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Effects of Pre- and Post-Harvest Factors on the Selected Elements Contents in Fruit Juices

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Abstract

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Pre- and post-harvest factors determine the levels of the selected risk elements in100% fruit juices. The juices samples closely followed the Brix international reference values. Fruit juices presented the following order of the elements mean concentrations: cadmium (1.597 µg/l), chromium (2.767 µg/l), lead (20.75 µg/l), nickel (73.37 µg/l), zinc (545.9 µg/l), and iron (1792 µg/l), measured by AAS. The pre-harvest factors (origin, fruit, and agriculture) and the post-harvest factors (blending, packaging, conservation, pasteurisation, and process) were evaluated according to the manufacturers information and were correlated with the elements concentrations of fruit juices. A strong relationship was detected between the fruit species used for the juice production (i.e. pre-harvest factor) and their elements concentrations. Furthermore, multiple correspondence analysis was used for reducing the data dimension by grouping the factors. The zinc concentration was detected as a potential proxy for the identification of the fruit juices manufacturing process.

Keywords: processing effects; trace metals; atomic absorption; chemometric

Mineral elements are nutrients that have a significant role in the regulation of the human body metabolism. Fruits and their juices contain various mineral elements in their composition, so they are important sources of minerals in human nutrition (Wall 2006; Ribeiro *et al*. 2009; Pennington & Fisher 2010).

Also, the intake of juices has been associated with the reduction of the risk of several types of cancer, thus increasing the public interest in these products (Chang *et al*. 2005).

The worldwide consumption of fruit juices has been increasing in recent years, requiring more extensive and better production efficiency, sustained by technological development. However, their equivalence to fresh fruits in health benefits has been questioned due to the high level of processing involved in the production of juices (AIJN 2010; Baker *et al*. 2012; Harmankaya *et al*. 2012).

The fruit juice products are obtained by two different types of processing: pure juices are produced directly by extraction from the fruit, without dilution; juices from concentrates are produced by adding an adequate amount of water to the concentrate, previously obtained by partial dehydration of the fruit juices (Ashurst 2005; Mobhammer *et al*. 2006; Keshani *et al*. 2010).

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The Portuguese legislation on the fruit juices products follows the Codex Alimentarius standards (FAO/WHO 2005), and best practices code of the Association of the Industry of Juices and Nectars (AIJN) which publishes a table that sets a Brix graduation reference for fruit juices (AIJN 2012).

The quality of the fruit juices depends primarily on the quality and variety of the fruits used for processing. Some varieties of the same fruit are more suited for the preparation of juice than others (Echeverria *et al*. 2002; Versari *et al*. 2002).

The processing operations introduce major changes in the fruits and will also be a determinant in the final product quality, particularly in view of the nutritional value provided by the chemical composition (Rodushkina & Magnusson 2005; Ros *et al*. 2007; Bevelacqua *et al*. 2011; Bhattacherjee *et al*. 2011; Tangahu *et al*. 2011; Fawole & Oto 2013).

The fruit cultivar, agricultural practice, and processing may be crucial factors determining the elemental composition of the juice, so that pre- and post-harvest factors determine the expected elemental concentration of fruit juices (Cisse *et al*. 2005; Nienaber & Shellhammer 2005; Léchaudel & Joas 2007; Jalbani *et al*., 2010; Fernandes, *et al*. 2011; Bragança *et al*. 2012).

In this work, elemental characterisation was made of some major industrial contaminating metals in 100% fruit juices available in the Portuguese market, which was correlated with the pre-harvest factors; origin (country), agriculture (conventional or organic), fruit (kind of fruit used), and the post-harvest factors; blending (fruits %), packaging (type of pack), conservation (refrigerated or ambient), pasteurisation (heat or high pressure), process (extraction or dilution), and Brix values.

MATERIAL AND METHODS

Three replicates were made for each sample measurement, considering as acceptable only the results with the coefficient of variation less than or equal to 10%. The water used in all laboratory procedures was 18.2 MΩcm (25°C) ultra-pure water Millipore Simplicity.

Sampling. A sample of 62 packs was collected of fruit juices (one pack for each different product of each producer) which were acquired randomly and as close as possible in time from major supermarkets in Portugal. All packs were kept refrigerated.

Determination of Brix graduation. In order to determine the sample dilution, the Brix graduation was measured with a refractometer model DBR45 (Tsingtao Unicom-Optics Instruments Co., Laixi, China), with automatic temperature compensation for aqueous solutions between 5°C and 40°C, with the temperature reading accuracy of ± 0.5°C, Brix range measuring was between 0 and 45 °Bx and Brix reading accuracy was ± 0.1 °Bx.

The device was calibrated, using ultra-pure water.

Sample preparation for elemental analysis. For separating materials in suspension, the juice samples were placed in polypropylene centrifuge tubes of approximately 50 ml and centrifuged at room temperature in a Hettich EBA12 centrifuge (Andreas Hettich GmbH, Tuttlingen, Germany) at a rotation speed of 6000 rpm for 60 minutes. The separated liquids were decanted into a sample holder and placed in a refrigerator for cooling.

The process of wet digestion was used applying with nitric acid in a closed system (Morte *et al*. 2008; Cindric *et al*. 2011). The samples of juices were placed in digestion tubes, of 16 mm diameter with screw caps, Merck COD Spectroquant. Into each tube 5 ml of sample and 0.5 ml of nitric acid (65% p.a.; Sigma-Aldrich, Steinheim, Germany) were added. Subsequently, after sealing the tubes digestion took place in a Spectroquant TR 320 Merck Thermoreactor (Merck, Darmstadt, Germany). The following temperature cycle was used in the digestion: 100°C/15 min, 120°C/15 min, and 148°C/15 min (Hseu 2004; Szymczycha-Madeja & Welna 2013). All samples appeared clear at the end of the digestion and with no suspended solids, the process being repeated if not. Finally, the samples were left to cool at room temperature, they the screw caps were carefully removed upon which all samples liberated pressurised gas indicating that all digestion procedures were performed well in a closed system.

The liquids obtained from the digestion flasks were placed in 10 ml volumetric flasks and the volume was made up with ultra-pure water. The storing in the refrigerator was made by transferring the contents of the volumetric flasks into glass tubes with screw caps which were properly identified.

Elemental analysis. Taking as reference the measurements made in wines by Rodrigues *et al*. (2011), and in fruit juices by Szymczycha-Madeja *et al*. (2014), the expected elemental concentration values of certain juices could be very low and in some cases even difficult to detect.

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The concentrations of Cd, Cr, Pb, and Ni were measured by graphite furnace atomic spectrometry, using an AAnalyst 300 Perkin Elmer, HGA 850 graphite furnace spectrometer with an A5 800 auto sampler.

In this system, as chemical modifiers were used (in aqueous solution), $NH_4H_2PO_4$, 40 g/l, for lead determination, and $Mg(NO_3)_2$, 0.3 g/l for the determination of cadmium, chromium, and nickel, respectively prepared from pa ammonium dihydrogen phosphate Riedel-de Haën and magnesium nitrate hexahydrate p.a. (Sigma-Aldrich). Also used were pirorevested graphite tubes, PerkinElmer HGA, with integrated platform.

The measuring operation began by introducing a known volume, in this case 20 µl of the sample, into the graphite tube chamber orifice, and the chamber was then subjected to a temperature multistep program, according to the analyte and sample matrix.

Still in this work, the concentrations of Zn and Fe were measured by flame atomic absorption (FAA), using an atomic absorption spectrometer Philips PU9100X (Bings *et al*. 2010).

In the two systems, FAAS and GFAAS, a solution of HNO_3 0.2% v/v, was used as: white, zero point calibration, and to dilutions. All operating conditions are described in Table 1.

Spiking tests were performed in four fruit juices samples. The % of recovery of each analyte was calculated in relation to the added element concentration and the range obtained was: Cd (94–115%), Cr (80–126%), Pb (98–109%), Ni (95–108%), Zn (84–127%), and Fe (96–106%). The % of recovery for all elements was always within the interval of 80–127%.

Statistical analysis. The statistical treatment of the data, namely multiple correspondence analysis, was done with IBM SPSS statistic software v. 20.

RESULTS

S***ample variables description***. Some sample information was obtained by examining the label, or by direct contact with the manufacturer.

Regarding the different countries (origin) in which the fruit juices were manufactured, they comprised: Spain (48%), Portugal (30%), South Africa (13%), France (4%), Germay (3%), and United Kingdom (2%). We assume that the manufacturing plant is geographically close to the cultivar at list in juice main fruit component.

Table 1. Operating conditions of GFAAS (Cd, Cr, Pb, Ni), and FAAS (Zn, Fe) measurements

Element	Cd	Cr	Pb	Ni	Zn	Fe
Wavelength (nm)	228.0	357.9	283.3	232.0	213.9	248.3
Maximum lamp current (mA)	15	10	20	25	10	15
Bandpass (nm)	0.7	0.7	0.7	0.2	0.5	0.2
Injection sample-modifier (µl)	20–10.	20-5.	20-5.	20-10.	–	–
Air/acetylene flame (l/min)	–	–	–	–	0.9–1.2	0.8–1.2
Calibration points (µg/l)	0; 0.5; 1; 5; 10	0; 5; 10; 15; 20; 25	0; 2.5; 5; 10; 25; 50	0; 5; 10; 25; 50; 100	0; 500; 1000; 1500; 2000	0; 1000; 2000; 3000; 4000
Standard solution 1.000 ± 0.002 (g/l)	Panreac	Panreac	Panreac	Panreac	Merck	Merck
Slope	0.03682	0.01746	0.00511	0.00389	0.00008	0.00002
Intercept	0.0280	0.0054	0.0118	0.0027	0.0156	0.0018
Coefficient of determination	0.99894	0.99796	0.99744	0.99924	0.99730	0.99591

Step	ramp time (s)	hold time (s)	internal gas flow (ml/min)	temperature (°C)					
1º Drying	5	20	250	120	120	120	120		
2º Drying	15	25	250	140	140	140	140		
3º Pyrolysis	10	20	250	850	1650	700	1400	–	
4º Atomisation	0	5	0	1650	2500	1800	2500		
5º Clean	1	3	250	2600	2600	2600	2600		

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Table 2. Elemental concentration mean, and 95% confidence interval of 100% fruit juices from the Portuguese market (in µg/l)

	Mean	95 % confidence interval of the mean	
		lower	upper
Cd	1.597	1.374	1.819
Cr	2.767	2.380	3.153
Pb	20.75	18.10	23.40
Ni	73.37	60.89	85.84
Zn	545.9	483.1	608.7
Fe	1792	1654	1930

As to the fruit species (fruit) in juices containing only onesort of fruit, following stand out: 22% of juices containing apple, 21% containing orange, and 21% containing other fruits. Two fruit species were contained in 31%, and a mixture of up to ten fruit species in 5%.

It was also possible to specify for each juice product its percentile (v/v) fruit composition, generally referred to in the ingredients label (blending set of factors: apple (%), orange (%), other fruits (%)).

Juices kept at ambient temperature with shelf life of twelve months (conservation) were packed mainly in Tetra Brik or Combiblock packs (packaging), along with glass bottles and Pure Packs. The juices kept under refrigeration with a shelf life of three months were packed either in HDPE or in PET bottles and only a small part in Pure Packs.

For the majority of juices (95%), the fruits were produced by conventional agriculture (agriculture) and only a small part of them (5%) by organic agriculture.

The pasteurisation treatment (pasteurisation) of juices was carried out in 81% by heat treatment, and in 19% by high pressure treatment.

The process of making the juice (process) was executed in 67% by concentrates dilution, and in 33% by direct extraction from fruits.

The Brix values of juices (Brix) ranged from 5.0 to 15.9. The average being 11.8 °Bx. In juices from mixed fruits, the Brix values were calculated, entering the weight of the amount of each fruit component in percentage of the mixture. It was verified that only 14% of the values found in the juice Brix graduation were below the minimum values of AIJN, but still very close to these values as all the rest of them.

Fruit juices elemental characterisation. The average concentrations of Cd, Cr, Pb, Ni, Zn, and Fe (Table 2) in the juices were estimated, calculating the intervals with a confidence level of 95%. The Kolmogorov-Smirnov test with Lilliefors correction and the Shapiro-Wilk test allowed to assume a normal distribution of these parameters, not rejecting the null hypothesis at the 0,05 significance level.

In the case of cadmium we estimated a mean value of 1.597 µg/l, the level rising up to 1.2 µg/l in the Brazilian juice market (Tormen *et al*. 2011).

Chromium, the amount of which we found to be 2.767 µg/l, was detected in the range of 0–17.61 µg/l in the Spanish juice market (Garcia *et al*. 1999).

As for lead, we found the mean value of 20.75 µg/l while in the Brazilian juice market, lead was detected in the range from 0.1 µg/l to 2.1 µg/l (Tormen *et al*. 2011) and even up to 129 µg/l (Froes *et al*. 2009). The Codex Alimentarius establishes as maximum lead content 0.3 mg/kg in orange juice.

We estimated the mean value of 73.37 µg/l for nickel whereas in the Brazilian juice market nickel was found in amounts of up to 180 µg/l (Simpkins *et al*. 2000;Froes *et al*. 2009; Tormen *et al*. 2011).

We found the mean value of 545.9 µg/l for zinc, which was detected in orange Australian juice in the range of 120–680 µg/kg (Simpkins *et al*. 2000), and in the juices in Brazil between 86.7 and 1122.5 µg/l (Nascentes *et al*. 2004). The limit set in the Codex Alimentarius for orange juice is 5 mg/kg.

Finaly, we estimated the mean value of 1792 µg/l for iron, which was detected in the juices of the Austral-

Table 3. Strong results of association from the Spearman correlation and correlation ratio

		Cd	Cr	b	Ni	Zn	Fe
Spearman	Pineapple (%)						0.475
	Red grape (%)					0.470	0.517
	Brix						0.446
Eta	Fruit	0.715	0.634	0.551	0.598	0.902	0.884
	Origin			0.482			
	Packaging				0.445		

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Table 4. Extracted matrix of main components, from correspondence analysis of multiple variables fruit, origin, agriculture, pasteurisation, packaging, conservation, and process

	Component	
	1	2
Fruit	0.725	**0.969**
Origin	0.654	**0.962**
Agriculture	0.034	**0.920**
Pasteurisation	**0.779**	0.103
Packaging	**0.963**	0.767
Conservation	**0.846**	0.012
Process	**0.849**	0.043
Eigenvalue	4.851	3.775
% of variance	69.296	53.936
Cronbach α	0.926	0.858

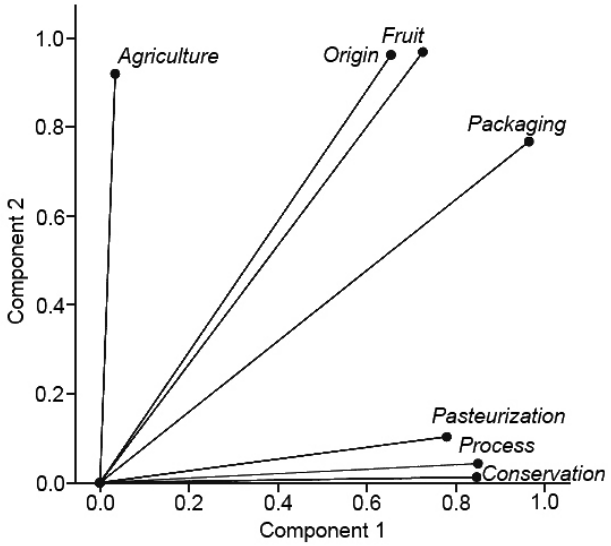

Figure 1. Representation of the variables: fruit, origin, agriculture, pasteurisation, packaging, conservation, and process in the components phase space

ian market in the range 20–1800 μg/kg (Simpkins *et al*. 2000) and in berry juices on the Serbian market in the range of 300–2100 μg/kg (Ristic *et al*. 2011). The limit fixed by the Codex Alimentarius for orange juice is 15 mg/kg.

Association between factors and elemental fruit juices concentrations. A bivariate correlation analysis was made, measuring the association between the factors and elemental concentrations by calculating the correlation coefficients (Table 3).

The variable Brix and the variables from the Blending set were not normally distributed, the bivariate Spearman correlation of these variables with the elemental concentrations of juices indicated a weak association between them.

The association of the qualitative variables (fruit, origin, agriculture, pasteurisation, packaging, conservation, process) with the elemental concentrations in the fruit juices, calculating the correlation ratio (Eta), was strong with the fruit variables.

Reducing data dimension. In order to reduce the number of variables (fruit, origin, agriculture, pasteurisation, packaging, conservation, process) a multiple correspondence analysis was made. Two components were extracted: component 1 and component 2 (Table 4) represented in two-dimensional space (Figure 1), showing the variables related to component 1, C1 = pasteurisation + packaging + conservation + process, and the variables related to component 2, C2 = fruit + agriculture + origin. The internal consistency of the components, measured by Cronbach's α, is very good (0.926) in the case of component 1 and good (0.858) in the case of component 2.

Two new variables were defined: pre-harvest and post-harvest, assigned respectively, to components C2

Table 5. Levene's test and *t*-test for Zn variable

		Levene's test for equality of variances		*t*-Test for equality of means						
				t	*df*	Sig. (2-tailed)	mean difference	std. error difference	95% confidence interval of the difference	
		F	Sig.						lower	upper
Equal variances	assumed	9.883	**0.003**	–2.180	65.000	0.033	–142.128	65.193	–272.327	–11.929
	not assumed			–2.558	61.589	**0.013**	–142.128	55.557	–253.199	–31.057

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and C1, while the former represents the agricultural production underlying the fruits gathering, the latter represents the industrial processing to obtain the juices.

The degree of association was measured between the new defined variables and the juices elemental concentrations. The Spearman correlation values indicated a weaker association of elemental concentration variables with the new group variables.

Differentiation of extraction and dilution processes. By the variance analysis, a comparison was made of the means of juice elemental concentration variables with the process variable group of extraction and group of dilution. First, the test was performed of homogeneity of variance (Levene's test) and then the Student *t*-test for independent sample.

Table 5 shows the result for the variable Zn, which was assigned a statistical difference between the two groups. In this case, H_0 was rejected in Levene test for the significance level $\alpha = 0.05$ since Sig. $P < 0.05$ and then the *t*-test result corresponding to equal variances, concluded not assumed, rejecting *t*-test H_0 of the variable Zn (to the significance level $\alpha = 0.05$) since Sig. (2-tailed) $P = 0.013 < 0.05$.

This result means that, statistically, the values of the Zn content of the dilution process are different from those of the extraction process, with 95% confidence level.

The average Zn concentration of juices obtained by direct extraction was 450.4 µg/l and of juices obtained by diluting the concentrate was 592.6 µ/g.

CONCLUSIONS

Fruit juices present in the Portuguese market are mostly manufactured in Spain, and they show the predominance of one of two kinds of fruits: apple or orange. The juices obtained by direct extraction are packed in HDFE bottles and preserved by refrigeration with a shelf life of three months. The juices obtained by dilution are packed mostly in Tetra Brik and are kept at room temperature with a shelf life of twelve months. At the present time, the organic juices still show a very small presence in this market, expressed by less than 5%, of the fruit juices.

Most juices follow the international recommendations on Brix graduation. As for the mineral elements present in fruit juice, the least abundant is cadmium, followed, respectively, in increasing order by chromium, lead, nickel, zinc, and finally iron.

A strong association was detected between juice elemental concentrations and the type of fruit used to make the juice.

In the second approach, after reducing the data matrix dimension no association was detected between the new group variables assigned to pre-harvest and post-harvest practices, and elemental fruit juice concentration, reinforcing the idea that just the fruit type, the fruit genotype, will have an important influence on the elemental concentration of juices.

As for the possible identification, by elemental analysis, of the process of obtaining the juices, i.e. by extraction from fruits or by diluting concentrates, the values of zinc concentration can provide an important contribution.

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