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Assessment of polycyclic aromatic hydrocarbons and derivatives in beer using a new cold fiber-solid phase microextraction system

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ABSTRACT

Sample preparation assisted by a cooling system including solid phase microextraction (SPME) has received an increased interest in recent years. In this work, a new cold fiber (CF-SPME) system using thermoelectric cooling is proposed. The system consists of three thermoelectric coolers (Peltier), a heat sink, a recirculation module to dissipate the heat generated on the hot side, and an electric power source to generate the necessary voltage for the system. The Peltier keeps the SPME fiber cool during the extraction process.. This system was used to analyze polycyclic aromatic hydrocarbons (PAHs), and their nitrated (nitro-PAHs) and oxygenated (oxy-PAHs) derivatives, in commercial beers by gas chromatography coupled to mass spectrometry (GC/MS). The analyte's extraction was optimized through Doehlert design. Optimal extraction conditions were obtained with a salt content of 0.6% w/v and beer volume of 700.0 μL. The validated method showed limits of detection (LOD) in the range of 0.003–0.128 μg L^{-1} and limits of quantification (LOQ) from 0.011 to 0.427 μg L^{-1}. Precision Coefficients of Variation values ranged from 3.0 to 18.7%, and recovery values of the analytes in beer varied between 80.1 and 100.3%. Among the PAHs derivatives evaluated in this study, only 9-fluorenone and 9-nitroanthracene were detected in some samples. Benzo[*b*]fluoranthene was the most detected analyte in the samples, and at least one PAH was detected in all samples. Principal component analysis (PCA) separated the samples into two groups, mainly due to the high relative concentrations of acenaphthene and chrysene. The cooling approach improved the extraction efficiency and the new system demonstrated high potential for portability and automation.

1. Introduction

Contaminants may be present in foods due to the various stages of their production, transportation, packaging, or storage. Environmental pollution and climate change have increased the of contaminants (Farre & Barcelo, 2013) including the polycyclic aromatic hydrocarbons (PAHs) and their nitrated (nitro-PAHs) and oxygenated derivatives (oxy-PAHs). These compounds have been found in several environmental matrices such as air, water, soil, sediments and biota (Adeyeye, 2020).

PAHs, which have two or more fused aromatic rings, are formed during incomplete combustion of organic matter (Alegbeleye et al., 2017) and are of special concern due to their carcinogenic and mutagenic potential (IARC, 2010). On the other hand, PAHs derivatives produce reactive oxygen species which are endocrine disruptors, and some have higher cytotoxicity than their parent PAHs (Walgraeve et al., 2010). These characteristics reinforce the importance of analyzing these contaminants in food.

PAHs have been detected in different types of beverages such as tea, coffee, distilled drinks, wine, and fruit juice (Singh et al., 2016). In spite of their toxicological importance, few studies describing the analysis of PAH derivatives in beverages have been published. Examples are one study aimed at determining nitro-PAHs in tea (Schlemitz & Pfannhauser, 1997) and another analyzing oxy-PAHs and nitro-PAHs in coffee (dos Santos et al., 2019).

Beer is the most consumed alcoholic beverage in the world (Anderson et al., 2019) and is also subject to PAHs contamination due to the drying and roasting of barley (Rascon et al., 2019). However, few studies have been focused on PAHs determination in beers (Okafor et al., 2020; Russo et al., 2016; Yoshioka et al., 2018) moreover, these studies did not

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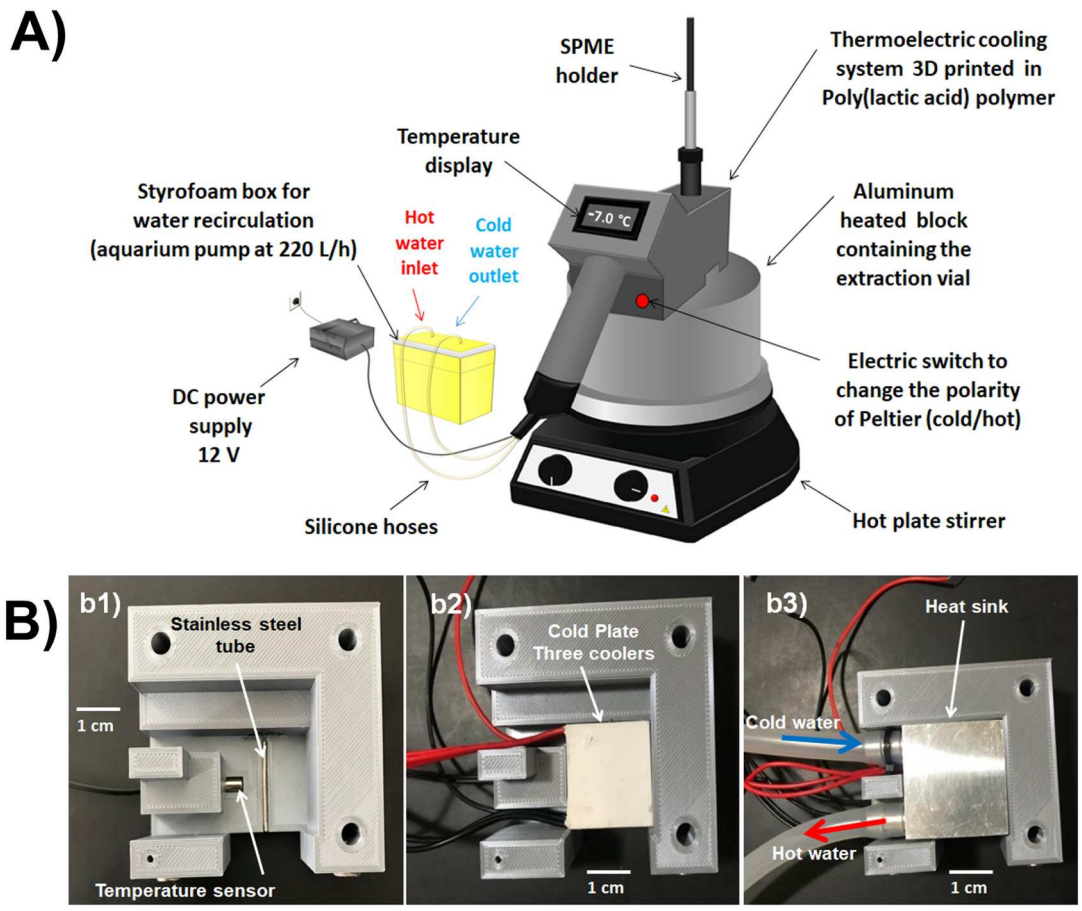

Fig. 1. Design of the thermoelectric cooling system for CF-SPME: (A) Schematic ilustration; (B) Picture of the disassembled internal parte: (b1) tube for SPME fiber and temperature sensor, (b2) cold/hot peltier plate, (b3) heat sink; 1 cm scale bar indicated.

make a proper comparison between light and dark beers. Beyond the limited number of works cited here, there are no studies that have analyzed PAHs derivatives in beer.

Although PAHs are usually found in low concentrations in food, they are one of the main factors that contribute to the onset of cancer in humans (Yoshioka et al., 2018). Therefore, it is of paramount importance to develop easy, fast, sensitive, and green methods for determination of these contaminants in beer. The solid phase microextraction (SPME) isa solvent-free sample preparation technique that allows one-step sampling and pre-concentration of analytes (Pawliszyn, 2000). SPME has already been used in the extraction of various analytes in food, biological and environmental matrices (J. Li et al., 2015; Xu et al., 2016) due to its well-known advantages which can be improved by cooling the fiber during extraction (Ghiasvand et al., 2006). Zhang and Pawliszyn proposed the first coold fiber solid phase microextraction (CF-SPME) device (Zhang & Pawliszyn, 1995).

The CF-SPME method involves simultaneously increasing the sample temperature and decreasing the fiber temperature. Heating the sample to elevated temperatures usually provides the energy necessary for the analytes to overcome interactions with the matrix, and therefore increases the vapor pressure and improves the mass transfer process (Tajik et al., 2017). On the other hand, due to the exothermic nature of sorption in the fiber, the increase in temperature reduces the extraction efficiency, so, through the reduction of the fiber temperature, significant extraction improvement is observed (Ghiasvand et al., 2016).

Cooling methods currently available for SPME include refrigerated fluid circulation, thermoelectric, and cryogenic systems. Cryogenic systems use liquid CO_2 or nitrogen (Ghiasvand et al., 2016). A simple liquid nitrogen cooling device has been proposed in previous studies (Menezes & Cardeal, 2011). However, the high maintenance cost of this system motivated the development of a new cooling method that does not require the use of cryogenics.

Several thermoelectric cooling systems have been proposed for extraction by CF-SPME (Banitaba, Davarani, & Movahed, 2014; Banitaba, Davarani, Ahmar, et al., 2014; Haddadi & Pawliszyn, 2009; Tajik et al., 2017). However, in these systems it is not mechanism for evaluating the temperature of the fiber, which normally compromises the control of thermal stability during the extraction process.

The objective of this study was assessment of PAHs, nitro-PAHs, and oxy-PAHs in beer using a new cooling system of the SPME fiber Thermoelectric Cooling System (TECS) to extraction, and a high performance gas chromatography-mass spectrometry (GC/MS) method to simultaneous determination of PAHs and derivatives. The method was optimized through Doehlert design and validated to analyze commercial samples of beer. The advantages of the developed system are easy handling, combined use of water and Peltier cooling, coupled with the polarity inversion. This feature allows focused and quick heating before fiber collection, thus increasing its lifetime.

2. Material and methods

2.1. Chemical and solutions

A PAH mix containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a] anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenzo[*a,h*]anthracene, and benzo[*g,h,i*] perylene at 2000.0 mg L^{-1} in methanol:methylene chloride

(1:1), was purchased from Supelco (Bellefonte, USA). 9,10-anthraquinone, 2-nitrofluorene, 5,12-naphthacenequinone, 3-nitrofluoranthene, 1-nitropyrene, 9-nitroanthracene, 2-methylanthraquinone, 9-fluorenone, and toluene HPLC-grade were obtained from Sigma-Aldrich (St. Louis, USA). Ethyl acetate, methylene chloride, ethanol, and acetonitrile HPLC-grade were purchased from JT Baker (Phillipsburg, USA). Sodium sulfate and hydrochloric acid were purchased from Synth (Diadema, Brazil). Ultrapure water was obtained by the ELGA system (High Wycombe, UK).

The saline solution 0.6% (w/v) was prepared from sodium sulfate salt. The synthetic beer consisted of a hydroalcoholic solution with 5% (v/v) ethanol at pH 4.0 (Russo et al., 2016). The ethanol content approximately corresponds to the average content of Brazilian beers, the same justification applies to the pH.

2.2. Gas chromatography/mass spectrometry

Chromatographic analysis were performed on a Finnigan Trace DSQ gas chromatograph coupled to a mass spectrometer analyzer, Polaris Q, manufactured by Thermo Scientific. A HP-5MS capillary column 30 m (0.25 μm × 0.25 mm) from Agilent Technology Inc. (Santa Clara, USA) was used with an oven temperature program, starting at 80 °C followed by heating at a rate of 30 °C min^{-1} to 150 °C, an increase to 210 °C at a rate of 10 °C min^{-1} held for 4 min, an increase to 240 °C at a rate of 15 °C min^{-1}, an increase to 280 °C at a rate of 10 °C min^{-1} held for 8 min. The carrier gas was helium (99.999%) at a flow rate of 2.5 mL min^{-1}. The injector was operated at 270 °C in splitless mode for 3 min.

The ion trap was operated in the electronic impact mode (EI) with 70 eV energy and positive mode. The temperature of the ion source was 250 °C and the interface temperature was 300 °C. The analysis was performed in Segment Scan mode (*m/z* 50-300).

2.3. CF-SPME system

Fig. 1 depicts the scheme of the new cooling system using the proposed Thermoelectric Cooler System (TECS). The TECS cooling module is made up of a support printed on a 3D printer, model Ender3, Creality (Shenzhen, China) in conventional poly (lactic acid) polymer (PLA). The cooling module is made up of three thermoelectric coolers (3.0 × 3.0 cm) set in series to increase the cooling capacity. The thermoelectric side in contact with the SPME fiber was cooled during the extraction and the heat generated on its back side was dissipated with an aluminum heat sink (4.0 × 4.0 cm) filled with cold water (4 ± 3 °C). The cold water was pumped with an aquarium pump Hbo-300 (Guangdong, China) immersed in 2.0 L of cold water with a gel bag Carbogel (São Paulo, Brazil) previously cooled (−10 ± 5) inside styrofoam box. This allows the water temperature to be maintained for up to 3 h. The temperature on the thermoelectric side in contact with the SPME fiber was monitored by a temperature sensor (thermocouple) and shown on an LCD display. Two on/off switches were used, one to turn on off all the TECS and the other to invert the polarity of the thermoelectric and the cooling/heating process. The use of a conventional power supply (12 V) was required to apply the required voltage to the thermoelectric coolers. This new TECS was used to cool the fiber during the extraction step (CF-SPME) in direct immersion (DI) mode. With this approach, the temperature measured by the thermocouples during the extraction was 6.0 °C (±0.5 °C).

2.4. Method optimization

The cooling efficiency of the TECS was evaluated using the fiber PDMS/DVB 65 μm into a SPME holder, both purchased from Supelco (Bellefonte, USA). The fiber (cooled and not cooled) was immersed in 20.0 mL of aqueous solution (n = 3) spiked with 2.5 μg L^{-1} of each PAHs and derivatives. The other conditions used in the extraction were 60 min, 70 °C, and constant agitation 1380 rpm.

Tests were performed to evaluate the effect of the addition of small amounts of different organic solvents as modifiers in the SPME extraction process. A volume of 100.0 μL of each solvent (acetonitrile and ethyl acetate) was added to 20.0 mL of an aqueous solution containing synthetic beer and the analytes, at a concentration of 2.5 μg L^{-1} (n = 3).

The salt content (NaCl, 0.5–1.7%, w/v) and beer volume (500-1500 μL) were selected as variables for optimization by Doehlert design, while the extraction temperature, agitation, extraction time, desorption temperature and desorption time were kept constant. At this stage, 9 experiments were performed containing 3 central points, as shown in Supplementary Table 1. Multivariate optimization was performed on the synthetic beer spiked with analytes at a concentration of 2.5 μg L^{-1}.

2.5. Samples

Twenty-six beer samples, including ten Pilsen and sixteen dark (ten roasted malt and six caramel), were purchased at local supermarkets.

2.6. Sample preparation

A volume of 700.0 μL of the beer sample was added to a 20 mL vial, along with 19.3 mL of saline (0.6%, w/v) and 100.0 μL of ethyl acetate. Then the vial was sealed and placed in an ultrasonic bath for 2 min. The PMDS/DVB fiber was inserted into the vial (direct immersion) and cooled during extraction using TECS. Extraction was performed for 45 min under constant temperature (70 °C) and stirring. After extraction, the analytes on the fiber was directly transferred to the GC/MS system for analysis.

2.7. Method validation

Performance parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and recovery were evaluated according to the recommendations of the Eurachem Guide (Eurachem, 2014).

The calibration curves for the analytes were made, in triplicate, at 7 concentration levels using synthetic beer. Synthetic beer was spiked with PAHs and PAHs derivative solutions for the validation study. Concentration levels were 0.5; 1.0; 2.0; 4.0; 8.0; 16.0 and 32.0 μg L^{-1}. LOQs and LODs were obtained by analysis of 10 blanks. Intraday precision was evaluated at concentrations of 2.0 and 8.0 μg L^{-1}, and 6 replicates were performed at each concentration. Interday precision was assessed on 2 consecutive days. Recovery was evaluated at a concentration of 4.0 μg L^{-1} through 10 replicates.

2.8. Statistics

Origin version 9.1 OriginLab Co (Northampton, USA) was used for analysis of variance, One-Way ANOVA. Bonferroni's post-test was used to compare mean values at 95% of confidence. Doehlert design were performed using Statistica 8.0 software (Tulsa, USA). MATLAB version 7.9.0.529 Mathworks (Massachusetts, USA) was used for processing the Principal Component Analysis (PCA). The data matrix was constructed for 26 samples and 14 variables. The data from this matrix was preprocessed (auto-scaled) before analysis by PCA.

3. Results and discussion

3.1. Thermoelectric Cooler System TECS

The use of 3D printing to make TECS components highlights additive technologies, which have been shown to be highly efficient and sustainable, since recyclable plastics can be used to manufacture the devices (Nesterenko, 2020). This work proposes a TECS of low cost and easy operation. Besides, this new TECS does not require the use of conventional cooling systems such as gas cylinders (Liao et al., 2006) or

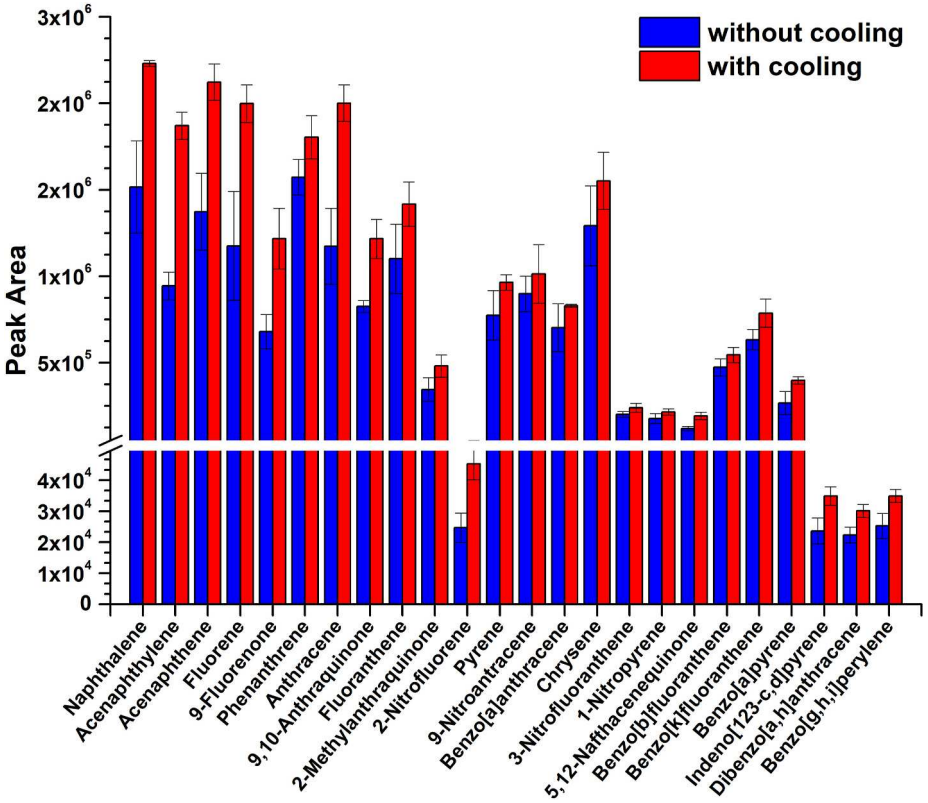

Fig. 2. Average chromatographic peak area (n = 3) of PAHs, nitro-PAHs and oxy-PAHs aqueous solution at 2.5 μg L^{-1} for SPME extractions without and with the cooling system of the fiber. Error bars represent the standard deviation.

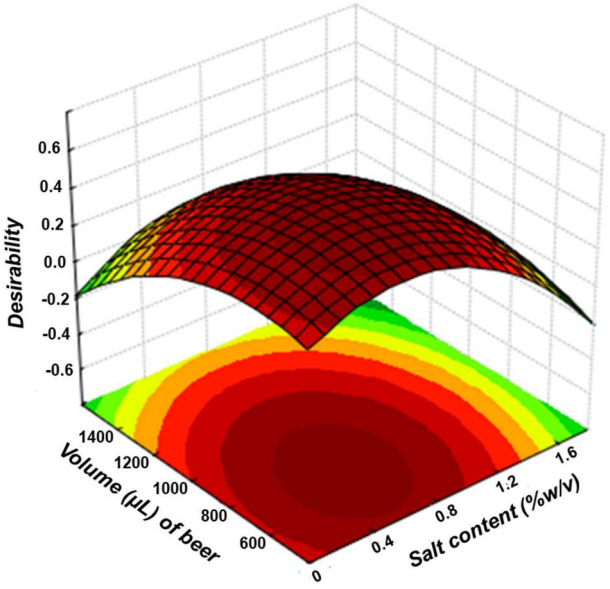

Fig. 3. Doehlert response surface obtained through desirability function from the evaluation of volume and salt content in the beer extraction of PAHs, nitro-PAHs and oxy-HPAs at 2.5 μg L^{-1} by TECS.

Dewar flasks with liquid nitrogen (Menezes & Cardeal, 2011). In addition, it is possible to monitor the fiber temperature through a display connected to the thermocouple (Fig. 1B). An important and innovative feature is the polarity inverter (cold/hot) (Fig. 1A). This switch, when in the hot position, allows the thermoelectric chip to be heated quickly so that only the needle may have a small expansion and thus SPME fiber to be retracted without damaging its structure. This damage is recurrent in CF-SPME contributing to decrease in the fiber's lifetime. The fiber damage occurs because the steel which makes up the SPME fiber protection needle has a volumetric coefficient for thermal expansion ($32x10^{-6}$ K^{-1} at 293.15 K) greater than the coefficient of fused silica ($9.1x10^{-6}$ K^{-1} at 293.15 K), where the polymeric coating is deposited. Therefore, during cooling the internal diameter of the steel needle presents a reduction greater than the fused silica reduction, challenging the fiber retraction into the needle cavity.

The TECS extraction efficiency was compared with the extraction without cooling. Fig. 2 shows the results from this preliminary test and it can be seen that there is a greater efficiency of the extraction for all analytes when the fiber is cooled, mainly for PAHs containing up to three rings, which, due to their higher vapor pressures, tend to desorb more quickly from the fiber without cooling.

3.2. Method optimization

Two of the main variables that may affect the extraction of analytes in SPME beer were evaluated through Doehlert design. The response surface obtained through Doehlert design and the desirability function is shown in Fig. 3. It can be observed that the optimal extraction conditions were obtained with a salt content of 0.6% w/v and beer volume of 700.0 μL. These results showed that higher amounts of beer and salt suppressed the analyte extraction. The addition of salt may decrease the solubility of the analytes in the aqueous phase and thus favor partitioning for the polymeric coating (Ishizaki et al., 2010). Using larger volumes of beer increases the amount of matrix interfering in contact with the SPME fiber. This required higher temperature and desorption times to minimize the possible carry-over peaks from previous injections and, consequently, damage the SPME fiber (Roychowdhury et al., 2020).

3.3. Method evaluation

The main merit parameters were evaluated for the proposed direct immersion cooled fiber method (DI–CF–SPME-GC/MS) for analysis of PAHs, nitro-PAHs and oxy-PAHs in beer. Supplementary Table 2 presents the determination coefficients (R^2), line equations, LODs, and LOQs of each analyte. The values of R^2 show that the proposed linear models are well adjusted. Due to the heteroscedasticity of the data, the equations of the lines were constructed by the weighted least squares method (WLSM). The values of LODs and LOQs obtained in this work were much lower than those obtained in another study of PAHs analysis in beer with GC/IT-MS (Russo et al., 2016) indicating a good sensitivity of the method.

Supplementary Table 3 presents the results obtained from the CV for evaluation of intraday and interday precision for concentration levels 2.0 and 8.0 μg L^{-1} and the percentage recovery for level 4.0 μg L^{-1}. Precision CV values ranged from 3.0 to 18.7%, indicating good method precision. The recovery values of the analytes in beer varied between 80.1 and 100.3%, which are similar to results found in other studies, 83.6% to 98.5% (Russo et al., 2016), and 80–111% (Yoshioka et al., 2018). Another important scientific contribution of this method is that there are no reports in the literature for the analysis of PAHs derivatives in beer, so the validation data presented herein will contribute to other studies in the future.

3.4. Method application to analyze the samples

Three types of different beers were analyzed using the validated DI–CF–SPME-GC/MS method. Table 1 shows the concentrations (μg L^{-1}) of PAHs, nitro-PAHs and oxy-PAHs in dark-roasted malt (n = 10), dark-caramel (n = 6) and Pilsen (n = 10) beers. The summations of the PAHs and nitro-PAHs concentrations in each beer type are shown in box plot Fig. 4. Oxygenated derivatives were not included in this figure since only 9-fluorenone can be quantified in a sample of dark-roasted and one of dark-caramel sample. ANOVA showed that there were no significant differences ($p < 0.05$) between the three types of beers evaluated in relation to ∑PAHs and ∑nitro-PAHs. However, Pilsen and dark-caramel samples presented significant differences ($p < 0.05$) between the ∑PAHs and ∑nitro-PAHs, while in the dark-roasted samples this was not observed. These results were similar to those observed in studies of PAHs and their derivatives in smoked meat (Chen et al., 2014) and tea (Schlemitz & Pfannhauser, 1997).

A higher content of PAHs can be expected in dark beers, both the caramel type and the roasted malt, but this was not observed for the roasted malt analyzed. The formation of PAHs during the roasting process depends mainly on the temperature and the roasting time. Temperatures below 220 °C and short roasting times are not favorable conditions for the formation of PAHs (Houessou et al., 2007).

In this study the ∑PAHs in Pilsen beers (6.34–35.84 μg L^{-1}) were lower than the ∑PAHs found in Italian and German beers (480.0–2820.0 μg L^{-1}) (Russo et al., 2016) and higher than the ∑PAHs found in the analysis of Spanish beers (1.14–3.05 μg L^{-1}) (Rascon et al., 2019).

This variation in the PAHs content of beers can be attributed to differences in ingredients and manufacturing processes in these countries. The type of caramel used in the dark-caramel beers analyzed in this study was the III (ANVISA, 2015) or INS 150c by the International Numbering System. This caramel is prepared by heating at 200 °C carbohydrates, with or without acid or alkaline substances, in the presence of ammonia compounds. Among the PAHs derivatives evaluated in this study, only 9-fluorenone and 9-nitroanthracene were detected in some samples. The 9-nitroantracene has been detected in other food samples like smoked pork (Chen et al., 2014) and peat malt (Dennis et al., 1984), while 9-fluorenone has been detected in soybean oil (Hua et al., 2016) in smoked meat (Chen et al., 2014), and in fried Chinese foods (G. Li et al., 2016; Zhao et al., 2017). Benzo[*b*]fluoranthene was the most detected

Table 1
Concentrations (μg L^{-1}) of PAHs and their derivatives in dark roasted (n = 10), dark-caramel (n = 6) and pilsen (n = 10) beers samples.

Analytes	Dark-roasted										Pilsen										Dark-Caramel					
	Concentration (μg L^{-1})[a]																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Naphthalene	nd	nd	2.55	nd	21.85	nd	nd	nd	nd	nd	3.66	nd	4.08	2.45	nd	nd	nd	nd	2.41	nd	nd	nd	nd	nd	1.89	nd
Acenaphthylene	nd	nd	nd	nd	1.08	nd	nd	nd	nd	nd	1.93	nd	nd	nd	nd	nd	nd	nd	0.35	nd	nd	nd	nd	nd	nd	nd
Acenaphthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.58	nd	nd	nd	nd	nd	nd	0.64	nd	nd	1.06	1.63	nd	nd	nd	nd
Fluorene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.96	nd	2.54	0.88	nd	nd	nd	nd	0.65	nd	nd	nd	nd	nd	nd	nd
Phenanthrene	19.06	2.07	2.20	nd	0.97	2.00	1.54	nd	0.86	2.28	8.16	0.93	2.25	1.51	2.84	nd	nd	2.82	2.38	3.18	1.71	2.44	0.85	1.70	3.22	nd
Anthracene	1.35	nd	1.40	nd	0.78	1.49	nd	nd	nd	1.58	6.95	nd	nd	0.78	nd	nd	nd	2.51	1.84	nd	1.67	1.85	nd	nd	2.59	nd
Fluoranthene	nd	nd	nd	nd	nd	0.50	nd	nd	nd	nd	1.30	nd	0.83	0.84	0.65	0.56	0.67	0.79	0.77	0.60	0.66	0.77	nd	0.85	0.83	nd
Pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.48	nd	1.34	0.89	nd	nd	0.50	1.08	0.73	0.98	0.68	0.83	nd	0.71	0.94	nd
Benzo [*a*] anthracene	0.96	nd	nd	nd	2.03	nd	nd	nd	nd	3.04	nd	nd	1.00	1.37	1.00	1.72	0.37	1.11	1.43	3.15	1.86	4.30	nd	1.80	0.76	2.98
Crysene	3.59	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.18	4.61	2.30	1.59	2.66	2.08	1.70	6.84	2.47	4.12	nd	2.48	2.54	2.71
Benzo [*b*] fluoranthene	2.44	3.87	2.70	3.23	2.37	4.18	nd	2.61	4.87	nd	5.82	5.41	0.40	4.30	6.62	5.70	4.95	3.62	3.46	nd	16.05	7.97	2.53	17.84	5.44	4.80
Benzo [*k*] fluoranthene	nd	nd	nd	nd	nd	nd	12.05	nd	nd	nd	nd	nd	nd	11.28	nd	nd	6.23	9.38	10.07	nd	nd	nd	2.83	nd	13.62	8.74
9-Nitroanthracene	nd	nd	3.31	nd	nd	2.72	4.04	3.86	nd	3.68	5.64	nd	nd	1.51	2.84	3.03	nd	nd	nd	nd	4.99	2.00	nd	nd	1.95	nd
9-Fluorenone	nd	nd	nd	nd	nd	nd	3.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.58	nd	nd	nd	nd	nd

[a] nd: not detected in sample.

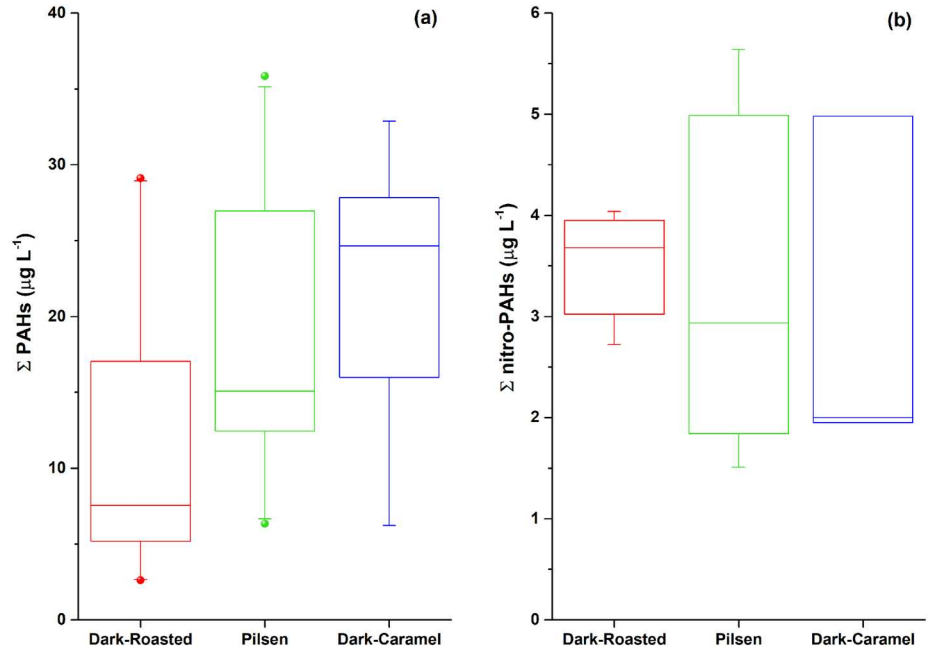

Fig. 4. Box plots of the sum of PAHs and nitro-PAHs concentrations (μg L^{-1}) for the three different types of beers studied: dark-roasted malt (n = 10), dark-caramel (n = 6), Pilsen (n = 10). Dots: outliers; Whiskers: 10th and 90th percentiles; Boxes: 25th and 75th percentiles; Lines inside the boxes: the median.

analyte in the samples and at least one PAHs was detected in all samples. The other compounds analyzed: 2-nitrofluorene, 3-nitrofluoranthene, 1-nitropyrene, 5,12-naphtacenequinone, 9,10-anthracenoquinone, 2-methylanthraquinone, benzo[*a*]pyrene, indene[1,2,3-*c*,d]pyrene, benzo[*g,h,i*]perylene and dibenzo[*a*,h]anthracene, were not detected in the samples analyzed; although benzo[*a*]pyrene and benzo[*g,h,i*]perylene have been detected in Italian beers (Russo et al., 2016).

There is no limit for PAHs in beer, but there are set limits for PAH4 (sum of benzo[*a*]pyrene, benzo[*a*]anthracene, chrysene, and benzo[*b*] fluoranthene in the European Union (EC, 2011). The stricter permitted maximum limits of PAH4 are set at 1.0 μg kg^{-1} in baby and young children's food. In this study, the PAH4 range ranged from 2.61 to 22.0 μg L^{-1} and considering the beer density as 1.0 g mL^{-1}, the PAH4 sum in the samples (2.61–22.12 μg kg^{-1}) was above the established limit. Therefore, these results indicated a potential health problem for consumers of these beers due to the carcinogenic characteristics reported for the PAH4 (EFSA, 2008). In addition, the PAHs derivatives that were detected in this study have high cytotoxicity, and therefore contribute to increase human exposure to these compounds. However, studies related to the bioaccumulation and biomagnification pathways of PAHs derivatives through the food chain are lacking (Sun et al., 2019).

A principal component analysis (PCA) was performed on the results of PAHs and derivative concentrations of the beer samples to verify similar profiles between the samples analyzed. The concentrations of PAHs and their derivatives present in the beer samples were arranged in a 26 x 14 matrix, in which the samples were arranged in the rows (n = 26), and the analytes in the columns (n = 14). The data from this matrix were pre-processed (auto-scaled) and the matrix was processed by the PCA method. Fig. 5A shows the PC1 and PC2 scores which explain 31.43 and 35.70% of the data variance, respectively.

In general, we can observe two groups of beers composed basically by the dark (dark-roasted malt) (Group B), and the other by the dark-caramel and Pilsen (Group A). This separation shows a difference between the beers where malt is used dry (Pilsen and dark-caramel), and the dark beers where it is roasted. The analytes that influenced this separation can be seen in Fig. 5B (PC1 x PC2 loadings). The separation of groups A and B occurred in PC2 and is due to the high relative concentrations of benzo[*a*]anthracene and chrysene in group A (dark-

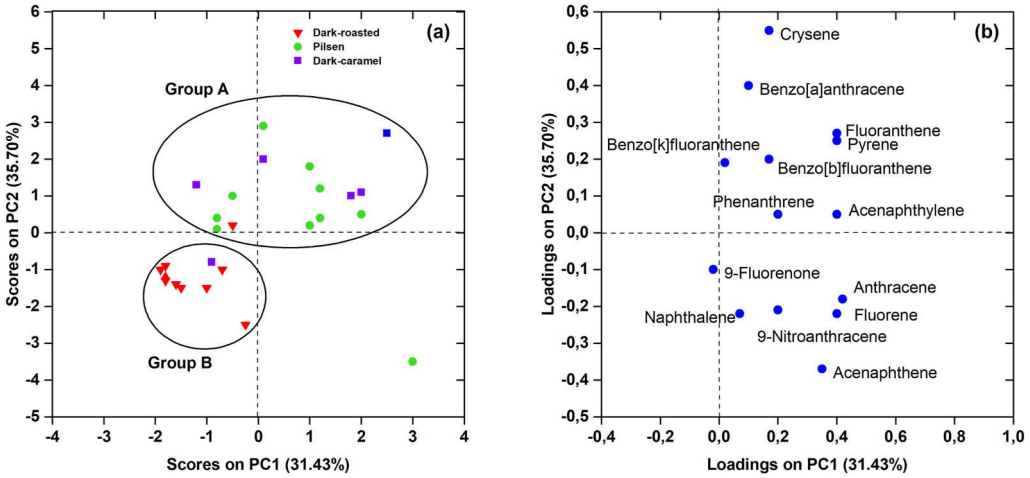

Fig. 5. Scoring (a) and loading (b) graphs for classes: dark-roasted malt (red), Pilsen (green) and dark-caramel (blue) beer samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

caramel and Pilsen), which are high molar mass PAHs, and the high relative concentrations of naphthalene, acenaphthene, fluorene, and 9-nitroanthracene in group B samples. The presence of chrysene in beers has already been detected by Russo et al. (Russo et al., 2016).

The separation between group A and B beers may be due to the type of malt processing. In the direct drying step, malt may come into contact with combustion products which facilitates the synthesis of PAHs from the simplest compounds, a situation similar to that which occurs in the contamination of vegetable oils (Russo et al., 2016). In the roasting process, only substances present in malt can degrade with the formation of PAHs by pyrolysis. It is important to consider that the roasted malt beers analyzed in this study may contain other PAHs that were not studied.

The presence of PAHs in beers varies significantly from beer types. The authors Yoshioka et al. (Yoshioka et al., 2018) analyzed 6 dark beer samples and did not detect the presence of PAHs whereas, in other studies, the presence of PAHs was reported in light beers (Rascon et al., 2019; Russo et al., 2016).

4. Conclusion

A new thermoelectric cooling system has been built to improve the efficiency of CF-SPME extraction. TECS components were produced by a 3D printing prioritizing additive technology, with highly efficient and sustainability. The TECS device for CF-SPME extraction is simple, low cost, allows the use of commercial holders without change and it has a polarity inverter to minimize the damage of SPME fibers. After optimization, the developed CF-SPME-GC/MS method using TECS was validated and demonstrated adequate analytical quality for the intended use in the range of interest. The method was successfully applied for the analysis of PAHs, nitro-PAHs and oxy-PAHs in commercial samples of light and dark beers. Benzo[*b*]fluoranthene was detected in all samples analyzed, while among the PAHs derivatives, only 9-fluorenone and 9-nitroanthracene were found in the samples analyzed. Principal component analysis allowed the separation of samples into two groups related to differences in their malt processing. The new TECS device presents high potential for automation contributing to improve CF-SPME sample preparation.

CRediT authorship contribution statement

Rosimeire Resende dos Santos: Methodology, Investigation, Validation, Writing – original draft. **Ricardo Mathias Orlando:** Methodology, Conceptualization, Writing – review & editing. **Zenilda de Lourdes Cardeal:** Data curation, Project administration, Resources. **Helvécio Costa Menezes:** Software, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2021.108104.

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