

Multi-Element Evaluation in Black Pepper (*Piper nigrum* L.) According to the Processing

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Black pepper is one of the most consumed spices worldwide and the evaluation of trace elements content is essential to promote consumer safety. In this study, concentrations of the elements As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Se, V and Zn was determined in samples of black peppercorn (n = 25) and ground black pepper (n = 25) to verify how processing affects element concentrations. The elements were determined by inductively coupled plasma mass spectrometry and flame atomic absorption spectrometry. A principal component analysis showed that Fe, Pb and V was influenced by the processing and ground black pepper samples showed higher levels for these elements than black peppercorn. Although specific legislation for spices is not available in Brazil, results indicated that Pb concentrations in two brands of the processed samples under investigation exceeded the maximum level established by Brazilian and European legislation for vegetables and fruits. These results point to the necessity to control the processing of the black pepper, especially the grinding step.

Keywords: trace elements, black pepper, PCA, processing

Introduction

Black pepper (*Piper nigrum* L.) is one of the most consumed spices worldwide. Its dry and ground beans are used in cooking for flavoring and increasing the shelf life of food products. Black pepper is also very much applied in natural medicine, due to its therapeutic properties.¹ Brazil is one of the world's largest pepper producers, and almost all of the pepper produced in the country is destined for the foreign market.² The Brazilian production of black pepper in the year of 2017 was about 79,371 Mt and in the first half of 2018, Brazil was the second largest exporter of this spice representing approximately 16% (31,000 Mt) of the total market.^{3,4}

The pepper culture has great social and economic importance for the country and the quality control of this spice is essential to promote consumer safety, evaluating the presence of potentially toxic contaminants, such as certain metals. Trace elements may be incorporated from the growing soil or by processing through drying, grinding, packaging and transport steps.⁵ During the drying process, aerial contaminant deposition may occur. The

grinding process may also contribute, due to equipment wear in commercial mills.⁶ In this context, the number of studies aiming at the determination of trace elements in plant samples, such as spices, herbs and medicinal plants, including black pepper, has increased in recent years.⁵⁻¹⁶

Different analytical procedures have been reported in the literature for the determination of trace elements in pepper. Özcan *et al.*¹⁷ reported the determination of 18 elements in black pepper samples after acid decomposition using inductively coupled plasma optical emission spectrometry (ICP OES). Baysal and Akman¹⁸ determined Pb and Cu through the direct analysis of black peppercorn using graphite furnace atomic absorption spectrometry (GF AAS). Karadaş *et al.*¹⁹ used inductively coupled plasma mass spectrometry (ICP-MS) to determine 13 elements in black pepper. De La Calle *et al.*²⁰ determined the concentrations of 10 elements in herbal and spice samples using ultrasonic assisted extraction in combination with X-ray fluorescence by total reflection. Jawad²¹ determined Pb, Cd, Cr, Fe, Mn and Cu concentrations in pepper samples after acid decomposition through flame atomic absorption spectrometry (FAAS).

Some of the afore mentioned studies reported concentrations of potentially toxic elements above the

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values established by local law bodies. The authors also pointed out possible sources of contamination, ranging from spice cultivation to processing and storage. However, no studies are available in the literature evaluating how sample processing affects trace element concentrations in black pepper samples. Therefore, the aim of this study was to determine trace element concentrations in commercial samples of black peppercorn and ground black pepper, evaluating the distribution trend of these elements as a function of sample processing.

Experimental

Instrumentation

ICP-MS analyses were carried out on a NexION™ 300 mass spectrometer (PerkinElmer, United States). Argon (99.9992% purity, Air Products Brasil Ltda, Brazil) was used for plasma maintenance/generation, sample aspiration and as auxiliary gas. Atomic absorption spectrometry analyses were conducted on an AAS ZEE nit 700 BU spectrometer (AnalytikJena, Germany). Hollow cathode lamps were used as the radiation source (AnalytikJena, Germany). An air/acetylene flame was used and background correction was performed by a deuterium lamp. Samples were prepared in a 402-D laboratory oven (Ethik Technology, Brazil), weighed on a ED224S analytical balance (± 0.0001 g precision) (Sartorius, Germany) and centrifuged in a microprocessed Q222TM204 tube centrifuge (Quimis, Brasil). The acidic decomposition of the samples was performed using a Multiwave GO microwave oven (Anton Paar, Austria).

Reagents and solutions

All solutions were prepared using ultra-pure water type 1+, with a specific resistivity of 18 M Ω cm (PURELAB Ultra Mk2, ELGA, UK), 65% m v⁻¹ HNO₃ (Vetec, São Paulo, Brazil) purified in a Sub-boiling Distillacid BSB 939 IR acid distiller (BERGHOF, Germany), and 30% m m⁻¹ hydrogen peroxide (Proquimios Comércio e Indústria Ltda, Rio de Janeiro, Brazil).

Analytical solutions for As, Cd, Co, Cu, Mn, Mo, Ni, Pb, Se and V were prepared by appropriate dilution of a multielementar PlasmaCAL QC Standard 3 140-102-051 solution (SCP Science, Quebec, Canada) in 5% v v⁻¹ HNO₃, containing elements at 100 mg L⁻¹ in 5% v v⁻¹ HNO₃. Iron and zinc were diluted from monoelementar standard solutions containing elements at 1000 mg L⁻¹ in 4% v v⁻¹ HNO₃ (SpecSol, São Paulo, Brazil).

Internal yttrium (Y) standard solutions were prepared by the appropriate dilution of monoelementar standard

solutions with 5% v v⁻¹ HNO₃ containing elements at 1000 mg L⁻¹ in 4% v v⁻¹ HNO₃ (SCP Science, Quebec, Canada).

Samples and certified reference material

The black pepper samples (peppercorn and ground) were acquired in March 2017 from markets in Espírito Santo, Brazil. The certified reference material (CRM) Agro C1003a (tomato leaves) was used to ensure the accuracy of the proposed method.

Sample preparation and procedures

All black pepper samples were previously dried at 60 °C for 72 h and crushed using a glass mortar and pestle. The samples were homogenized by manual shaking and the quartering was performed. About 200 mg of each sample were decomposed in triplicate with a mixture of 1 mL concentrated nitric, 1 mL hydrogen peroxide and 6 mL ultrapure water in a microwave digestion system. The heating program according to the equipment cookbook (method Organic B) was used. The program consisted of two steps: a heating ramp of 10 °C min⁻¹ up to 100 °C, a 10 min hold, a heating ramp of 18 °C min⁻¹ up to 180 °C, another 10 min hold and cooling to 50 °C. The tube contents were transferred to 15 mL polypropylene volumetric tubes and diluted with ultrapure water. The final solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and As, Cd, Co, Cu, Mn, Mo, Ni, Pb, Se and V concentrations were determined whereas Fe and Zn were determined by flame atomic absorption spectrometry (FAAS). Instrumental parameters are displayed in Table 1.

Statistical analysis

The variability of each element according to its origin (peppercorn or ground samples) was investigated. Due to the high number of elements determined, a principal component analysis (PCA) was applied to reduce the dataset size and to identify similarities between the analyzed samples. The reduction of the data set size by a PCA is performed by maximizing the variance of the data matrix $\mathbf{X}_{(n,m)}$, in this case formed by $n = 50$ samples and $m = 12$ variables (concentration of the determined elements), consisting in two modes: samples and variables.²²

Before the construction of the PCA model, data are self-scaled, since the variables that compose the matrix \mathbf{X} present different units and orders of magnitude. Calculations were performed using the MATLAB²³ version 8.1 R2013a software.

Table 1. Instrumental parameters

Parameter ICP-MS	Operating condition	
Plasma gas flow rate / (L min ⁻¹)	16	
Nebulizer gas flow rate / (L min ⁻¹)	1.2	
Auxiliary gas flow rate / (L min ⁻¹)	1.2	
Sample uptake rate / (mL min ⁻¹)	1.1	
RF power / W	1500	
Spray chamber	quartz cyclone	
Nebulizer type	concentric (Meinhard type)	
Torch	quartz EasyGlide TM	
Number of replicates	3	
Isotopes	⁷⁵ As, ¹¹¹ Cd, ⁵⁹ Co, ⁶³ Cu, ⁵⁵ Mn, ⁹⁸ Mo, ⁶⁰ Ni, ²⁰⁸ Pb, ⁸² Se, ⁵¹ V	
Internal standard	⁸⁹ Y (As, Mo, Se)	
Parameter F AAS	Operating conditions	
	Fe	Zn
Wavelength / nm	248.3	213.9
Slit / nm	0.2	0.5
Lamp current / mA	6.0	5.0
Flame	C ₂ H ₂ /air	C ₂ H ₂ /air
Burner height / mm	6	6
Fuel flow / (L h ⁻¹)	75	50
Oxidant flow / (L h ⁻¹)	225	–
Nebulizer rate / (mL min ⁻¹)	5	5

ICP-MS: inductively coupled plasma mass spectrometer; RF: radio frequency; FAAS: flame atomic absorption spectroscopy; C₂H₂: acetylene.

Results

Analytical figures of merit

First, the main analytical figures of merit regarding sample element determinations were evaluated. Analytical curves presented determination coefficient (R^2) higher than 0.999, indicating a good linearity of the method used. The limits of detection (LOD) and of quantification (LOQ), were calculated using International Union of Pure and Applied Chemistry (IUPAC) recommendation. The within-day precision (repeatability) was expressed as relative standard deviation (RSD). The results are shown in Table 2.

The accuracy of the analytical methods was verified through addition and recovery tests and the analysis of the tomato leaf Agro C1003a certified reference material. The additions were done prior to sample decomposition in order to obtain solutions with 5, 10 and 100 $\mu\text{g L}^{-1}$ of the elements to be determined by ICP-MS and 0.25 and 2.5 mg L^{-1} of the elements to be determined by F AAS. The recovery values obtained for each element are in accordance with the criteria suggested by the AOAC²⁴ for the concentration levels evaluated herein (Table 2). The values measured using the methodology adopted herein and the certified values were not significantly different as evaluated by the Student's *t*-test with a significance level of 95% (Table 3).

Trace element determination

Considering that the analysis methodology has adequate sensitivity and accuracy, all 12 elements were determined in 50 commercial pepper samples. Five black

Table 2. Figures of merit obtained using the proposed procedures for determination of elements in black pepper samples

Element	LOD for the method / ($\mu\text{g L}^{-1}$)	LOQ for the sample / ($\mu\text{g kg}^{-1}$)	Repeatability (RSD) / %	Recovery range / %
As	0.056	14.03	11	98-104
Cd	0.007	1.80	4.4	91-93
Co	0.004	1.12	6.5	96-102
Cu	0.451	113	2.6	79-109
Fe	0.085 ^a	21.3 ^b	5	96-110
Mn	0.116	29.14	4.1	99-107
Mo	0.013	3.34	3.9	92-99
Ni	0.134	33.53	3.9	91-98
Pb	0.009	2.32	11	81-89
Se	0.235	59.00	6.6	94-112
V	0.081	20.32	10	88-93
Zn	0.023 ^a	5.72 ^b	6	91-106

^a mg L^{-1} ; ^b mg kg^{-1} ; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

Table 3. Evaluation of the accuracy: certified and measured values of the tomato leaves certified reference material Agro C1003a (mean \pm standard deviation, n = 3)

Element	Concentration / (mg kg ⁻¹)	
	Certified value	Measured value
As	19.0 \pm 2.8	18.3 \pm 1.7
Cd	26.6 \pm 2.1	24.3 \pm 2.3
Co	0.33 \pm 0.14	0.25 \pm 0.02
Cu	1130 \pm 140	1250 \pm 121
Fe	1120 \pm 190	1370 \pm 31
Mn	470 \pm 69	538 \pm 59
Ni	3.16 \pm 0.87	2.80 \pm 0.14
Pb	3.67 \pm 0.55	2.91 \pm 0.25
Zn	37.5 \pm 5.0	36.03 \pm 1.65

peppercorn samples and 5 ground samples from each brand were evaluated, to verify the possibility of a trace element source during processing. The samples comprised five different brands: A, B, C, D and E. Each sample was chosen from a different lot in order to obtain a greater variability of the set of samples commercialized in the study region.

The results for each element in 25 samples of black peppercorn and 25 samples of ground black pepper are shown in Figures 1 and 2. In this representation, the asterisks indicate the elements which the concentrations shown significant differences between the peppercorn and ground samples when the Student's *t*-test was applied with 95% confidence. Based on these results, we have an indication that the processing can change the concentrations of the elements in the samples. For Fe, the range obtained for the commercially ground samples showed values higher (69.8-1147 mg kg⁻¹) than the range obtained for samples commercialized in grain (16.6-137 mg kg⁻¹). A similar behavior was observed for Pb (peppercorn: 10.9-88.4 μ g kg⁻¹; ground: 21.3-947 μ g kg⁻¹) and V (peppercorn: <20.3-141.7 μ g kg⁻¹; ground: 64.1-1072 μ g kg⁻¹). On the other hand, As showed lower concentrations in ground samples (peppercorn: <14.0-2623 μ g kg⁻¹; ground: 40.2-580 μ g kg⁻¹).

The increase in concentrations of Fe, Pb and V during processing is, in fact, quite critical, however, it is noteworthy that the difference between the concentrations in the peppercorn and ground samples depends directly from the manufacturer. The mean concentrations of Fe obtained in the samples processed by

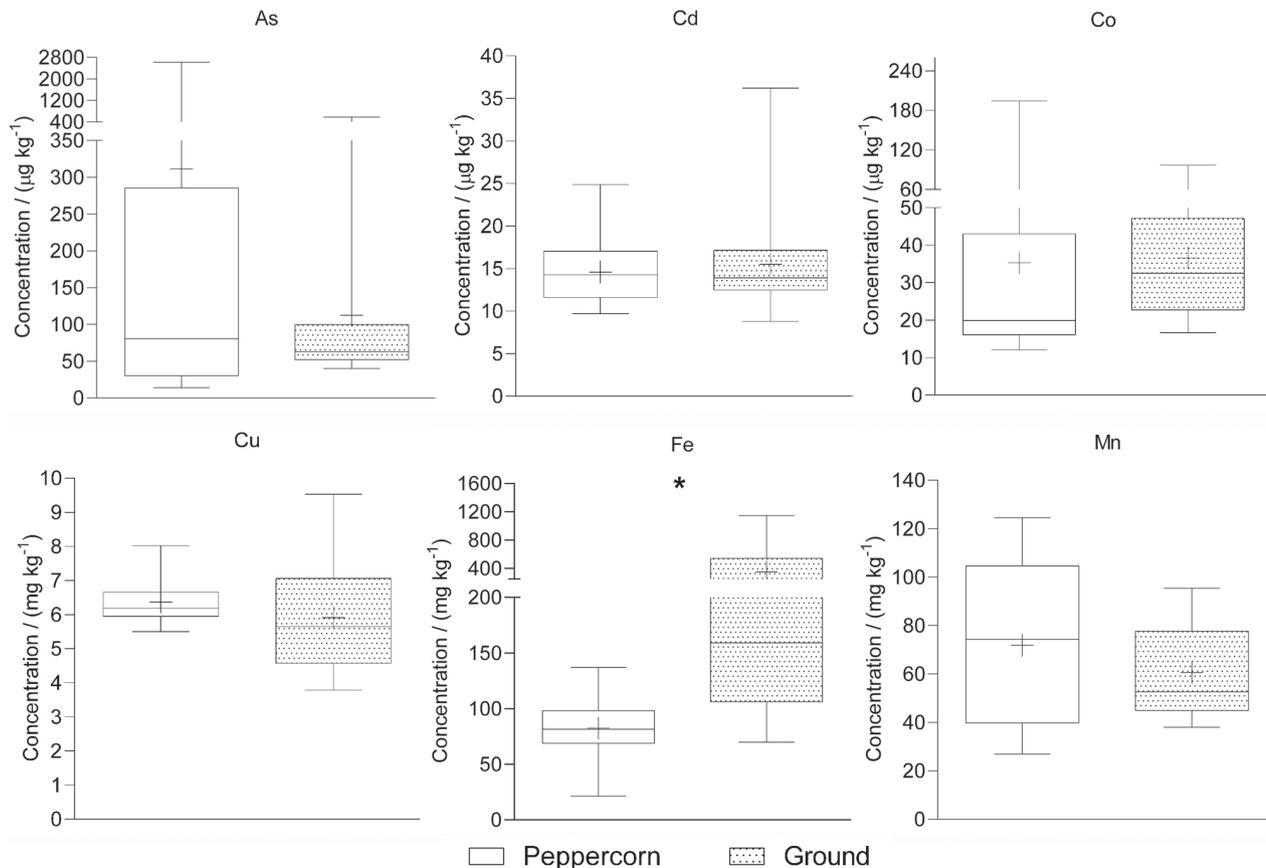


Figure 1. Boxplot of As, Cd, Co, Cu, Fe and Mn concentration in the peppercorn and ground pepper samples. * $p < 0.05$.

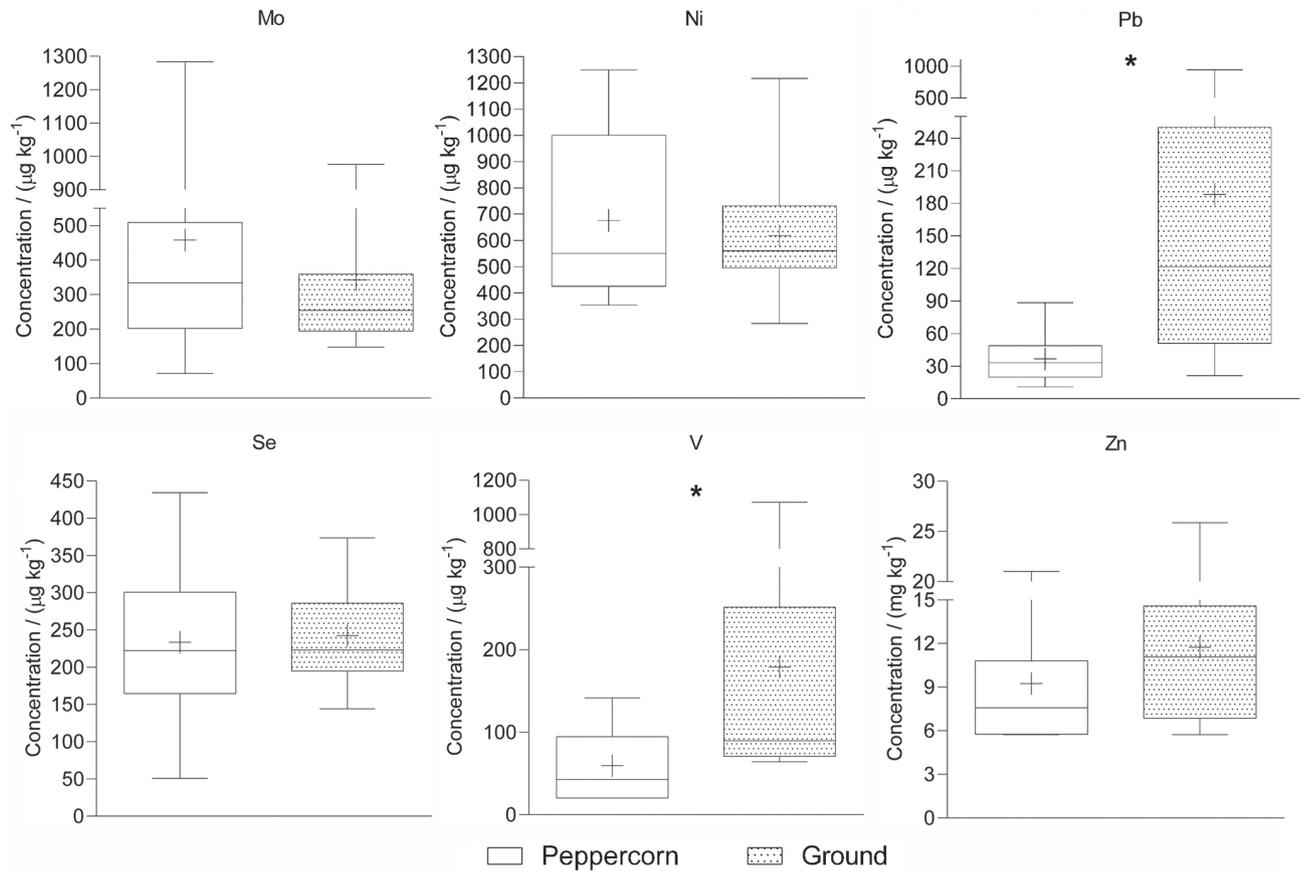


Figure 2. Boxplot of Mo, Ni, Pb, Se, V and Zn concentration in the peppercorn and ground pepper samples. * $p < 0.05$.

the manufacturers A (peppercorn: $92.29 \pm 15.50 \text{ mg kg}^{-1}$, ground: $94.78 \pm 36.83 \text{ mg kg}^{-1}$) and B (peppercorn: $83.74 \pm 40.25 \text{ mg kg}^{-1}$, ground: $131.8 \pm 24.2 \text{ mg kg}^{-1}$), for example, showed no significant difference in relation to the peppercorn samples. However, the samples processed by the manufacturers C (peppercorn: $70.37 \pm 11.37 \text{ mg kg}^{-1}$, ground: $217.4 \pm 105.5 \text{ mg kg}^{-1}$), D (peppercorn: $83.76 \pm 32.80 \text{ mg kg}^{-1}$, ground: $593.8 \pm 365.8 \text{ mg kg}^{-1}$) and E (peppercorn: $81.28 \pm 18.87 \text{ mg kg}^{-1}$; ground: $714.9 \pm 287.0 \text{ mg kg}^{-1}$), showed significant differences in Fe concentrations as compared to unprocessed samples (95% confidence level). Regarding the As element concentrations, when the Student's *t*-test was performed at 95% confidence, it was not possible to observe significant differences after processing. This is due to the great variability between the batches of analyzed samples that is evidenced by the high standard deviations obtained for this element. The median and range concentrations can be found in Table S1 of the Supplementary Information (SI) section.

Principal component analysis

The results presented here were even more evident when the PCA model was applied. The PCA scores plot

(Figure 3) indicate that the pepper processing stage did not significantly affect element concentrations in samples from brands A, B and C. However, a larger dispersion in samples from brands D and E according to their origin (peppercorn or ground) was observed.

Independent of processing, brands A, B and C are grouped, indicating that the applied grinding process did not lead to significant modifications in the composition of the evaluated elements for these brands. However, for the D and E brands, the ground samples presented higher dispersion, indicating that the processing influences the element concentrations, especially for Fe, Pb and V, as indicated by the PCA loading plot (Figure 3).

The PCA model was built from all results obtained in the analysis of 50 black pepper samples. Figure S1 of the SI section shows the explained variance for the first 10 main components of the self-scaled data. The first two main components explain 44.52% of the total data variability. This percentage is expected, due to variations among different brand batches and adopted pre-processing.

Discussion

According to the results, the processing adopted by

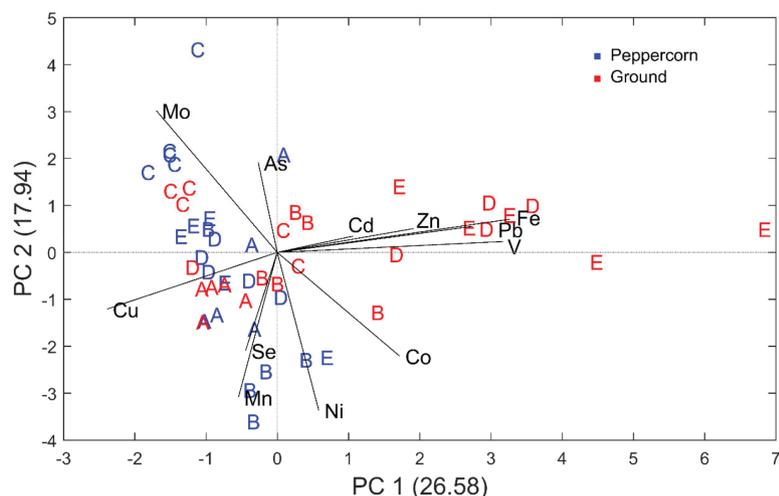


Figure 3. Principal component analysis (PCA) biplot: scores and loading.

some of the manufacturers (D and E) is incorporating trace elements to the final product, whereas some manufacturers are able to obtain the processed pepper without risk and without loss of quality. More critically, it was possible to observe an increase in Fe, Pb and V concentrations.

The higher concentration of Fe found in the processed samples can be attributed to contamination during the grinding process. Panduwawala *et al.*,⁶ compared the iron content present in samples grinded in different mills: mortar and pestle, grinding stone, food blender and commercial mills. The author observed that spice grinding in commercial mills can lead to the incorporation of 3 to 5 times more Fe, due to the wear of the grinding equipment used in this process. High levels of Fe have also been reported by De La Calle *et al.*²⁰ ($1264 \pm 216 \text{ mg kg}^{-1}$), Jawad²¹ ($481.28 \pm 2.37 \text{ mg kg}^{-1}$), Karadaş *et al.*¹⁹ ($158 \pm 19 \text{ mg kg}^{-1}$), Matloob⁸ ($194.1 \pm 39.03 \text{ mg kg}^{-1}$), Özcan *et al.*¹⁷ ($89.24 \pm 11.43 \text{ mg kg}^{-1}$), Seddigi *et al.*⁷ ($144 \pm 9.2 \text{ mg kg}^{-1}$), Singh and Garg¹⁴ (76.0 mg kg^{-1}), Soliman²⁵ ($620.02 \pm 541.36 \text{ mg kg}^{-1}$) and Soylak *et al.*¹² ($281.8 \pm 20.5 \text{ mg kg}^{-1}$).

The low concentrations of Pb detected in the peppercorn samples may reflect the uptake of this element by the plant from the contaminated soil.²⁶ Meanwhile, the high levels of Pb found in the samples after processing may indicate contamination during this process.

Intentional adulteration of spices has been widely reported worldwide in recent decades and is considered a public health problem in some countries.²⁶ The culinary consumption of spices from India has raised the blood lead level (BLL) of American children.^{27,28} Some studies have drawn attention to the high concentrations of Pb in black pepper samples as related by Abou-Arab *et al.*¹⁰ ($1.1 \pm 0.6 \text{ mg kg}^{-1}$), Baysal and Akman²⁹ ($0.88 \pm 0.59 \text{ mg kg}^{-1}$), Jawad²¹ ($5.97 \pm 1.09 \text{ mg kg}^{-1}$),

Özcan *et al.*¹⁷ ($0.88 \pm 0.14 \text{ mg kg}^{-1}$), Baysal and Akman¹⁸ ($0.88 \pm 0.59 \text{ mg kg}^{-1}$) and Soylak *et al.*¹² ($1.44 \pm 0.15 \text{ mg kg}^{-1}$).

Evidence shows that some manufacturers add certain substances to condiments in order to add flavor, to enhance its weight and color. These substances may contain trace elements, such as Pb.²⁶ According to Cowell *et al.*,²⁶ adulteration of turmeric with lead chromate (PbCrO_4) and ground paprika with lead oxide (Pb_3O_4) is a concern in India and Bangladesh. This same work mentions that producers reported that merchants use lead chromate during boiling and polishing to make spices brighter and to hide the marks of pest attacks in order to attract buyers.²⁶

Although paprika and turmeric contamination are more common, manufacturers generally use the same grinding mill for all spices which can lead to cross-contamination. In addition, it is very common for spices to be marketed in the form of blends.

Another element that showed an increase in its concentration after processing was vanadium. The results also indicated a possible contamination during the processing. Although sources of contamination are not easily identifiable, studies show significant amounts of this element are apparently introduced by mechanical means during food processing.³⁰

Although high concentrations of certain elements were detected, it is important to estimate the amount of black pepper that can be ingested without risk to consumers. As there are no specific laws to the maximum amount of Co, Cu, Fe, Mn, Mo, Ni, Se, V and Zn allowed in the peppercorns, the levels found were compared with National Academy of Sciences (NAS) of the United States of America recommendations on tolerable daily intakes.

The NAS establishes Recommended Dietary Allowances (RDAs) for Co ($2\text{--}3 \mu\text{g day}^{-1}$), Cu (0.9 mg day^{-1}),

Fe (8 mg day⁻¹ for men and 18 mg day⁻¹ for women), Mo (45 µg day⁻¹ for men and women), Se (55 µg day⁻¹ for men and women), Zn (11 mg day⁻¹ for men and 8 mg for women) and Mn (2.3 mg day⁻¹ for men and 1.8 mg day⁻¹ for women), besides the Tolerable Upper Intake Levels (ULs) established for Ni (1.0 mg day⁻¹) and V (1.8 mg day⁻¹).³¹ In order to surpass these values, a very high daily consumption of black pepper would be necessary. Although data on daily consumption for black pepper are not available, it is unlikely that a person consumes high amounts of this spice daily to cause toxic effects on consumer health. However, would be interesting that investigation about the bioavailability of trace elements in spices be conducted.

There are different legislations worldwide on contaminants in foodstuffs that set maximum levels for lead and cadmium in order to protect public health. Commission Regulation (EC) No. 1881/2006³² recommends the maximum limit of 0.10 mg kg⁻¹ for Pb (vegetables category) and Commission Regulation (EC) No. 488/2014³³ recommends the maximum limit of 0.05 mg kg⁻¹ for Cd (vegetables and fruit category). According to the Brazilian National Health Surveillance Agency, ANVISA,³⁴ the maximum limit for Cd and Pb is, respectively, 0.050 and 0.10 mg kg⁻¹ (fruits vegetables other than the family Cucurbitaceae category). Considering these limits, Cd levels obtained in black peppercorns and ground black pepper are accepted. However, Pb contents are above the guidelines in some ground black pepper samples, indicating possible contamination during the processing of this spice. It is important to notice that the guidelines used as reference do not show limits for black pepper and this way it is difficult to do more considerations about the higher levels found in ground black pepper. This situation points out to the necessity to have a specific guideline to spices, considering that some studies have shown that the lead present in spices can be easily absorbed. Lin *et al.*²⁷ obtained an average bioaccessibility value of Pb in a spice mixture equal to 49% and Gleason *et al.*³⁵ obtained an average bioaccessibility in turmeric of 42.9%. In black pepper, Cowell *et al.*²⁶ found a bioavailability value of 70%. These studies report the risk of Pb intoxication due to its high bioavailability.

Conclusions

The influence of the processing on trace elements levels was evaluated in black pepper samples and high Fe, Pb and V concentrations were observed in ground black pepper for two brands investigated. These increased levels suggest that there is contamination during the processing. Although specific legislation for spices is not available in Brazil,

some samples presented Pb levels above to the safe limits for human consumption in accordance with Brazilian and European legislation for vegetables and fruits.

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

Acknowledgments

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