± 4 (60)
S_2	Nectar	Orange	B	214 ± 5 (20)	184 ± 4 (20)
S_3	Nectar	Orange	C	24.1 ± 0.8 (3)	246 ± 3 (60)
S_4	Nectar	Orange	D	61.7 ± 4.5 (3)	117 ± 1 (40)
S_5	Nectar	Mango	A	321 ± 12 (20)	2036 ± 2 (500)
S_6	Nectar	Mango	B	231 ± 12 (30)	690 ± 10 (60)
S_7	Nectar	Mango	C	253 ± 3 (20)	814 ± 1 (200)
S_8	Nectar	Mango	D	199 ± 6 (20)	771 ± 2 (200)
S_9	Nectar	Passion fruit	B	72.1 ± 3.8 (20)	116 ± 2 (20)
S_{10}	Nectar	Passion fruit	C	96.4 ± 1.4 (5)	312 ± 5 (80)
S_{11}	Nectar	Peach	A	184 ± 4 (20)	246 ± 3 (80)
S_{12}	Nectar	Peach	B	244 ± 18 (20)	208 ± 1 (20)
S_{13}	Nectar	Peach	C	183 ± 15 (20)	330 ± 2 (80)
S_{14}	Nectar	Peach	D	207 ± 5 (20)	247 ± 12 (80)
S_{15}	Nectar	Grape	A	321 ± 1 (40)	1033 ± 12 (300)
S_{16}	Nectar	Grape	B	259 ± 3 (30)	812 ± 10 (100)
S_{17}	Nectar	Grape	C	243 ± 2 (20)	636 ± 18 (100)
S_{18}	Nectar	Grape	D	606 ± 3 (40)	851 ± 6 (300)
S_{19}	Juice	Orange	A	215 ± 6 (40)	558 ± 5 (200)
S_{20}	Juice	Orange	B	466 ± 7 (40)	331 ± 5 (40)
S_{21}[a]	Juice	Grape	A	334 ± 1 (25)	2855 ± 27 (750)
S_{22}[a]	Juice	Grape	B	990 ± 3 (25)	3296 ± 18 (2000)

[a]These samples were analyzed after their microwave-assisted acid digestion. [b]Dilution factors applied to the samples are between parentheses. The results (μg L^{-1}) are expressed as mean ± standard deviation of two independent determinations.

Given that the metals analyzed are essential to human health, a preliminary comparison between the determined analytes concentration and the Dietary Reference Intakes (DRI) can be performed. As mentioned in the Introduction, DRI for Cu and Mn are 900 μg day^{-1} and 2.3 mg day^{-1}, respectively [4]. Therefore, only one cup (200 mL) of grape juice, for instance, would be able to supply 15% and 27% of Cu and Mn DRI, respectively. However, it should be taken into account that the purpose of this work was to determine the total concentration of the metals. So, to have more nutrition information about the analyzed juices, it would be necessary to perform a more detailed study regarding the bioavailability of the metals in the samples.

4. Conclusions

The results obtained from the developed method showed that is possible to perform the direct determination of Cu and Mn in most of nectars and fruit juices by GF AAS after minimal pretreatment of the samples by dilution with a 0.70 mol L^{-1}

HNO$_3$ solution. However, for the grape juice, the acid digestion in a microwave oven was needed, since the nonspecific interferences could not be eliminated even when the optimal conditions for GF AAS measurements were employed.

Twenty-two samples of nectars and juices of different flavors and brands were analyzed. The influence of the flavor and the brand on the concentrations of the analytes in nectars was verified through the two-way analysis of variance (ANOVA), and both factors significantly impacted the concentrations of Cu and Mn in this kind of sample. The grape juice was the beverage that presented the highest concentration of both Cu and Mn. Also, the brands A and B showed the highest concentrations of both analytes.

Data Availability

The data used to support this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] ABIR-Associação Brasileira das Indústrias de Refrigerantes e de Bebidas não Alcoólicas: http://www.abir.org.br. 2019.

[2] L. Tormen, D. P. Torres, I. M. Dittert, R. G. O. Araújo, V. L. A. Frescura, and A. J. Curtius, "Rapid assessment of metal contamination in commercial fruit juices by inductively coupled mass spectrometry after a simple dilution," *Journal of Food Composition and Analysis*, vol. 24, no. 1, pp. 95–102, 2011.

[3] B. E. Tvermoes, A. M. Banducci, K. D. Devlin et al., "Screening level health risk assessment of selected metals in apple juice sold in the United States," *Food and Chemical Toxicology*, vol. 71, pp. 42–50, 2014.

[4] ANVISA-Agência Nacional de Vigilância Sanitária. Resolution RDC no 269, of September 22nd, 2005. Technical regulation on the recommended daily intake (RDI) of protein, vitamins and minerals.

[5] M. Olalla, J. Fernández, C. Cabrera, M. Navarro, R. Giménez, and M. C. López, "Nutritional study of copper and zinc in grapes and commercial grape juices from Spain," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 9, pp. 2715–2720, 2004.

[6] I. J. Cindrić, M. Zeiner, M. Kröppl, and G. Stingeder, "Comparison of sample preparation methods for the ICP-AES determination of minor and major elements in clarified apple juices," *Microchemical Journal*, vol. 99, no. 2, pp. 364–369, 2011.

[7] A. Dehelean and D. A. Magdas, "Analysis of mineral and heavy metal content of some commercial fruit juices by inductively coupled plasma mass spectrometry," *The Scientific World Journal*, vol. 18, Article ID 215423, 6 pages, 2013.

[8] R. E. S. Froes, W. B. Neto, N. O. C. e Silva, R. L. P. Naveira, C. C. Nascentes, and J. B. B. da Silva, "Multivariate optimization by exploratory analysis applied to the determination of microelements in fruit juice by inductively coupled plasma optical emission spectrometry," *Spectrochimica Acta Part B: Atomic Spectroscopy*, vol. 64, no. 6, pp. 619–622, 2009.

[9] M. Harmankaya, S. Gezgin, and M. M. Özcan, "Comparative evaluation of some macro- and micro-element and heavy metal contents in commercial fruit juices," *Environmental Monitoring and Assessment*, vol. 184, no. 9, pp. 5415–5420, 2012.

[10] S. Kiliç, S. Yenisoy-Karakaş, and M. Kiliç, "Metal contamination in fruit juices in Turkey: method validation and uncertainty budget," *Food Analytical Methods*, vol. 8, no. 10, pp. 2487–2495, 2015.

[11] A. Szymczyzha-Madeja and M. Welna, "Evaluation of a simple and fast method for the multi-elemental analysis in commercial fruit juice samples using atomic emission spectrometry," *Food Chemistry*, vol. 141, no. 4, pp. 3466–3472, 2013.

[12] D. S. Velimirović, S. S. Mitić, S. B. Tošić, B. M. Kaličanin, AN. Pavolović, and M. N. Mitić, "Levels of major and minor elements in some commercial fruit juices available in Serbia," *Tropical Journal of Pharmaceutical Research*, vol. 12, no. 5, pp. 805–811, 2013.

[13] M. Welna and A. Szymczycha-Madeja, "Effect of sample preparation procedure for the determination of As, Sb and Se in fruit juices by HG-ICP-OES," *Food Chemistry*, vol. 159, pp. 414–419, 2014.

[14] V. L. C. Bragança, P. Melnikov, and L. Z. Zanoni, "Trace elements in fruit juices," *Biological Trace Element Research*, vol. 146, no. 2, pp. 256–261, 2012.

[15] G. C. Brandão, M. O. Aureliano, M. C. S. Sauthier, and W. N. L. Santos, "Photo-oxidation using UV radiation as a sample preparation procedure for the determination of copper in fruit juices by flame atomic absorption spectrometry," *Analytical Methods*, vol. 4, no. 4, pp. 855–858, 2012.

[16] D. Cautela, F. Santelli, F. Boscaino, B. Laratta, L. Servillo, and D. Castaldo, "Elemental content and nutritional study of blood orange juice," *Journal of the Science of Food and Agriculture*, vol. 89, no. 13, pp. 2283–2291, 2009.

[17] S. L. C. Ferreira, E. G. P. da Silva, L. A. Portugal et al., "Evaluation and application of the internal standard technique for the direct determination of copper in fruit juices employing fast sequential flame atomic absorption spectrometry," *Analytical Letters*, vol. 41, no. 9, pp. 1571–1578, 2008.

[18] Y. Liu, B. Gong, Z. Li, Y. Xu, and T. Lin, "Direct determination of selenium in a wild fruit juice by electrothermal atomic absorption spectrometry," *Talanta*, vol. 43, no. 7, pp. 985–989, 1996.

[19] A. Szymczyzha-Madeja, M. Welna, D. Jedryczko, and P. Pohl, "Developments and strategies in the spectrochemical elemental analysis of fruit juices," *Trends in Analytical Chemistry*, vol. 55, pp. 68–80, 2014.

[20] C. Velasco-Reynold, M. Navarro-Alarcon, H. López-GaDe La Serrana, and M. C. Lopez-Martinez, "Copper in foods, beverages and waters from South East Spain: influencing factors and daily dietary intake by the Andalusian population," *Food Additives & Contaminants: Part A*, vol. 25, no. 8, pp. 937–945, 2008.

[21] T. A. Eisele and S. R. Drake, "The partial compositional characteristics of apple juice from 175 apple varieties," *Journal of Food Composition and Analysis*, vol. 18, no. 2-3, pp. 213–221, 2005.

[22] A. D. Peuke, "Nutrient composition of leaves and fruit juice of grapevine as affected by soil and nitrogen fertilization," *Journal of Plant Nutrition and Soil Science*, vol. 172, no. 4, pp. 557–564, 2009.

[23] P. Pohl and B. Prusisz, "Fractionation of calcium and magnesium in honeys, juices and tea infusions by ion exchange and flame atomic absorption spectrometry," *Talanta*, vol. 69, no. 5, pp. 1227–1233, 2006.

[24] M. A. Z. Arruda, M. Gallego, and M. Valcárcel, "Determination of aluminum in slurry and liquid phase of juices by flow injection analysis graphite furnace atomic absorption spectrometry," *Analytical Chemistry*, vol. 65, no. 22, pp. 3331–3335, 1993.

[25] A. P. Oliveira, J. A. G. Neto, J. A. Nóbrega, P. R. M. Correia, and P. V. Oliveira, "Determination of selenium in nutritionally relevant foods by graphite furnace atomic absorption spectrometry using arsenic as internal standard," *Food Chemistry*, vol. 93, no. 2, pp. 355–360, 2005.

[26] C. S. Nomura, C. S. d. Silva, and P. V. Oliveira, "Análise direta de sólidos por espectrometria de absorção atômica com atomização em forno de grafite: uma revisão," *Química Nova*, vol. 31, no. 1, pp. 104–113, 2008.

[27] S. L. R. Ellison, V. J. Barwick, and T. J. D. Farrant, *Practical Statistics for Analytical Scientist: A Bench Guide*, Royal Society of Chemistry, Cambridge, UK, 2nd edition, 2009.

[28] Brazilian Table of Food Composition, *Núcleo de Estudos e Pesquisas em Alimentação*, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil, 4 edition, 2011.

[29] W. W. Fischer, J. Hemp, and J. E. Johnson, "Manganese and the evolution of photosynthesis," *Origins of Life and Evolution of Biospheres*, vol. 45, no. 3, pp. 351–357, 2015.

[30] M. M. Najafpour, G. Renger, M. Hołyńska et al., "Manganese compounds as water-oxidizing catalysts: from the natural water-oxidizing complex to nanosized manganese oxide structures," *Chemical Reviews*, vol. 116, no. 5, pp. 2886–2936, 2016.

[31] World Health Organization (WHO), *Trace Elements in Human Nutrition and Health*, World Health Organization (WHO), Switzerland, Geneva, 1996.

[32] Ministry of Health of Brazil, Law no. 55871, Brazil, 1965.