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Canned sardines commercialized in Brazil: Packaging and inorganic contaminants evaluation

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ABSTRACT

The main objectives of this work were to study electrolytic chromium/chromium oxide coated steel (ECCS) packaging used in canned sardines commercialized in Brazil and to determine the presence of inorganic contaminants in this food with two different conserves. Aluminum(Al), arsenic(As), cadmium(Cd), chromium(Cr), iron(Fe), lead(Pb) and tin(Sn) were determined by ICP OES. The packaging analysis revealed that the quality parameters established for ECCS packaging were satisfied. Element levels varied between (mg kg^{-1}): Al (not detected (ND) and 13.24); As (0.66 and 6.44); Cd (ND and 0.43); Cr (ND and 0.13); Pb (ND and 0.09); Sn (ND and 0.26); Fe (14.0 and 42.0). For inorganic contaminants, levels above the Brazilian and MERCOSUR regulation thresholds were found in sardine samples: As, Cd and Cr in 78%, 12% and 8%, respectively. Daily sardine can consumption may also contribute up to 9.3% of $BMDL_{0.5}$ for inorganic arsenic and 13% of PTMI for cadmium.

1. Introduction

Preservation of fish in conserves is one of the most adopted procedures in the world. Concerning with public health, several studies have been carried out with canned foods in order to determine the content of trace elements – especially with those whose essential body function is not known. Inorganic contaminants were studied in canned fish (tuna and sardines) in Austria (Suppin, Zahlbruckner, Krapfenbauer-Cermak, & Hassan-Hauser, 2005); in Brazil (De Paiva, Milani, & Morgano, 2017; Lazarini, Milani, & Morgano, 2019; Medeiros et al., 2012); in Spain (Olmedo et al., 2013); in the United States (Shiber, 2011); Ghana (Okyere, Voegborlo, & Agorku, 2015); in Italy (Storelli, Barone, Cuttone, Giungato, & Garofalo, 2010); in Iran (Hosseini et al., 2013); Libya (Abolghait & Garbaj, 2015); in Niger (Babalola et al., 2014), in Portugal (Afonso et al., 2015); in Czech Republic (Kral, Blahova, Sedlackova, Kalina, & Svobodova, 2017); in Serbia (Novakov et al., 2017) and in Turkey (Yabanli, 2013).

In general, inorganic contaminants As, Cd, Pb, Cr and Sn have been associated with several carcinogenic and neurotoxic effects on the human organism (FAO/WHO, 2019). Agriculture and industry are the main sources for these contaminants which can be accumulated in the marine environment and incorporated into fish tissues (Andayesh, Hadiani, Mousavi, & Shoeibi, 2015). Inorganic contaminants levels are specie and fishing area dependent (Mendil, Demirci, Tuzen, & Soylak, 2010) and may also be influenced by the metallic material used for packaging (Mol, 2011).

In Brazil, sardines are the most commercialized fish species as conserves in electrolytic chromium / chromium oxide coated steel (ECCS) packaging and may contain inorganic contaminants (Demirayak, Kutbay, Kılıc, Bilgin, & Huseyinova, 2011) with known toxic effects: arsenic (Khaniki & Zazoli, 2005) and hexavalent chromium (Stellman, 2011) with carcinogenic potential; tin that may lead to renal failure and gastrointestinal disorders and lead that can cause hematological, neurological, and behavioral changes. Chronic exposure to cadmium may result in lung damage (emphysema) and renal dysfunction (FAO/WHO, 2019; Mehri & Marjan, 2013; Stellman, 2011), while aluminum may lead to acute renal dysfunction (Mehri & Marjan, 2013; Peto, 2010).

Although sardine consumption represents about 78% of all fish commercialized in canned form in Brazil (Gonçalves, 2011), few studies have reported the evaluation of inorganic contaminants as well as the packaging characterization used for this food. In this context, the aims of this study were: i) to verify and to evaluate possible modifications in the ECCS packaging; ii) to validate an analytical method using inductively coupled plasma optical emission spectrometry (ICP OES) for aluminum, arsenic, cadmium, chromium, iron, lead and tin quantification in canned sardine samples commercialized in Campinas, SP, Brazil; iii) to evaluate the exposure to these contaminants by the

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consumption of canned sardines.

2. Material and methods

2.1. Samples

Fifty canned sardine samples were acquired in local markets from Campinas, Brazil considering the five main brands available (A, B, C, D and E) with five lots of each brand, totalizing 25 samples with oil conserve and 25 samples with tomato sauce conserve. Samples were purchased according to the commercial availability, considering the first months of packaging and the four-year date of expiration. For elements evaluation, the usual consumption of this food was considered (Ferreira, Barbosa, & Santos, 2017) and drained content was homogenized in domestic processor. All analyses were performed in analytical triplicate with blanks experiments. For packaging evaluation, 16 samples were purchased from a single brand, considering both oil and tomato sauce conserves. In this study we observed that all brands available used the same can manufacture. Sardines cans were opened, the internal content was removed and the ECCS packaging were washed using neutral detergent, soft sponge and purified water for rinsing. After the visual evaluation, the packaging test specimens were prepared.

2.2. Reagents and standards

Water and nitric acid were purified using a reverse osmosis system (18.2 MΩ cm, Gehaka, São Paulo, Brasil) and a sub-boiling distiller (Berghof, Eningen, Germany), respectively and 30% (m/v) hydrogen peroxide (Merck, Darmstadt, Germany) was also employed for microwave assisted digestion. Analytical curves were prepared using multi-elementar standard solutions (1000 mg L^{-1}) (Merck, Darmstadt, Germany) in 2.5% (v/v) HNO_3 solution. For quality assurance (validation) were used Standard Reference Materials (SRM): *Lobster Hepatopancreas* (NRC TORT-2) for Al, As and Cd and *Oyster Tissue* (NIST SRM 1566b) for Cr, Pb and Fe. Potassium dichromate ($K_2Cr_2O_7$), zinc sulfate ($CuSO_4$) and acetone p.a. (Synth, Diadema, Brazil) were used for packaging characterization.

2.3. Packaging analysis

In packaging characterization, the can components body (one-piece part that comprises the bottom and wall) and top (closure) were evaluated with respect to steel thickness, superficial Rockwell hardness and chromium layer (oxide and metallic) determinations. The cans were visually evaluated using a light microscopy (stereomicroscope M165C, Leica, Heerbrugg, Switzerland) and classified according to the appearance and degree of rusting, according to ASTM. American Society for Testing and Materials (2012). The thickness of the steel sheets (body and top) was determined directly using a spherical face micrometer (Mitutoyo 395-271, São Paulo, Brazil), according to ABNT. Brazilian Association of Technical Standards (2008) and the superficial Rockwell surface hardness at 30 T scale was determined using a durometer hardness testing (Wilson Instruments/Instron 503S, Norwood, USA), according to ABNT. Brazilian Association of Technical Standards, 2009bABNT. Brazilian Association of Technical Standards (2009b). Metallic chromium internal coating and chromium oxide in inner steel sheets were determined according to ABNT. Brazilian Association of Technical Standards (2006), 2009a) using a coulometric and colorimetric method, respectively. For coulometric measures, a potentiostat/galvanostat electrochemical system (Autolab, PGSTAT 302 N) was used. For these tests, three measurements were performed in each test specimen.

Internal coating was evaluated considering the dry layer and porosity (Dantas, Anjos, Segantini, & Gatti, 1996) and rating adhesion (ASTM. American Society for Testing & Materials, 2017). For dry layer and porosity, a gravimetric method based on the chemical deposition of copper was applied, consisting in the contact between the internal coating and $CuSO_4$ solution in acid medium for 10 min. Rating adhesion was determined using tape test with 6 × 6 grid at 90° angle, spare by 1 mm.

2.4. Determination of inorganic contaminants in canned sardine samples

An ICP OES (5100 VDV, Agilent Technology, Tokyo, Japan) equipment equipped with a double-step nebulization camera and a seaspray nebulizer was used for the determination of Al, As, Cd, Cr, Fe, Pb and Sn. The liquid argon with 99.996% purity (Air Liquide, São Paulo, Brazil) was used in order to generate plasma, nebulizing gas and auxiliary gas. The optimized operational conditions of the equipment were: power of the radiofrequency generator (1200 W); sample flow rate (0.50 L min^{-1}), argon flow from the nebulizer (0.60 L min^{-1}); auxiliary argon flow rate (1.00 L min^{-1}); main argon flow rate (12.0 L min^{-1}); axial mode of vision; number of replicates (n = 3) and the following wavelengths: Al (308.215 nm); As (193.696 nm); Cd (214.439 nm); Cr (267.716 nm); Fe (259.940 nm); Pb (220.353 nm) and Sn (189.925 nm).

The digestion of canned sardine samples was performed using a microwave digester (Start E, Milestone, Sorisole, Italy) under conditions adapted from De Paiva et al. (2017): 1 g of sample (or 0.25 g of lyophilized powder SRM) was weighed into a PTFE flask followed by the addition of 4 mL of concentrated nitric acid and kept overnight. Then, 4 mL of deionized water and 2 mL of hydrogen peroxide were added; the flasks were sealed and transferred to the microwave digester, applying 1000 W of power with 2 stages of digestion: (1) room temperature to 170 °C for 15 min and (2) 170 °C for 25 min. Digested samples were transferred to a 25 mL graduated flask with 5% (v/v) nitric acid solution and the measures were performed in an ICP OES using analytical curves ranging from 0.0025 to 1.0 mg L^{-1}.

2.5. Quality assurance and statistical analysis

The method for inorganic contaminants was validated considering the National Institute of Metrology, Quality and Technology recommendations (INMETRO, 2016). The figures of merit evaluated were: accuracy (using SRMs), analytical curves linearity, limits of detection (LOD) and quantification (LOQ) and precision. The results were evaluated by one-way ANOVA and Tukey test, with a significance level of 95% ($p < 0.05$), using the XLSTAT program (Addinsoft, Paris, France).

3. Results and discussion

3.1. Validation of the method for inorganic contaminants in canned sardines

In Table 1 are shown the results obtained in the validation for inorganic contaminants method in canned sardine by ICP OES.

For accuracy, two SRM were employed (Lobster Hepatopancreas and Oyster Tissue) and the recovery values (ratio between the value obtained and the certified one) ranged from 89 to 100%, being considered satisfactory according to AOAC - Association of Official Agricultural Chemists (2013) recommendation (80 and 110%). For Sn, recovery tests (spike) were performed since the available SRMs did not present certified values. Two levels (25 mg kg^{-1} and 100 mg kg^{-1}) were tested, considering the linear range of the analytical curve and the thresholds established by Brazil, Agência Nacional de Vigilância Sanitária (2013) and MERCOSUR (2011) regulations. Recovery values were 82 and 93%, respectively, within AOAC - Association of Official Agricultural Chemists (2013) recommendations.

Limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times the standard deviation of 10 analytical blanks,

Table 1
Quality assurance results for inorganic contaminants method in canned sardines.

Figures of merit	Aluminum*	Arsenic*	Cadmium*	Chromium**	Lead**	Iron**
Certified value (mg kg^{-1})	197.2 ± 6.0	7.65 ± 0.65	2.48 ± 0.08	0.77 ± 0.15	0.35 ± 0.13	105 ± 13
Obtained value (mg kg^{-1})	175.7 ± 2.8	7.3 ± 0.4	2.5 ± 0.1	0.75 ± 0.02	0.34 ± 0.01	105 ± 4
Recovery (%)	89 ± 1	96 ± 6	100 ± 3	97 ± 3	98 ± 3	100 ± 3
LOD (mg kg^{-1})[a]	0.5	0.05	0.003	0.006	0.01	0.2
LOQ (mg kg^{-1})[b]	0.8	0.08	0.005	0.01	0.02	0.3
Precision (%)[c]	11.0	1.7	1.5	1.0	2.3	9.0

[a] LOD (3 s) = detection limit (n = 10) e.
[b] LOQ (10 s) = quantification limit (n = 10); LOD (3 s) and LOQ (10 s) reported were multiplied by the dilution factor (25x).
[c] Precision = variation coefficient (n = 16); Certified reference materials.
* *Oyster Tissue* (NIST SRM 1566b).
** *Lobster Hepatopancreas* (NRC TORT-2).

respectively, and multiplied by the dilution factor (25x). The values ranged between 0.003 and 0.8 mg L^{-1} and were considered adequate to the thresholds stablished by Brazilian and MERCOSUR regulations (Brazil, Agência Nacional de Vigilância Sanitária, 1965, 2013; MERCOSUR, 2011).

The precision of the method was evaluated using the coefficients of variation from 16 analytical replicates (8 replicates / day). Values ranged from 1.0% to 11% for all elements, within AOAC - Association of Official Agricultural Chemists (2013) recommendations (< 11%). The linearity of the analytical curves was evaluated by the coefficient of determination; with $r^2 \geq 0.999$ for all elements studied.

3.2. Canned sardines packaging evaluation

The packaging was evaluated by visual inspection and light microscopy, in which no apparent corrosion was observed. The bottom and the lid of the cans were evaluated to check their thickness and superficial Rockwell hardness (Table 2).

According to Table 2, thicknesses of the steel sheets from body and top probably have nominal values of 0.19 mm and 0.20 mm, respectively. These conclusions considered the specification of low carbon steel produced by single or double reduction: tolerances of ± 2.0% (± 0.004 mm) or ± 2.5% (± 0.005 mm), respectively. Superficial Rockwell hardness results indicated that T65 hardness was used to the can top and body (wall and bottom) and both are in agreement with ABNT. Brazilian Association of Technical Standards (2014) requirements: variation < 4 Rockwell units for single-reduction steel sheets.

Metallic chromium internal coating and chromium oxide in inner steel sheets were determined using an optimized method from ABNT. Brazilian Association of Technical Standards (2009a) with a determination coefficient, $R^2 = 0.9999$. The results are presented in Table 3.

From Table 3, the metallic chromium internal coating from bottom and top (closure) did not present variation, in agreement with the ABNT. Brazilian Association of Technical Standards (2014) specification for mass of metallic chromium between 50 and 140 mg/m^2. Values of chromium oxide in inner steel sheets presented a wide range and mean values were lower than the threshold established by ABNT. Brazilian Association of Technical Standards (2014), 5 to 15 mg/m^2.

Table 2
Thickness and superficial Rockwell hardness results in packaging used for canned sardines (n = 12).

Conserve		Thickness (mm)	Superficial Rockwell hardness (30 T Scale)
		Mean ± SD	Mean ± SD
In Oil	Top	0.198 ± 0.002	64.5 ± 0.8
	Body	0.191 ± 0.001	63.9 ± 0.6
Tomato Sauce	Top	0.198 ± 0.001	66.1 ± 0.5
	Body	0.189 ± 0.002	62.9 ± 0.7

SD = Standard Deviation; **Top** = can closure; **Body** = can wall and bottom.

This fact is probably due to the adhesion of the chromium oxide to the coating layer – removed for test specimen preparation.

Results for internal coating evaluation indicated that the body of these cans received two coating layers. This conclusion is based in the values of dry layer of the bottom being about twice the one of top, 12.7 g/m^2 and 47.6 g/m^2, respectively). This procedure guarantees the metallic material resistance to the various stages of production process of this type of packaging. In porosity test, no deposition of metallic copper was observed in both conserves (in oil and tomato sauce) – indicating that internal coating minimized the development of the corrosion in the cans. Rating adhesion test results were “grade zero” in all test specimen, in agreement to ABNT. Brazilian Association of Technical Standards (2008) that recommend values up to “grade 1”.

3.3. Inorganic contaminants in canned sardine samples with oil and tomato sauce conserves

The results for Al, As, Cd, Cr, Fe, Pb and Sn for the different canned sardine samples are presented in Table 4.

The elements levels in canned sardine samples in oil conserve ranged from < 0.5 to 13.24 mg kg^{-1} for Al; 0.77 to 6.44 mg kg^{-1} for As; < 0.003 to 0.13 mg kg^{-1} for Cd; < 0.006 and 0.13 mg kg^{-1} for Cr; < 0.01 and 0.08 mg kg^{-1} for Pb; < 0.07 and 0.26 mg kg^{-1} for Sn and 13.30 and 41.40 mg kg^{-1} for Fe. For the samples in tomato sauce, inorganic contaminants levels varied between < 0.5 and 13.20 mg kg^{-1} for Al; 0.66 and 4.18 mg kg^{-1} for As; < 0.01 and 0.43 mg kg^{-1} for Cd; < 0.006 and 0.07 mg kg^{-1} for Cr; < 0.01 and 0.09 mg kg^{-1} for Pb; < 0.07 and 0.26 mg kg^{-1} for Sn; 15.00 e 42.00 mg kg^{-1} for Fe. Although similar levels were observed for Fe, statistical differences were found at 95% confidence level agreeing with Zuchi et al. (2015) study, which authors investigated the mechanism of dependence between the biofortification of iron and sulfur in tomatoes crops.

The highest levels of Al, As and Cr were found in canned sardines in oil conserve: 13.24 mg kg^{-1}; 6.44 mg kg^{-1} and 0.13 mg kg^{-1}, respectively. For Cd, Pb and Fe, the highest levels were observed in samples with tomato sauce: 0.43 mg kg^{-1}; 0.09 mg kg^{-1} and 42.00 mg kg^{-1}, respectively, while the concentration of Sn were low in both samples. Regarding the brands, only “C” samples exhibited a significant difference at 95% confidence level for As and Pb, indicating low variation in different brands of canned sardines.

With respect to the inorganic elements which are part of packaging material (Cr, Fe and Sn), their contents in samples with oil conserve and tomato sauce did not present a significant difference (at 95% confidence level). The concentrations in both conserves ranged from < 0.006 to 0.13 mg kg^{-1}, 13.30 to 41.40 mg kg^{-1}, < 0.07 to 0.26 mg kg^{-1} for Cr, Fe and Sn, respectively.

Inorganic contaminants regulation from Brazil and MERCOSUR established maximum limits for As, Cd, Pb and Sn in fish are: 1.0 mg kg^{-1}; 0.1 mg kg^{-1}; 0.3 mg kg^{-1} and 250 mg kg^{-1}, respectively (Brazil, Agência Nacional de Vigilância Sanitária, 2013; MERCOSUR, 2011) and

Table 3
Chromium (III) oxide, metallic chromium, total chromium (n = 5) and dry layer (n = 6) results in packaging used for canned sardines.

Test specimen		Chromium (mg/m²)			Dry layer (g/m²)
		Cr (III) Oxide	Metallic	Total	
Bottom	Mean ± SD	3.3 ± 1.7	93.3 ± 3.8	96.6 ± 4.6	28.0 ± 3.4
	Range	1.3–5.7	88.9–98.8	90.2–102.6	24.3–47.6
Top (closure)	Mean ± SD	3.4 ± 0.7	94.1 ± 6.8	97.5 ± 6.6	14.2 ± 0.5
	Range	2.2–4.5	81.0–100.1	84.6–103.5	12.7 –18.2

SD = Standard Deviation.

0.1 mg kg^{-1} for Cr in any food (Brazil, Agência Nacional de Vigilância Sanitária, 1965). For some elements, levels above these thresholds were found for As, Cd and Cr in 78%, 12% and 8% of the samples analyzed, respectively. Although As levels may present a potential risk for human, Schaeffer, Soeroes, Fodor, and Thomaidis (2005) reported that only 10% of total arsenic content in fish corresponds to the highest toxic species such as inorganic arsenic (i-As).

Regarding chromium, some samples presented higher Cr levels than the threshold established by Brazilian regulation (0.1 mg kg^{-1}). These results indicate an apparent migration from chromium sheets, whose objective is to give resistance to the corrosion processes to the steel used in the packaging production for canned foods. Cr levels were similar to those reported by Ikem and Egiebor (2005) in samples from Georgia and Alabama (USA), which found levels of 0.015 mg kg^{-1} for Cr. On the other hand, studying the canned sardines from Nigeria, Iwegbue, Nwajei, Arimoro, and Eguavoen (2009) found higher Cr concentrations (between 0.01 and 0.1 mg kg^{-1}), when compared to the results from the present work.

In literature, some studies reported inorganic contaminants levels in canned sardine: for arsenic, Cano-Sancho et al. (2015) verified similar levels (1.78 mg kg^{-1}) in their study with sardines commercialized in Catalonia (Spain) while Okyere et al. (2015) found values between < 0.01 and 1.44 mg kg^{-1} for lead and lower than 0.01 mg kg^{-1} for cadmium in 55 and 64 samples from Ghana, respectively. Vieira, Morais, Ramos, Delerue-Matos, and Oliveira (2011) reported the presence of cadmium, lead and arsenic in sardines with concentrations ranging from 0.0017 to 0.0151 mg kg^{-1} for Cd, 0.0029 to 0.0560 mg kg^{-1} for Pb, 0.8116 to 1.3362 mg kg^{-1} for As.

Babalola et al. (2014) studied canned sardines samples from various regions of the world and the authors observed Cd concentration around 0.001 mg kg^{-1} in samples in vegetable oil conserve from Morocco; 0.001 mg kg^{-1} and 0.01 mg kg^{-1} in samples in sunflower oil conserve from Portugal and United Kingdon, respectively and 0.012 mg kg^{-1}, 0.001 mg kg^{-1} and 0.025 mg kg^{-1} in canned sardines with tomato sauce from Morocco, Argentina and South Africa, respectively. Mol (2011) evaluated 30 canned sardine samples from Turkey and found high contents for Fe, Cd and Pb: 0 to 2.875 mg kg^{-1}; 0 to 89 mg kg^{-1} and 0 to 0.113 mg kg^{-1}, respectively and low Sn levels (0 to 0.158 mg kg^{-1}) similar to those found in this study.

3.4. Al, As, Cd, Cr, Fe, Pb and Sn exposure estimative by the canned sardine intake

For Al, As, Cd, Cr, Fe, Pb and Sn exposure estimative by the daily consumption of one portion of canned sardines (drained weight = 84 g), the mean values shown in Table 4 were considered as well as the PTWI (Provisional Tolerable Weekly Intake) of 2 mg kg^{-1} body weight for Al; $BMDL_{0.5}$ (Benchmak Dose Lower Limit) of 3 μg kg^{-1} body weight for i-As (inorganic arsenic); PTMI (Provisional Tolerable Monthly Intake) of 25 μg kg^{-1} body weight for Cd; the PTDI (Provisional Tolerable Daily Intake) of 0.8 mg kg^{-1} body weight for Fe; the PTWI (Provisional Tolerable Weekly Intake) of 14 mg kg^{-1} body weight for Sn (FAO/WHO, 2019); TDI (Tolerable Daily Intake) of 0.3 mg kg^{-1} body weight for Cr (III) (EFSA, 2014); the $BMDL_{0.1}$ (Benchmak Dose Lower Limit) of

Table 4
Mean values (range) for inorganic contaminants evaluation in canned sardines (n = 50).

Brand	Type of conserve	Elements (mg kg^{-1})						
		Al	As	Cd	Cr	Pb	Sn	Fe*
A	Oil	1.56^{a}	1.00^{a}	0.02^{a}	0.01^{a}	0.02^{a}	0.18^{b}	17.20^{a}
		(< 0.5–3.70)	(0.77–1.30)	(0.01–0.03)	(< 0.006–0.03)	(< 0.01–0.03)	(0.14–0.26)	(14.00–17.90)
	Tomato sauce	4.20^{A}	0.90^{A}	0.02^{A}	0.01^{A}	0.03^{A,B}	0.20^{B}	28.10^{A,B}
		(< 0.5–10.92)	(0.66–1.55)	(0.01–0.04)	(< 0.006–0.04)	(< 0.01–0.06)	(0.16 – 0.23)	(23.0–33.60)
B	Oil	4.38^{a}	1.31^{a}	0.04^{a}	0.08^{a}	0.02^{a}	0.02^{b}	25.14^{a}
		(< 0.5–13.24)	(1.13–1.83)	(0.01–0.11)	(0.05–0.11)	(< 0.01–0.08)	(< 0.07 – 0.12)	(17.60–32.20)
	Tomato sauce	0.64^{A}	1.51^{A}	0.05^{A}	0.04^{A}	0.01^{A}	0.09^{A}	17.32^{A}
		(< 0.5–2.24)	(1.12–1.95)	(0.04–0.07)	(0.02–0.07)	–	(0.09 – 0.13)	(15.0–18.80)
C	Oil	3.76^{a}	3.21^{b}	0.04^{a}	0.05^{a}	0.02^{a}	0.09^{a,b}	23.84^{a}
		(< 0.5–7.70)	(1.79–6.44)	(0.01–0.07)	(0.02–0.11)	(< 0.01–0.06)	(< 0.07–0.21)	(13.50–41.40)
	Tomato sauce	5.12^{A}	3.03^{B}	0.18^{A}	0.04^{A}	0.05^{B}	0.14^{A,B}	32.88^{B}
		(1.92–13.20)	(1.69–4.18)	(< 0.003–0.43)	(0.01–0.06)	(< 0.01–0.09)	(< 0.07–0.18)	(17.70–42.00)
D	Oil	1.60^{a}	2.50^{a,b}	0.06^{a}	0.02^{a}	0.02^{a}	0.18^{a}	17.76^{a}
		(< 0.5–2.70)	(2.01–3.59)	(< 0.003–0.13)	(0.01–0.06)	(< 0.01–0.03)	(0.10–0.22)	(13.30–22.40)
	Tomato sauce	1.93^{A}	1.93^{A,B}	0.05^{A}	0.02^{A}	0.01^{A}	0.20^{B}	25.70^{A,B}
		(1.20–2.70)	(1.09–2.53)	(< 0.003–0.19)	(< 0.006–0.05)	(< 0.01– 0.02)	(0.17–0.26)	(20.30–38.60)
E	Oil	2.14^{a}	1.93^{a,b}	0.02^{a}	0.03^{a}	0.004^{a}	0.16^{b}	18.54^{a}
		(< 0.5–3.08)	(0.84–2.74)	(< 0.003–0.08)	(< 0.006–0.13)	(< 0.01–0.02)	(0.12–0.21)	(16.00–22.10)
	Tomato sauce	1.13^{A}	1.28^{A}	0.02^{A}	0.03^{A}	0.02^{A,B}	0.21^{B}	22.38^{A,B}
		(< 0.5–2.10)	(0.66–2.69)	(< 0.003–0.04)	(< 0.006–0.05)	(< 0.01–0.05)	(0.12 – 0.24)	(20.50–22.80)

[a,b] same letters in the same column for oil conserved samples show no significant difference at 95% confidence level (one-way ANOVA and Tukey test).
[A,B] same letters in the same column for tomato sauce conserved samples show no significant difference at 95% confidence level (one-way ANOVA and Tukey test).
[*] significant difference between the type of conserves at 95% confidence level (one-way ANOVA and Tukey test).

Table 5
Al, As, Cd, Cr, Fe, Pb and Sn exposure estimative for daily intake of canned sardines with tomato sauce and oil conserve by an adult (60 kg bw).

Element	Type of conserve	Mean ($mg\ kg^{-1}$)		Exposure Estimative		
Daily						
i-As*	Oil	0.20	0.28 $\mu g\ kg^{-1}$ bw		9.3% $BMDL_{0.5}$	
	Tomato sauce	0.17	0.24 $\mu g\ kg^{-1}$ bw		8.1% $BMDL_{0.5}$	
Cr	Oil	0.05	0.0001 $mg\ kg^{-1}$ bw		0.02% TDI	
	Tomato sauce	0.04	0.0001 $mg\ kg^{-1}$ bw		0.02% TDI	
Fe	Oil	20.5	0.029 $mg\ kg^{-1}$ bw		3.6% PTDI	
	Tomato sauce	25.3	0.035 $mg\ kg^{-1}$ bw		4.4% PTDI	
Pb	Oil	0.03	0.04 $\mu g\ kg^{-1}$ bw		0.33% $BMDL_{0.1}$	
	Tomato sauce	0.03	0.04 $\mu g\ kg^{-1}$ bw		0.33% $BMDL_{0.1}$	
Weekly						
Al	Oil	3.36		0.033 $mg\ kg^{-1}$ bw		1.6% PTWI
	Tomato sauce	3.07		0.030 $mg\ kg^{-1}$ bw		1.5% PTWI
Sn	Oil	0.15		0.0015 $mg\ kg^{-1}$ bw		0.01% PTWI
	Tomato sauce	0.18		0.0017 $mg\ kg^{-1}$ bw		0.01% PTWI
Monthly						
Cd	Oil	0.04		1.85 $\mu g\ kg^{-1}$ bw		7.4% PTMI
	Tomato sauce	0.08		3.36 $\mu g\ kg^{-1}$ bw		13% PTMI

* **i-As** = inorganic arsenic (10% do total arsenic content); **bw** = body weight for an adult (60 kg) and a sardine can (drained weight = 84 g); **PTWI** = Provisional Tolerable Weekly Intake; **PTDI** = Provisional Tolerable Daily Intake; **BMDL** = Benchmak Dose Lower Limit; **PTMI** = Provisional Tolerable Monthly Intake; **TDI** = Tolerable Daily Intake.

12 $\mu g\ kg^{-1}$ body weight for Pb (EFSA, 2010) and an adult body weight of 60 kg.

Schaeffer et al. (2005) reported that about 90% of total arsenic found in fish, including sardines, is in the organic forms, such as arsenobetaine, arsenocoline, arsenic sugars and/or dimethylarsinic acid. As the organic species present low toxicity, it was considered that only 10% of total arsenic levels correspond to the inorganic species with toxicity potential. In Table 5 is presented the exposure estimative to the trace elements by the daily consumption of a canned sardine.

According to the results, the elements that most contributed to the exposure to sardine consumers are i-As and Cd. Higher values for inorganic arsenic were found in the samples in oil conserve (up to 9.3% $BMDL_{0.5}$), while the greater exposure to cadmium was observed in the canned sardines with tomato sauce (up to 13% PTMI). Although the inorganic contaminants levels in canned sardines conserved with tomato sauce suggest an influence of the acid environment present in this type of conserve in the migration from the packaging to the fish (Quintaes, Amaya-Farfan, Morgano, & Mantovani, 2002), it is important to point out that the inorganic contaminants levels in the tomato sauce and in sardines before their contact with the cans also may have influenced the high levels observed in this study.

For Al and Sn, low exposures estimative were observed for the weekly intake of canned sardines being 1.6% and 0.01% PTWI, respectively. Although Fe may be considered inorganic contaminant in high levels, the daily intake of canned sardines is low, contributing with 3.6% and 4.4% PTDI for oil conserve and tomato sauce, respectively.

4. Conclusions

- The characterization of the canned sardines packaging (ECCS) showed that the quality parameters were completely satisfied according to ASTM and ABNT (Brazil).
- No significant difference was observed, at 95% confidence level, between Al, As, Cd, Cr, Pb and Sn levels in canned sardine samples in oil and tomato sauce conserves.
- Levels above the maximum limits allowed by Brazilian and MERCOSUR regulations were observed for arsenic, cadmium and chromium.
- Considering the daily consumption of a sardine can by an adult (60 kg bw), inorganic arsenic and cadmium presented the highest contributions in the exposure to the inorganic contaminants.

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