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Fresh and pasteurized orange juice analysis by TXRF

Análise de suco de laranja in natura e pasteurizado por TXRF

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Abstract

Orange is considered the main product of the Brazilian citrus agro-industrial complex. However, in the end of 2016, the ANVISA pointed out orange as a risky product due to contamination using pesticides in its cultivation. Therefore, in this context, an analysis of the chemical elements present in fresh and pasteurized orange juices becomes extremely relevant. Thus, this work aims to quantify the elements that are present in fresh and pasteurized orange juices, using the TXRF technique. Samples of fresh orange juice of Pêra variety were acquired in a store in the city of Londrina-PR, were analysed; three samples of oranges were purchased at a store in Itápolis, São Paulo and five more samples of Pêra orange were obtained in the rural area. Samples of pesticides used in orange cultivation were quantified, and three different trademarks of pasteurized juice were analysed. In some of the samples that were collected in Itápolis-SP market, lead (Pb) element was quantified, however its concentration was within the limit established by Brazilian legislation. The aluminium (Al) element was detected in all samples of pasteurized orange juice, showing the influence of the packaging on the elemental concentration of the juice. All pesticides quantified in this work showed a significant concentration of some micro-contaminants, but when the pesticide was diluted in deionized water, those micro-contaminants could not be quantified.

Keywords: XRF. Orange juice. Pesticides. Contamination. Elementary concentration

Resumo

A laranja é considerada o principal produto do complexo agroindustrial cítrico brasileiro. Porém, no final de 2016, a ANVISA apontou a laranja como um produto de risco devido à contaminação por agrotóxicos em seu cultivo. Nesse contexto, a análise dos elementos químicos presentes nos sucos de laranja in natura e pasteurizados torna-se extremamente relevante. Desse modo, este trabalho visa quantificar os elementos que estão presentes em sucos de laranja in natura e pasteurizados, através da técnica de TXRF. Foram analisadas, amostras de suco de laranja in natura da variedade Pêra, adquiridas em loja da cidade de Londrina-PR; três amostras de laranja foram adquiridas em uma loja em Itápolis, São Paulo e cinco amostras de laranja Pêra foram obtidas na área rural. Foram quantificadas amostras de agrotóxicos utilizados no cultivo da laranja e analisadas três marcas diferentes de suco pasteurizado. Em algumas das amostras coletadas no mercado de Itápolis-SP foi quantificado o elemento chumbo (Pb), porém sua concentração ficou dentro do limite estabelecido pela legislação brasileira. O elemento alumínio (Al) foi detectado em todas as amostras de suco de laranja pasteurizado, mostrando a influência da embalagem na concentração elementar do suco. Todos os pesticidas quantificados neste trabalho apresentaram uma concentração significativa de alguns micro-contaminantes, mas quando diluído em água deionizada, esses micro-contaminantes não puderam ser quantificados.

Palavras-chave: TXRF. Suco de laranja. Agrotóxicos. Contaminação. Concentração elementar.

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Introduction

The global pursuit for a healthier lifestyle has brought a considerable increase in the consumption of natural juices, among which orange juice stands out. Consequently, this fruit production sector has been gaining market shares in several countries (FERRAREZI, 2008).

Until 2010, Brazil held more than 50% of the world production of orange juice, being responsible for 85% of the world exports of the product (NEVES *et al.*, 2010). São Paulo State is the region that concentrated just over 70% of national orange culture (FERNANDES, 2010).

Brazil, currently the largest global exporter of orange juice, shipped approximately 870 thousand tons of the product considering the 2018/2019 harvest, which resulted in an income of US $ 1.6 billion according to National Association of Citrus Juice Exporters (CITRUSBR, 2019).

However, at the end of 2016, the Brazilian National Health Surveillance Agency (ANVISA) released a report on the Program for Analysis of Pesticide Residues in Food (PARA), pointing out orange as a product with highly contamination propensity, observed at risk rates in 11% of the analysed samples. Some types of contamination related to the application of pesticides, such as drift, cross contamination and the soil itself, can result in the presence of residues in the orange (FERNANDES, 2010).

Within this perspective, this work aims to quantify the elements which are present in fresh and pasteurized orange juices, through the technique of Total Reflection X-ray Fluorescence (TXRF), in order to verify the presence or absence of elements coming from the pesticides used in orange cultivation. Thus, also check if juices are contaminated by these harmful elements. The contamination level will be established based on the comparison of the elements concentration with the limits established by the Brazilian legislation (ANVISA, 1965).

Materials and methods

To achieve the proposed objective, three samples of fresh orange juice of Pêra variety were analysed, the oranges were acquired in a store in the city of Londrina in Parana; three samples of oranges of the same variety were purchased at a store in Itápolis, São Paulo; five more samples of Pêra orange were obtained in the rural area, also located in the city of Itápolis-SP. In this same farm in the countryside of São Paulo, samples of pesticides used in orange cultivation were acquired: Carfentrazone Ethyl (herbicide), Metarhizium (insecticide), Propargito (acaricide) and Piridabem (acaricide). Subsequently, a diluted sample of the Piridabem pesticide was quantified. This pesticide has a high elemental concentration of lead and it was utillized in the same proportion used by a farmer, which is, 1 ml of the pesticide for each litre of deionized water.

Finally, three different trademarks of pasteurized juice were analysed, and for each trademark, three boxes (three samples) of the product were investigated. For each sample, three repetitions were prepared, and each repetition was measured in triplicate, resulting, therefore, in a total of nine spectra. The average and its standard deviation were calculated using the total gathered data.

The main stages of orange processing in the production of pasteurized juice are receiving, washing, selection and classification, extraction, finishing, pasteurization, cooling and storage (FERRARA, 2003).

The harvested oranges are transported in carts to the companies' yard, where they are stored at the reception docks. Successively, at the top, the oranges are washed with chlorinated water, on mats equipped with nozzles, while at the bottom, rotating brushes are responsible for the fruit mechanical cleaning and movement.

The extraction of the juice is carried out by equipment that operate by compressing the whole fruit, in order to allow the juice to escape through a cylinder, preventing the incorporation of foreign material inside the mechanism (TOCCHINI; NISIDA; MARTIN, 1995).

The pasteurization step consists of a moderate thermal treatment used for foods to annihilate vegetative microorganisms, such as pathogens, and inactivation of enzymes (JOGEN, 2002). The orange pasteurization process takes place at a minimum temperature of 95 ºC, for 20 seconds, to then be cooled and packaged (TRIBESS, 2003).

For this work, a standard model for TXRF PicofoxBruker equipment was used, which is an acrylic cylindrical disc sample holder with three centimeters in diameter. A volume of 180 μl of each sample was pipetted into an Eppendorf and mixed with 20 μl of a standard solution containing Gallium, resulting in a concentration of 1 ppm of Ga. A volume of 5 μl of the resulting solution was pipetted on one side of the acrylic disc and dried in an oven at a temperature of 80 ºC, for approximately 20 minutes, in order to produce a thin film on the substrate, before the TXRF analysis.

The samples were analysed using an S2 Picofox system from Bruker S.A., irradiated for a period of 500 seconds, at a voltage of 50 kV and a current of 602 μA, resulting in a power of 30 W. In all the analysed tests, the bottom of the sample holder was measured for future decontamination. In all spectra, background radiation was subtracted before the sample concentration was established.

Results and discussions

Fresh juice samples

Table 1 illustrates the average concentration of each element, as well as its standard deviation. The analyses were based on fresh juice samples acquired in commercial establishments in the cities of Londrina-PR and Itápolis-SP and Itapolis' rural area.

According to ANVISA (1965), the maximum level for Cr, Cu, Zn and Pb elements established by Brazilian legislation are: 0.1 mg/l, 30 mg/l, 50 mg/l and 0.5 mg/l, respectively.

In all orange samples gathered from purchases at both Londrina-PR and Itápolis-SP market, the presence of Pb element was identified in a concentration below the regulated level by the Brazilian legislation.

Zn and Cu elements were also found in all samples collected in the city of Itápolis-SP. However, the concentration of these elements does not pose a risk to human health.

Another relevant fact is the presence ofChromium (Cr) element in samples from Itápolis' markets. These were concentrated in a level 10 times higher than that acceptable by ANVISA. Chromium is an essential element for human health, considered a trace element, however it can act as a micro-contaminant when found in excessive concentrations in the body. In excess, this element can cause side effects, such as tiredness, loss of appetite, tendency to bruises, nausea, headaches, dizziness, urinary changes, nasal bleeding and skin reactions like hives (RUPPENTHAL, 2013).

The concentration of the element Fe for this group of samples, samples from Itápolis' markets, has a higher value when compared to samples from rural areas, which may indicate a difference in the cultivated soil. It is important to mention that, even though, pesticides are used on the fruit collected in Itápolis rural area, the presence of Pb element was not detected.

Figure 1 shows one of the spectra obtained for one of the samples collected in the commercial area of Itápolis. This spectrum shows the presence of the element Cr for the analyzed sample, being highlighted by the arrow. The blue line in the spectrum corresponds to the ROI, which represents the region of interest for analysis, while the pink line in the spectrum corresponds to all measured elements. When selecting the ROI option for the region of interest, the software will ignore the others.

Pasteurized juice samples

Table 2 shows the results obtained for the samples of pasteurized orange juice.

Note that, in Table 2, all pasteurized orange juice samples have aluminium (Al) as a common element, an element that was not detected in fresh orange juice samples. This shows the influence of the storage packaging on the elemental composition of the juice.

The excess of aluminium in the human body can cause serious damage to the health and, according to the literature, the excess of this element directly impacts the occurrence of neurodegenerative diseases, especially Alzheimer's, autoimmune diseases and even breast cancer (FERREIRA *et al*., 2008). Although aluminium is considered a micro-contaminating element, the Brazilian legislation does not provide any information on the acceptable limit for this element.

Strontium (Sr), has also been detected in two different juice trademarks. This element is usually used in industrial sectors, such as oral hygiene. For instance, hexahydrate strontium chloride salt is applied in toothpastes for teeth sensitivity. Regarding metal oxide adsorption in the soil (CHIANG *et al*., 2010), point out the great influence of Sr adsorption on Fe and Al oxides on Sr2+ mobility.

Chromium was also detected in Trademark 1 juice samples. While Pb element was detected in all samples, Trademark 1, 2 and 3, it was below the concentration established by Brazilian legislation.

Figure 2 shows one of the spectra obtained for one of the samples of pasteurized juice analyzed in this work. The arrow highlights the presence of the Al element in the sample.

Standard deviation in the pesticide's samples

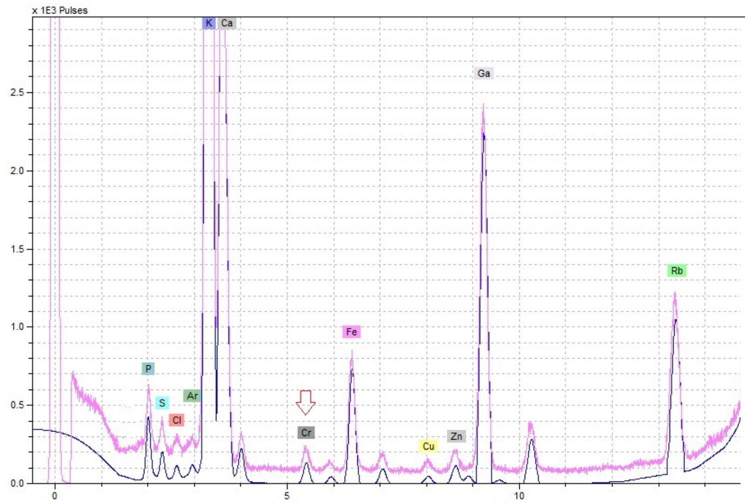
Table 3 demonstrates the average concentration of each element, as well as its quantified standard deviation in the pesticide's samples. S element is quantified in a higher concentration in Propargite and Piridabem acaricides. The amount of chlorine found in Carfentrazone herbicide is approximately 12 times higher than in Metarhizium and Propargito pesticides. Potassium is also highly measured on Metarhizium insecticide.

Table 1 – Average concentration of each element, as well as its standard deviation for fresh orange juice.

	Average Concentration (mg/l)		
Elements	**Londrina-PR market**	**Itápolis-SP market**	**Itápolis-SP rural area**
P	15 ± 4	41 ± 5	5 ± 2
S	1.7 ± 0.2	7 ± 1	2 ± 1
Cl	2.6 ± 0.4	26 ± 5	2.3 ± 0.7
K	309 ± 60	593 ± 32	196 ± 12
Ca	10 ± 1	13 ± 1	4.2 ± 0.5
Ti	0.58 ± 0.04	1.4 ± 0.4	-
Cr	-	1.0 ± 0.4	-
Mn	0.084 ± 0.004	0.09 ± 0.05	-
Fe	4 ± 1	9 ± 4	1.3 ± 0.4
Cu	-	0.07 ± 0.01	0.06 ± 0.02
Zn	0.103 ± 0.004	0.7 ± 0.4	0.04 ± 0.01
Br	0.024 ± 0.002	0.090 ± 0.009	0.015 ± 0.005
Rb	1.4 ± 0.5	0.39 ± 0.08	0.13 ± 0.05
Pb	0.10 ± 0.03	0.2000 ± 0.001	-

Source: The authors.

Figure 1 – Spectrum obtained for one of the samples from the Itápolis-SP trade. The pink line of the spectrum corresponds to the element's measurement, while the blue line corresponds to the ROI.



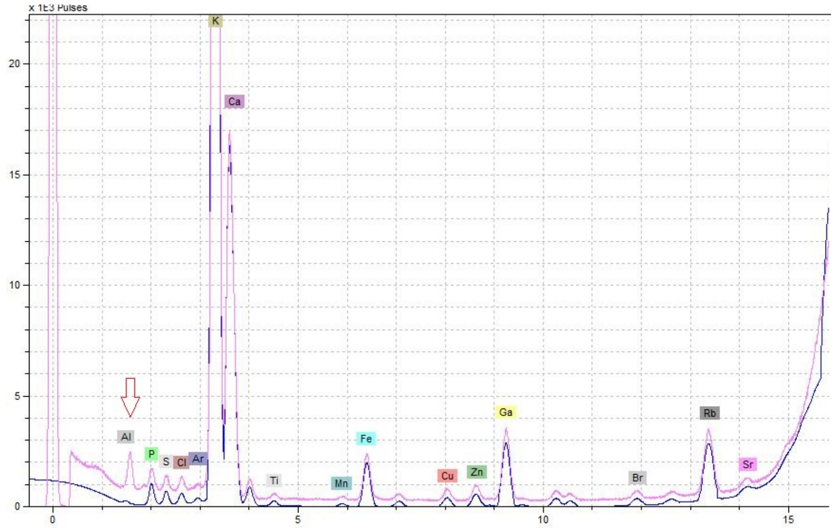
Source: The authors.

Table 2 – Average concentration of each element, as well as its standard deviation for pasteurized orange juice.

	Average Concentration (mg/l)		
Elements	**Trademark 1**	**Trademark 2**	**Trademark 3**
Al	0.30 ± 0.09	0.9 ± 0.1	1.6 ± 0.5
P	31 ± 7	32 ± 2	14 ± 3
S	8 ± 2	7.9 ± 0.5	4.1 ± 0.9
Cl	14 ± 3	3.9 ± 0.4	1.9 ± 0.7
K	461 ± 87	380 ± 35	280 ± 20
Ca	67 ± 18	15 ± 1	13 ± 2
Ti	0.60 ± 0.06	0.46 ± 0.05	0.6 ± 0.2
Cr	0.022 ± 0.004	-	-
Mn	0.19 ± 0.03	0.10 ± 0.01	0.13 ± 0.03
Fe	1.0 ± 0.4	1.2 ± 0.1	2.1 ± 0.8
Cu	0.04 ± 0.01	0.06 ± 0.02	-
Zn	0.20 ± 0.01	0.11 ± 0.01	0.4 ± 0.1
Br	0.07 ± 0.02	0.08 ± 0.01	0.038 ± 0.004
Rb	0.60 ± 0.06	0.62 ± 0.08	0.62 ± 0.08
Sr	0.5 ± 0.1	0.06 ± 0.02	-
Pb	0.015 ± 0.009	0.015 ± 0.006	0.015 ± 0.006

Source: The authors.

Figure 2 – Spectrum obtained for one of the 3 trademark's. The pink line of the spectrum corresponds to the element's measurement, while the blue line corresponds to the ROI.



Source: The authors.

Table 3 – Average concentration of each element and its respective standard deviation for pesticides samples.

	Average Concentration (mg/l)				
Elements	**Carfentrazona**	**Metarhizium**	**Propargito**	**Piridabem**	**Dilluted Piridabem**
P	-	5588 ± 285	-	795 ± 68	-
S	916 ± 47	6 ± 2	3878 ± 343	5082 ± 444	5.8 ± 0.1
Cl	12774 ± 1265	410 ± 82	176 ± 16	6785 ± 609	5.7 ± 0.2
K	12 ± 1	9340 ± 628	6.1 ± 0.1	983 ± 87	0.101 ± 0.004
Ca	2.6 ± 0.6	72 ± 18	125 ± 4	517 ± 35	0.88 ± 0.03
Ti	0.6 ± 0.2	15 ± 2	8 ± 2	41 ± 5	-
Cr	-	-	-	17 ± 2	-
Fe	2 ± 1	42 ± 14	13 ± 7	144 ± 15	0.042 ± 0.005
Cu	0.20 ± 0.04	-	-	-	-
Zn	8 ± 3	33 ± 11	-	5 ± 1	-
Br	0.60 ± 0.08	-	-	-	-
Rb	-	1.3 ± 0.1	-	-	-
Sr	1.16 ± 0.03	-	-	-	-
Pb	0.35 ± 0.04	3 ± 1	0.8 ± 0.2	13 ± 1	-

Source: The authors.

Ti and Fe element concentration are much higher in Piridabem acaricides, even higher than the concentration of these elements in the other analysed acaricide (Propargite). Copper was detected only in Carfentrazon defensive, in a very small concentration. Zinc was measured in greater concentration in Metarhizium insecticide. Br and Sr elements were found exclusively in Carfentrazona defensive, while Rb was found only in Metarhizium insecticide sample.

For the diluted Piridabem sample, the quantified elements were S, Cl, K, Ca and Fe. All elements were detected at a concentration well below than when the sample was prepared without dilution.

The elements Ti, Pb, P, Cr and Zn were not quantified in the post-dilution pesticide. These components are present in the elemental pesticide composition and after the dilution process, it was not possible to quantify them, as their concentration level was measured below the detection limit for the equipment used. This suggests that Pb and Ti elements found in some samples of juice in this work come from other sources and not from pesticides.

Conclusions

The TXRF technique proved to be effective when it comes to analysing elements in orange juice samples and pesticides. With this technique, it was possible to correlate elements from different samples of orange juice, as well as samples of pesticides. Furthermore, it aided to identify a possible contamination in orange juice consumed by the general population.

All pesticides quantified in this work showed a significant concentration of some micro-contaminants, such as Cr and Pb, but when the pesticide was diluted in deionized water in the proportion used by the farmer, those micro-contaminants could not be quantified because they measured at a concentration rate below detection limit of the equipment used. It is possible that differences in the composition of the soil directly influence the elemental composition of the juice.

In some of the samples that were collected in Itápolis-SP market, Pb element was quantified, however its concentration was within the limit established by Brazilian legislation. Cr element was quantified at a concentration rate above the established limit.

Considering the first measurement step, aluminium element was detected in all samples of pasteurized orange juice, clearly showing the influence of the packaging on the elemental concentration of the juice.

Finally, taking into account the detection limit of the equipment used, it is not possible to affirm that the micro-contaminating elements present in the analysed orange juice samples come from pesticides, since Pb element was not quantified in any of the orange samples collected in the rural area of Itápolis.

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