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Evaluation of Free- and Bound-Carbonyl Compounds in Craft Beers

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Carbonyl compounds (CC) can be formed during the craft beer production process, which influence the sensory properties, in addition to their toxicity. The formation of bound-CC, in craft beers, is favored by the high reactivity of these substances. This work aimed to quantify 15 carbonyl compounds, in free and bound forms, in craft beers using hydrolysis (bound-CC only) and derivatization reactions. Acetaldehyde concentrations ranged from 8.83 to 466.1 μg L^{-1} (free fraction) and 22.47 to 1665 μg L^{-1} (bound fraction). Other compounds found were acrolein (free + $bound_{max.}$: 2897 μg L^{-1}), benzaldehyde (free + $bound_{max.}$: 1326 μg L^{-1}), heptanal (free + $bound_{max.}$: 1140 μg L^{-1}) and formaldehyde (free + $bound_{max.}$: 97.73 μg L^{-1}). Craft beers showed a proportion of up to 76% for CC in the bound form, which can be related to undesirable flavors in beverages. The consumption of craft beers containing free- and bound-CC (especially acrolein) could pose a risk to the health of frequent consumers.

Keywords: carbonyl compounds, craft beer, derivatization, 2,4-DNPH, HPLC-DAD

Introduction

Beer is an alcoholic beverage produced from the fermentation of cereals. Its original formula contains barley malt, drinking water, hops and yeast as its main ingredients, and other cereals, classified as brewing adjuncts, can be added to the formulation, replacing part of the barley malt up to 45% by mass. In craft beers, ingredients such as fruits, fruit juices, condiments and others can be added, as long as they do not change the original composition of the beverage.[1,2] The beer manufacturing process can be described in several stages, according to the formulation used. However, the main stages of the beverage production are (*i*) malting, (*ii*) milling, (*iii*) mashing, (*iv*) filtration, (*v*) boiling, (*vi*) fermentation, (*vii*) maturation and (*viii*) bottling.[3-7]

According to the Brazilian Beer Industry Association (CERVBRASIL),[8] Brazil is the third largest producer of the beverage, with an annual production of around 14 billion liters. In addition to production, the country stands out as a major consumer of the beverage, occupying the 17th position in the world.[8] An advance in the growth of the beer market has been observed over the last few years. A significant part of this growth is related to new consumption trends, with highlight on the increase in craft breweries.[9]

Craft beers are identified as a product with high added value and their main characteristics are the variety of colors, aromas and flavors.[10] In the absence of an official definition, craft beers have been considered as those produced on a small scale and are associated with a slow fermentation process, without the addition of preservatives, stabilizers, dyes or that contain any difference compared to industrialized beers. They can be produced with small amounts of natural products and are modified in a particular way by each producer until they have specific organoleptic properties.[2,11-14]

Fermentation, environmental conditions, yeast strain and raw materials (mainly malt and hops) are factors that contribute to the beer chemical profile, promoting the

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formation of compounds that contribute to the composition of the aroma and flavor characteristic of the beverage. However, the combination of these factors can also promote the formation of compounds with undesirable characteristics, such as carbonyl compounds (CC), furan derivatives (furfural and furfuryl alcohol), biogenic amines, among others.[3,15,16]

The presence of carbonyl compounds in beer significantly influences the sensory properties, especially the flavor and aroma of the beverage.[17] Additionally, the ingestion of high concentrations of carbonyl compounds can promote mutations in the human body and increase the risk of some types of cancer.[18,19] Thus, in the classification list of the International Agency for Research on Cancer (IARC), some carbonyl compounds are among those with carcinogenic characteristics.[20] Ingestion of carbonyl compounds may also be associated with multiple sclerosis, Alzheimer's disease, cardiovascular disease, hepatotoxicity, and nephrotoxicity.[21] Another effect caused mainly by acetaldehyde, is veisalgia (better known as "next day hangover"). Symptoms of the hangover include headache, nausea, drowsiness, fatigue and vomiting.[22]

In addition to fermentation, aging is one of the main stages in the formation of carbonyl compounds in beers, which are products of oxidative and non-oxidative reactions. Among the carbonyl compounds formed during beer aging, those with higher molecular weight such as 3-methylbutanal, 2-methylbutanal, (*E*)-2-nonenal, heptanal, benzaldehyde, among others, stand out. Carbonyl compounds formed during aging are mainly responsible for the rancid taste of beer.[23]

The main pathways of the formation of carbonyl compounds in beers can include the Strecker degradation of amino acids, the oxidation of alcohols and the autoxidation of fatty acids.[24] Carbonyl compounds can be present in beer in two forms (free and/or bound). In free form, they contribute significantly to the taste of the drink. In the bound form, these compounds are neither evaporated nor transformed into their corresponding alcohols, which makes it difficult to perceive them from an analytical and sensory point of view.[25]

The formation of bound-carbonyl compounds is favored by the high reactivity of these substances, which can react with several other components present in beer. The clear mechanism of how these interactions occur has not yet been fully elucidated. However, as cysteine and bisulfite are well-known intermediaries in brewing products, the bonding of carbonyl compounds to these species to form bound-carbonyl compounds is quite usual.[26,27] On the other hand, carbonyl compounds (especially aldehydes) can react with SO_2 from yeast metabolism or any exogenous sulfite added before the bottling step to form α-hydroxysulfonates.[28] As proposed by Trueba *et al*.,[26] a schematic representation of the chemical reactions for the formation of bound-carbonyl compounds in beers is represented in Figure 1.

In view of the above, this work aimed to identify and quantify 15 carbonyl compounds, in free and bound forms, in real samples of craft beers using hydrolysis reactions and derivatization with 2,4-dinitrophenylhydrazine and subsequent analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD).

Experimental

Standards, reagents and samples

The analytical standards of carbonyl compounds, in the form of their respective 2,4-dinitrophenylhydrazones (2,4-DNPHo), used in this study were formaldehyde-2,4-DNPHo; acetaldehyde-2,4-DNPHo; acrolein-2,4-DNPHo; propionaldehyde-2,4-DNPHo; crotonaldehyde-2,4-DNPHo; methacrolein-2,4-DNPHo; butyraldehyde-2,4-DNPHo; benzaldehyde-2,4-DNPHo; valeraldehyde-2,4-DNPHo; cyclohexanone-2,4-DNPHo; hexaldehyde-2,4-DNPHo; heptanal-2,4-DNPHo; octanal-2,4-DNPHo; nonanal-22,4-DNPHo; decanal-2,4-DNPHo, all purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA), with purity ≥ 99%.

Other reagents used were 2,4-dinitrophenylhydrazine (2,4-DNPH), 97% purity (Sigma-Aldrich Co., Saint Louis, MO, USA), phosphoric acid (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil), sodium hydroxide (Dinâmica

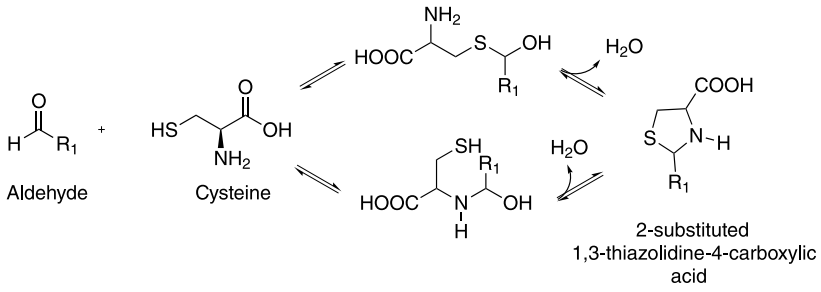

Figure 1. Schematic representation of chemical reactions involving the formation of bound-aldehydes in beers (adapted from Trueba *et al.*[26]).

Química Contemporânea Ltda, São Paulo, SP, Brazil), pesticide grade dichloromethane (Merck KGaA, Darmstadt, Germany), HPLC grade acetonitrile (J.T. Baker Chemical Company, Radnor, PA, USA), fuming hydrochloric acid (Synth, Diadema, SP, Brazil) and ultrapure water (Milli-Q, Darmstadt, Germany).

Stock solutions of the carbonyl compounds hydrazones were prepared individually, in acetonitrile, at the following concentrations: formaldehyde (510 mg L^{-1}); acetaldehyde (511 mg L^{-1}); acrolein (480 mg L^{-1}); propionaldehyde (410 mg L^{-1}); crotonaldehyde (400 mg L^{-1}); methacrolein (510 mg L^{-1}); butyraldehyde (400 mg L^{-1}); benzaldehyde (400 mg L^{-1}); valeraldehyde (444 mg L^{-1}); cyclohexanone (410 mg L^{-1}); hexaldehyde (510 mg L^{-1}); heptanal (400 mg L^{-1}); octanal (410 mg L^{-1}); nonanal (500 mg L^{-1}) and decanal (444 mg L^{-1}). From the respective stock solutions, an intermediate solution was prepared (10 mg L^{-1}) containing the mixture of all carbonyl compounds. All solutions were stored in amber bottles under refrigeration.

Craft beer samples (n = 13) were acquired in commercial establishments located in the city of Salvador, Bahia, Brazil. To obtain the samples, different brands, styles and commercial availability were considered. Information about the craft beers used in this study can be found in Table 1.

All samples of craft beers were submitted to the decarbonation process, according to the methodology described by the Instituto Adolfo Lutz (IAL), for the removal of CO_2, before being submitted to the procedure of derivatization of carbonyl compounds.[29] After decarbonation, the samples were kept closed and stored under refrigeration, in the original packaging, properly closed, until the time of analysis.

Before carrying out the derivatization reaction of the cabonyl compounds, in the samples of craft beers, a solution of 0.5% 2,4-DNPH (in acetonitrile) was prepared from the recrystallized and purified derivatizing agent (using liquid-liquid extraction), according to the procedure described by Cardozo *et al*.[30] This step is very important for this type of analysis because it minimizes the presence of possible contamination of the derivatizing agent, which can compromise the analytical quality of the data obtained by liquid chromatography.

The preparation of samples for the analysis of free and bound-carbonyl compounds was carried out through adaptations of the procedures proposed by Cardozo *et al*.[30,31]

Preparation of the craft beer samples

Analysis of the free-carbonyl compounds

To 50 mL of craft beer samples, 5 drops of fuming hydrochloric acid were added to reduce the pH to < 2. Then, 10 mL of 0.5% 2,4-DNPH solution, previously purified, were added to the respective acidified samples and the mixture was subjected to an ultrasound bath for 20 min. After the derivatization reaction, the samples were pre-concentrated by means of solid phase extraction (SPE), using Sep-pack® C18 cartridges (Waters Co., Milford, MA, USA), previously conditioned with 2 mL of methanol. A polytetrafluoroethylene (PTFE) filter (0.45 μm) was connected to the end of the cartridge and the carbonyl compounds retained in the adsorbent were eluted with 1 mL of acetonitrile and, therefore, filtered directly into the respective 2.0 mL vials and injected into the chromatographic system.

Analysis of the bound-carbonyl compounds

Initially, the pH of the samples was adjusted to 11, to allow the hydrolysis of bound-carbonyl compounds to

Table 1. Information on the analyzed craft beer samples

Sample	Style/type of beer	Adjuncts/ingredients	Origin country
01	Witbeer	coriander, allspice and orange	Belgium
02	Premium Lager	herbal	Jamaica
03	Lager	wheat	Brazil
04	Weiss	ginger	Brazil
05	Pilsen	wheat	Argentina
06	Amber Lager	caramel	Argentina
07	Weiss	orange and coriander	Argentina
08	Honey Wheat Ale	bee's honey	Brazil
09	American Lager	orange	Brazil
10	American India Pale Ale	passion fruit	Brazil
11	Ale Fruit Beer	cinnamon and berries	Brazil
12	Weissbeer	cloves and bananas	Brazil
13	Pilsen	fruity cereals	Brazil

substances present in craft beers, in order to release them in solution. For this, 1 mol L^{-1} sodium hydroxide was added to 50 mL of craft beer samples and stirred for 30 min. After stirring, 10 mL of 0.5% 2,4-DNPH solution were added to the samples, together with 5 drops of fuming hydrochloric acid, and they were subjected to an ultrasound bath for 20 min. After derivatization, the samples were percolated in Sep-pack® C18 cartridges (Waters Co., Milford, MA, USA), previously conditioned with 2 mL of methanol, for pre-concentration of the analytes. Then, the samples were eluted from the cartridges (1 mL of acetonitrile) and filtered directly into the respective 2.0 mL vials and injected into the chromatographic system. In this way, the total concentration or $CC_{free+bound}$ ($CC_{free+bound} = CC_{free} + CC_{bound}$) of each CC individually present in the craft beers samples were analyzed.

Thus, the bound form of each carbonyl compound was analyzed indirectly, through the quantification of free form (CC_{free}) and of total portion ($CC_{free+bound}$) of each CC individually present in the craft beers samples. The concentration of each bound-CC was then obtained by the difference between the concentrations of $CC_{free+bound}$ and CC_{free} present in the samples ($CC_{bound} = CC_{free+bound} - CC_{free}$). Thus, a chemical fractioning of the carbonyl compounds present in craft beers was carried out.

Chromatographic analysis

The analyzes of free- and bound-carbonyl compounds were performed in a high-performance liquid chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a diode array detector (SPD-M20A), a quaternary pump (LC-20AT); communication interface (CBM-20A); automatic injector (SIL-20A); and column oven (CTO-20A). The chromatographic separation of the analytes was performed using a Shim-pack VP-ODS Shimadzu column (250 mm × 4.6 mm; 5 µm), coupled to a Shim-pack GVP-ODS Shimadzu pre-column (10 mm × 4.6 mm; 5 µm).

Water (solvent A) and acetonitrile (solvent B) were used as the mobile phase. Elution gradient was 0-2.0 min (45% B to 75% B); 2.0-10.0 min (75% B); 10.0-23.0 min (75% B to 100% B); 23.0-25.0 min (100% B); 25.0-27.0 min (100% B to 50% B); 27.0-33.0 min (50% B to 45% B); 33.0-33.5 min (45% B). The mobile phase flow was 1.0 mL min^{-1}. The oven temperature was 40 °C. The injection volume for the standard solutions and the samples was 20 µL. Chromatographic runs were performed at a wavelength of 365 nm. It is worth mentioning that the chromatographic conditions used in this work were adapted from Cardozo *et al.*[31]

The quantification of the free- and bound-carbonyl compounds in craft beer samples was performed by the external standardization method. Analytical curves were constructed through successive dilutions of the intermediate solution (10 mg L^{-1}) containing the hydrazones of the respective carbonyl compounds, in acetonitrile, at concentrations ranging from 5.0 to 300 µg L^{-1}. The samples that presented analytes with concentrations above the linear range were diluted in acetonitrile. All samples and standard solutions were analyzed in triplicate.

To attest the performance of the analytical method, the following figures of merit were evaluated: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability (intraday precision) and intermediate precision (interday precision).[32-34]

Exposure risk assessment for consumption of craft beers containing carbonyl compounds

The determination of the estimated daily intake (EDI) for assessmenting the exposure to carbonyl compounds ($CC_{free+bound}$) found in the analyzed craft beer samples was performed according to equation 1, as suggested by Hernandes *et al.*[35]

$$\text{EDI } (\mu g\ kg^{-1}\ BW\ day^{-1}) = \frac{\left(\begin{matrix}CC_{free+bound}\ \text{concentration}\\ (\mu g\ mL^{-1})\end{matrix}\right) \times \left(\begin{matrix}\text{craft beer consumption}\\ (mL\ day^{-1})\end{matrix}\right)}{\text{body weight (kg)}} \quad (1)$$

For EDI calculations, the sums of individual concentrations of free- and bound-carbonyl compounds ($CC_{free+bound}$) identified and quantified in craft beers were taken into account (as mentioned in "Analysis of the bound-carbonyl compounds" sub-section). To obtain the EDI values, the following considerations were made: (*i*) average daily craft beer consumption of 300 mL for women and 600 mL for men, considering a moderate consumption; (*ii*) average body weight (BW) of 70.3 kg for Brazilian women and 80.7 kg for Brazilian men.[35-37]

The ratio between the benchmark dose's lower one-sided confidence limit (BMDL) and predicted human consumption/exposure of the same substance is known as the margin of exposure (MOE) (equation 2). As mentioned by Hernandes *et al.*,[35] since the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has not established levels for safe ingestion of genotoxic compounds (such as formaldehyde, acetaldehyde and acrolein), MOE can be used for risk characterization for the consumption of craft beers containing these compounds. MOE is typically used to compare the health risks of various chemicals and, as a result, to prioritize risk management efforts. The lower the

MOE, the greater the risk to people; typically, a value of less than 10000 is used to indicate health risk.[38]

$$\text{MOE} = \frac{\text{BMDL}_{10}\ (\mu g\ kg^{-1}\ BW\ day^{-1})}{\text{EDI}\ (\mu g\ kg^{-1}\ BW\ day^{-1})} \quad (2)$$

$BMDL_{10}$ values considered in our work, for formaldehyde, acetaldehyde and acrolein, were the same mentioned by Hernandes *et al.*[35]

Results and Discussion

Validation of the method

Chromatographic profile of the carbonyl compounds

The chromatographic separation was satisfactory for the analyzed carbonyl compounds. The chromatogram shown in Figure 2 is the result of the injection of a standard solution of 50 μg L^{-1}, containing the fifteen carbonyl compounds studied, and of one craft beer sample. However, only fourteen peaks were observed in the chromatogram of the mixture of carbonyl compounds using the proposed chromatographic method, since there was coelution of the compounds valeraldehyde and cyclohexanone. These compounds, when analyzed individually, presented very close retention times (14.127 and 13.974 min, respectively) which may have resulted in the overlapping of the peaks during the simultaneous injection. Thus, the identification and quantification of these two compounds were performed considering the sum of both them, which did not compromise the analytical quality of the results for the other analytes.

Figures of merit

Table 2 shows the figures of merit obtained to attest the analytical quality of the data, through the validation of the method.

Selectivity

In the chromatographic conditions used, it was possible to observe that the samples of craft beers did not present chemical species interfering in the retention times of the studied carbonyl compounds. This fact was observed by comparing the chromatogram of a sample of craft beer without the addition of the standard solution of carbonyl compounds and that of the same sample fortified with the standards of carbonyl compounds at a concentration of 100 μg L^{-1} (Figure S1, Supplementary Information (SI) section).

Linearity

It was possible to observe that the method presented good linearity for the concentration levels considered as working range, being 5 to 100 μg L^{-1} for acrolein;

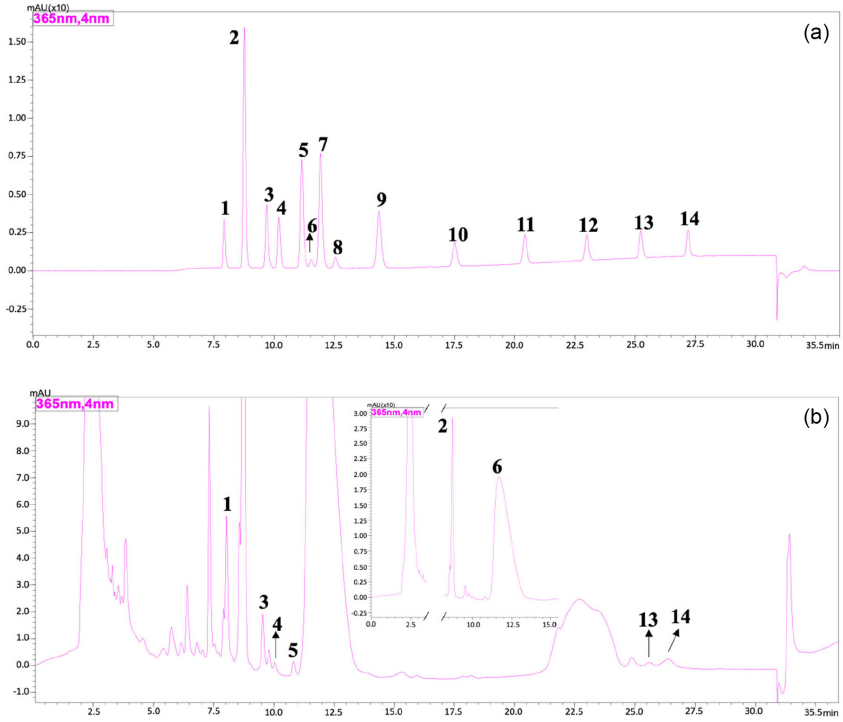

Figure 2. Chromatograms obtained, by HPLC-DAD (365 nm), for (a) a standard mixture of carbonyl compounds (CC), at a concentration of 200 μg L^{-1}, and for (b) a sample of craft beer ($CC_{free+bound}$ analysis). Peak identification: 1-formaldehyde; 2-acetaldehyde; 3-acrolein; 4-propionaldehyde; 5-crotonaldehyde; 6-methacrolein; 7-butyraldehyde; 8-benzaldehyde; 9-valeraldehyde+cyclohexanone; 10-hexaldehyde; 11-heptanal; 12-octanal; 13-nonanal; 14-decanal.

Table 2. Figures of merit obtained for free- and bound-carbonyl compounds analysis in craft beers

Carbonyl compound	t_R / min	Linear range / (μg L^{-1})	R^2	LOD / (μg L^{-1})	LOQ / (μg L^{-1})	Repeatability (intraday precision) RSD / %				Intermediate precision (interday precision) RSD / %			
						5 μg L^{-1}	25 μg L^{-1}	50 μg L^{-1}	200 μg L^{-1}	5 μg L^{-1}	25 μg L^{-1}	50 μg L^{-1}	200 μg L^{-1}
Formaldehyde	7.875	5.00-200	0.9996	2.90	9.66	12.7	12.6	13.8	4.03	–	6.83	3.91	4.09
Acetaldehyde	8.701	5.00-200	0.9997	2.37	7.89	2.63	4.45	4.41	2.04	2.56	1.34	0.68	0.67
Acrolein	9.613	5.00-100	0.9994	2.11	7.02	11.6	3.29	3.47	2.06	10.1	4.14	1.30	0.44
Propionaldehyde	10.116	5.00-200	0.9996	2.93	9.68	14.2	2.86	4.94	2.14	8.97	4.77	1.90	0.48
Crotonaldehyde	11.037	5.00-200	0.9997	2.58	8.61	9.07	1.67	1.74	1.13	7.28	1.6	1.23	0.95
Methacrolein	11.418	25.0-300	0.9996	6.13	20.5	–	16.9	4.93	2.97	–	14.5	8.26	2.08
Butyraldehyde	11.790	5.00-200	0.9996	2.68	8.94	9.28	3.78	3.29	1.72	7.94	4.02	1.71	0.35
Benzaldehyde	12.361	10.0-200	0.9990	5.52	18.4	–	13.0	6.78	2.78	–	11.6	10.2	1.42
Valeraldehyde + Cyclohexanone	14.089	5.00-200	0.9995	3.08	10.3	10.5	4.60	2.74	1.86	7.82	3.69	1.73	0.66
Hexaldehyde	17.303	5.00-200	0.9992	3.99	13.3	12.9	12.2	4.59	2.18	16.8	16.9	3.32	1.21
Heptanal	20.122	5.00-200	0.9991	4.12	13.7	16.5	6.40	5.21	2.02	13.7	10.9	3.47	0.94
Octanal	22.721	5.00-200	0.9999	1.69	5.62	15.0	6.35	6.19	1.59	7.67	8.08	3.73	0.97
Nonanal	24.912	5.00-200	0.9995	3.28	10.9	15.5	7.36	5.74	1.91	14.1	5.74	3.33	1.04
Decanal	26.989	5.00-200	0.9996	2.91	9.71	12.1	6.60	3.42	2.00	11.2	7.34	3.75	0.96

t_R: retention time average; R^2: determination coefficient; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

25 to 300 μg L^{-1} for methacrolein; 10 to 200 μg L^{-1} for benzaldehyde and 5 to 200 μg L^{-1} for the other carbonyl compounds. These intervals showed the best responses in relation to the coefficients of determination (R^2), whose values ranged from 0.9990 to 0.9999 (Table 2). These values indicate a good dispersion of the points and an ideal fit of the data to the regression line, since the R^2 values remained greater than 0.99, as recommended.[32,34]

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were obtained through the parameters of the analytical curves.[32,34] The values found for the limits of detection ranged between 1.69 μg L^{-1} (octanal) and 6.13 μg L^{-1} (methacrolein). While, for the limits of quantification, the variation was from 5.62 μg L^{-1} (octanal) to 20.45 μg L^{-1} (metacrolein) (Table 2). For the limits of detection, the values found in the present work were lower when compared to the values found by Zhao *et al.*[39] (16 μg L^{-1}, for formaldehyde) considering the same analytical technique (HPLC-DAD) used in both studies. Hernandes *et al.*[35] obtained limits of detection ranging from 0.03 to 0.3 μg L^{-1} for the analysis of formaldehyde, acetaldehyde, acrolein, furfural, acetylfuran and 5-methylfurfural in beers using HS-SPME/GC-MS (headspace solid phase microextraction followed by gas chromatography coupled to mass spectrometry), after derivatization of the analyzes with 2,2,2-trifluoroethylhydrazine (TFEH). For the analysis of free-CC (formaldehyde, acetaldehyde, acrolein and furfural) in craft beers, LOD values between 0.01 and 0.5 μg L^{-1} were obtained using derivatization with TFEH, followed by analysis by HS-SPME/GC-MS.[15] Nevertheless, the low LOD and LOQ obtained in our work attest the good sensitivity of the proposed method for determining free- and bound-CC in craft beers.

Precision

Repeatability and intermediate precision were the precision parameters evaluated in this work and the concentration levels tested were 5.0, 25, 50 and 200 μg L^{-1}. In the intraday precision (repeatability), the values of relative standard deviations found were between 1.13% (crotonaldehyde), at a concentration of 200 μg L^{-1}, and 16.9% (metacrolein), at a concentration of 25 μg L^{-1}. For the interday precision (intermediate precision), the values varied between 0.35% (butyraldehyde), at a concentration of 200 μg L^{-1}, and 16.9% (hexaldehyde), at a concentration of 25 μg L^{-1} (Table 2). For these parameters, the acceptance criteria for the relative standard deviation (RSD) can be from 1 to 2%, for the quantification of macro quantities, and up to 20% for trace analysis.[34] In this work, the determinations of free- and bound-carbonyl compounds were performed at trace levels, obtaining relative standard deviations below 20% for all tested concentration levels, attesting that the precision for the proposed method was satisfactory.

Free- and bound-carbonyl compounds analysis in craft beers

The results obtained for the free- and bound-carbonyl compounds identified and quantified in the craft beer samples are shown in Table 3. The concentrations of free carbonyl compounds ranged from 7.30 (acrolein, sample 10) to 2897 μg L^{-1} (acrolein, sample 1). For the bound-carbonyl

Table 3. Free- and bound-carbonyl compounds concentration in craft beers

Carbonyl compounds	Concentration[a] / (μg L^{-1})													
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7	
	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}
Formaldehyde	< 2.90	32.72 ± 0.95	15.77 ± 0.24	< 9.66	< 9.66	28.69 ± 3.07	< 2.90	14.02 ± 0.06	< 2.90	19.00 ± 0.14	< 2.90	14.02 ± 0.06	< 9.66	< 2.90
Acetaldehyde	460.0 ± 11.3	165.7 ± 3.5	8.831 ± 0.556	22.47 ± 4.68	34.04 ± 0.95	142.9 ± 0.1	242.1 ± 2.6	159.0 ± 0.8	142.1 ± 4.0	1665 ± 9	242.1 ± 2.6	159.0 ± 0.8	< 2.37	< 7.89
Acrolein	2897 ± 11	< 7.02	2153 ± 19	713.4 ± 52.3	1400 ± 10	ND	2570 ± 1	ND	< 7.02	12.09 ± 0.58	2570 ± 1	ND	< 2.11	< 2.11
Propionaldehyde	ND	< 9.68	ND	ND	ND	< 9.68	ND	< 2.93	1182 ± 28	ND	ND	< 2.93	362.9 ± 4.7	ND
Crotonaldehyde	ND	ND	173.6 ± 8.0	464.0 ± 20.9	< 8.61	ND	ND	< 2.58	ND	< 2.58	ND	< 2.58	ND	< 2.58
Methacrolein	ND	ND	128.4 ± 12.6	ND	ND	< 6.13	ND	ND	ND	ND	ND	ND	ND	1124 ± 53
Butyraldehyde	ND	ND	ND	ND	ND	< 8.94	ND	ND	ND	25.54 ± 0.26	ND	ND	ND	ND
Benzaldehyde	ND	ND	ND	ND	< 18.4	1326 ± 23	10.33 ± 2.07	1282 ± 90	< 5.52	158.7 ± 5.2	< 5.52	508.5 ± 3.7	ND	ND
Valeraldehyde + Cyclohexanone	12.11 ± 0.51	ND	< 3.08	< 3.08	ND	ND	ND	< 3.08	< 3.08	< 3.08	ND	< 3.08	ND	ND
Hexaldehyde	ND	< LOD	ND	< LOD	113.8 ± 7.9	ND	165.2 ± 1.4	ND	ND	< LOD	165.2 ± 1.4	ND	ND	ND
Heptanal	1040 ± 31	ND	1140 ± 25	ND	445.4 ± 7.9	ND	127.1 ± 1.8	ND	ND	ND	127.1 ± 1.8	ND	ND	ND
Octanal	ND	ND	ND	18.99 ± 3.89	ND	< 5.62	< 1.69	< 1.69	ND	ND	< 1.69	< 1.69	ND	ND
Nonanal	ND	ND	ND	ND	ND	ND	< 3.28	< 3.28	< 10.9	ND	< 3.28	< 3.28	ND	< 3.28
Decanal	ND	< 2.91	ND	ND	ND	13.09 ± 2.37	< 2.91	< 9.71	ND	ND	< 2.91	< 9.71	ND	< 2.91
CC_{total}[b] / (mg L^{-1})	4.61		4.84		3.51		4.25		3.20		3.79		1.49	
	Sample 8		Sample 9		Sample 10		Sample 11		Sample 12		Sample 13			
	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}	CC_{free}	CC_{bound}		
Formaldehyde	< 2.90	12.71 ± 0.85	< 2.90	< 2.90	< 2.90	< 9.66	< 2.90	19.28 ± 0.76	< 9.66	97.73 ± 3.00	< 2.90	< 9.66		
Acetaldehyde	252.5 ± 5.8	814.9 ± 40.9	69.43 ± 1.31	303.1 ± 11.0	466.1 ± 10.1	675.3 ± 0.6	142.0 ± 5.1	1048 ± 60	10.41 ± 0.23	302.6 ± 11.0	57.05 ± 1.02	1303 ± 0		
Acrolein	< 2.11	< 7.02	1.416 ± 28	ND	7.30 ± 0.26	ND	1316 ± 37	ND	1812 ± 44	ND	853.2 ± 2.7	ND		
Propionaldehyde	925.7 ± 10.1	ND	ND	< 2.93	617.3 ± 10.0	ND	ND	< 2.93	ND	2270 ± 15	ND	< 2.93		
Crotonaldehyde	ND	333.9 ± 12.7	148.2 ± 6.1	ND	ND	ND	ND	ND	ND	ND	ND	ND		
Methacrolein	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
Butyraldehyde	ND	ND	ND	ND	< 2.68	< 2.68	ND	ND	ND	ND	ND	ND		
Benzaldehyde	ND	ND	ND	ND	< 5.52	< 5.52	ND	ND	ND	ND	ND	ND		
Valeraldehyde + Cyclohexanone	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08	< 3.08		
Hexaldehyde	< 3.99	< 3.99	< 3.99	< 3.99	< 3.99	< 13.3	< 3.99	19.93 ± 0.78	< 3.99	< 3.99	< 3.99	< 13.3		
Heptanal	< 4.12	< 4.12	72.51 ± 5.43	ND	ND	< 13.7	ND	< 13.7	< 4.12	< 4.12	< 13.7	< 13.7		
Octanal	< 1.69	< 1.69	< 1.69	< 1.69	ND	11.20 ± 1.36	561.6 ± 27.4	ND	ND	ND	564.6 ± 5.9	ND		
Nonanal	222.1 ± 33.1	ND	ND	ND	< 10.9	< 3.28	ND	ND	ND	ND	ND	18.03 ± 0.16		
Decanal	ND	16.64 ± 0.52	ND	ND	ND	22.34 ± 1.99	ND	ND	ND	ND	ND	27.64 ± 0.11		
CC_{total}[b] / (mg L^{-1})	2.56		2.01		1.78		3.11		4.49		2.80			

[a]Mean ± standard deviation; [b]CC_{total}: sum of individual concentrations of free- and bound-CC (CC_{free} + CC_{bound}). ND: not detected. Analytes indicated as ND represent concentrations that could be below the instrumental limits of detection, different from the limits of detection of the method, estimated in this work.[32]

compounds, the variation was from 11.20 (octanal, sample 10) to 2270 μg L^{-1} (propionaldehyde, sample 12).

It is worth mentioning that data related to the determination of free- and bound-carbonyl compounds in different types of beverages are scarce. Most works present only the determination of the free fraction of carbonyl compounds, which may be underestimating CC concentrations in different types of beverages. One of the main carbonyl compounds found in fermented and distilled beverages is acetaldehyde (Table 4). In this work, the acetaldehyde was identified and quantified in 92% of the analyzed craft beer samples. In 69% of the craft beer samples, the bound-acetaldehyde fraction was higher than the free-acetaldehyde fraction. In general, acetaldehyde concentrations ranged from 8.83 to 466.1 μg L^{-1} (free fraction) and from 22.47 to 1665 μg L^{-1} (bound fraction), in the craft beer samples (Table 3).

The concentrations of acetaldehyde in the free form can decrease after the maturation step (1.5 μg L^{-1}), especially in craft ale-type beers, with a reduction in the concentration of approximately 90% for this compound, compared to previous steps in the production process (15.2 μg L^{-1}). Aldehydes (including acetaldehyde, formaldehyde and acrolein) can bind to phenolic compounds, in addition to other substances, due to the electrophilic and nucleophilic character of these compounds, respectively.[15] Phenolic compounds such as catechin, epicatechin, caffeic acid, rutin and formononetin have been identified and quantified in different types of craft beers.[13] This could explain the higher concentrations of acetaldehyde, formaldehyde and others carbonyl compounds in the bound fraction, compared to the free fraction, for most craft beer samples analyzed in our work, with emphasis on samples 8 and 10, which are craft beers with ale-type fermentation.

In addition to acetaldehyde, other more relevant compounds found in craft beer samples were formaldehyde

Table 4. (Free-) carbonyl compounds analysis in different types of beverages

Matrix	Carbonyl compounds	Derivatizing agent	Instrumental technique	LOD	Concentration range	Reference
Craft beer	formaldehyde; acetaldehyde; acrolein; furfural	TFEH	HS-SPME/ GC-MS-SIM	0.01 to 0.5 μg L^{-1}	< 1.0 to 24.8 μg L^{-1}	15
Wine	formaldehyde; acetaldehyde; acrolein; furfural	TFEH	HS-SPME/ GCxGC-ToFMS	0.5 to 3.0 μg L^{-1}	8.4 to 1.715 μg L^{-1}	19
Wine	formaldehyde; acetaldehyde; acrolein; furfural; 5-methylfurfural	not informed	HS-SPME/ GC-MS-SIM	< 1.5 μg L^{-1}	< 1.5 to 227.9 μg L^{-1}	21
Mineral water	formaldehyde; acetaldehyde; acrolein; acetone; propionaldehyde; crotonaldehyde; methacrolein; isobutyraldehyde; butyraldehyde; valeraldehyde; hexaldehyde; benzaldehyde; *o*-tolualdehyde; and *m*-tolualdehyde	2,4-DPNH	UFLC-MS	0.6 to 4.0 ng mL^{-1}	< LOD to 125 ng mL^{-1}	31
Beer	formaldehyde; acetaldehyde; acrolein; furfural; acetylfuran; 5-methylfurfural	TFEH	HS-SPME/ GC-MS-SIM	0.03 to 0.3 μg L^{-1}	< 1.0 to 4264.3 μg L^{-1}	35
Beer	formaldehyde	EAHC	HPLC-DAD	0.016 mg L^{-1}	0.17 to 0.62 mg L^{-1}	39
Sugar cane spirit	furfural acrolein	2,4-DPNH	HPLC	0.02758 mg/100mL	4.28 to 39.78 mg/100mL up to 7.45 mg/100mL	40
Sugar cane spirit	acrolein	2,4-DNPH	HPLC	0.0516 mg/100 mL	up to 25.95 mg/100mL	41
Beer	formaldehyde	2,4-DNPH	HPLC-UV	0.6 ng mL^{-1}	172 to 385 ng mL^{-1}	42
White wine Red wine	acetaldehyde	2,4-DNPH	HPLC-DAD/MS	5 μg L^{-1}	30 to 70 mg L^{-1} 4 to 6 mg L^{-1}	43
Beer	furfural	2,4-DNPH	HPLC-DAD	19 μg L^{-1}	205 to 687 μg L^{-1}	44
Mineral water	formaldehyde acetaldehyde	2,4-DPNH	LC-MS/MS	–	2.6 to 31.4 μg mL^{-1} 5.3 to 144 μg mL^{-1}	45
Wine	formaldehyde; acetaldehyde; acrolein; furfural	TFEH	HS-SPME/ GCxGC-ToFMS	0.5 to 3.0 μg L^{-1}	8.4 to 1715 μg L^{-1}	46
Craft beer	formaldehyde; acetaldehyde; acrolein; propionaldehyde; crotonaldehyde; methacrolein; butyraldehyde; benzaldehyde; valeraldehyde; cyclohexanone; hexaldehyde; heptanal; octanal; nonanal; decanal	2,4-DPNH	HPLC-DAD	1.69 to 6.13 μg L^{-1}	7.30 to 2897 μg L^{-1} (free-CC) 11.20 to 2270 μg L^{-1} (bound-CC)	this work

2,4-DNPH: 2,4-dinitrophenylhydrazine; EAHC: ethoxyamine hydrochloride; TFEH: 2,2,2-trifluoroethylhydrazine; LOD: limit of detection; HS-SPME/GC-MS-SIM: headspace solid phase microextraction followed by gas chromatography coupled to mass spectrometry in selective ion monitoring mode; GC-MS: gas chromatography coupled to mass spectrometry; UFLC-MS: ultra-fast liquid chromatography with diode-array detection; HPLC-DAD: high performance liquid chromatography with diode-array detection; HS-SPME/GCxGC-ToFMS: headspace solid phase microextraction followed by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometer.

(free: < 9.66 to 15.77 μg L^{-1}; bound: < 9.66 to 97.73 μg L^{-1}) and acrolein (free: < 7.02 to 2897 μg L^{-1}; bound: < 7.02 to 713.4 μg L^{-1}), which were found predominantly in the bound and free fraction, respectively.

Hernandes *et al.*[15] reported that acetaldehyde, acrolein and formaldehyde were found in their respective free forms in all stages of fermentation to produce craft beers of the ale and lager types. Additionally, the boiling and fermentation processes appear to be important steps in the formation of acrolein and acetaldehyde in ale-type beers. It is noteworthy that these authors analyzed carbonyl compounds only in free forms, at different stages of the craft beers production process; in addition to having analyzed a reduced number of analytes, when compared to our work, with emphasis on the analysis of low molecular weight carbonyl compounds.

Other analytical techniques have been used to determine CC in beers. The work of Hernandes *et al.*[35] showed the use of HS-SPME/GC-MS for the identification and

quantification of CC such as formaldehyde, acetaldehyde, acrolein and furfural in ale and lager beers, in concentrations that varied from 1.3 to 4264 μg L^{-1}, in their respective free forms. Substances from other chemical classes were also analyzed such as ethyl carbamate, furfuryl alcohol and other four furan-containing compounds. The authors point out that acrolein (concentrations found of up to 5.4 μg L^{-1}) was present in concentration capable of causing health risk.

The carbonyl compounds are chemical species whose characteristics make their individual quantification difficult through classical methods. The complex nature of carbonyl compounds requires, in addition to the use of an adequate instrumental technique, a pretreatment of the samples before analysis.[30,31,47] In this sense, derivatization using 2,4-dinitrophenylhydrazine as a derivatizing agent has been a common treatment, followed by analysis by high performance liquid chromatography (Table 4). The main advantage of using this derivatizing reagent is the ability to analyze a complex mixture of several aldehydes and ketones simultaneously.[24]

In addition, the method proposed in this work showed adequate sensitivity to analyze carbonyl compounds in free and bound forms in craft beers, since limits of detection (1.69 to 6.13 μg L^{-1}) were compatible with previously published papers (Table 4).

It is worth mentioning the scarcity of data in the literature regarding the analysis of carbonyl compounds with higher molecular weights in beers and, consequently, these substances have not been evaluated in their free and bound forms in the beverage in a discriminated way (Table 4). As shown in Table 3, in this work, the carbonyl compounds with the highest molecular weights identified in craft beer samples, with the highest analytical frequency, were crotonaldehyde ($free_{max}$: 199.5 μg L^{-1}; $bound_{max}$: 464.0 μg L^{-1}); benzaldehyde ($free_{max}$: < 18.39 μg L^{-1}; $bound_{max}$: 1326 μg L^{-1}); hexaldehyde ($free_{max}$: 364.9 μg L^{-1}; $bound_{max}$: < 13.29 μg L^{-1}); heptanal ($free_{max}$: 1140 μg L^{-1}; $bound_{max}$: < 13.74 μg L^{-1}); octanal ($free_{max}$: 564.6 μg L^{-1}; $bound_{max}$: 18.99 μg L^{-1}).

Carbonyl compounds present in beers can be classified into different groups according to their precursors such as (*i*) group 1: Strecker aldehydes which have amino acids and higher alcohols as precursors (methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde, phenylacetaldehyde); (*ii*) group 2: aldehydes formed from saturated fatty acids (hexanal, heptanal, octanal); (*iii*) group 3: aldehydes formed from unsaturated fatty acids ((*E*)-2-nonenal, (*E*)-2-octenal, (*E*)-butenal); and (*iv*) group 4: the precursors of 2-furfural, which are saccharides. It is noteworthy that some of these aldehydes have been shown to be directly proportional to the unpleasant flavors associated with aging beer (oxidized, rancid, sweet mouldy-musty).[48] Thus, aldehydes from different precursor groups were identified and quantified in free and bound forms in our work. Benzaldehyde (group 1) was found mainly in bound form in concentrations ranging from 10.33 to 1326 μg L^{-1}. Aldehydes that have saturated fatty acids as precursors (group 2) were found predominantly in the free fraction (hexaldehyde or hexanal: 113.8-165.2 μg L^{-1}; heptanal: < 13.7-1040 μg L^{-1}; octanal: < 5.62-561.6 μg L^{-1}; nonanal: < 10.9-222.1 μg L^{-1}), except for the decanal that was identified only in the bound fraction (< 9.71-27.64 μg L^{-1}). Crotonaldehyde ((*E*)-2-butenal) and methacrolein (methylpropenal) have unsaturated fatty acids as precursors in their formation (group 3), having been found predominantly as bound-CC in the analyzed craft beers, with concentrations that varied from 333.9-464.0 μg L^{-1} and < 20.5-1124 μg L^{-1}, in the respective bound forms.

If the average concentrations of CC, mainly of aldehydes, are calculated based on a "standard drink", it is possible to observe that, when consuming a 600 mL bottle of craft beer, a person will be ingesting up to 58.6 μg of formaldehyde, 1084 μg of acetaldehyde and 1738 μg of acrolein, approximately, considering the sum of the free and bound fractions of both compounds. Compared to other groups of beverages (such as carbonated mineral water, wine, fortified wine, and spirits) these results are significantly higher, even considering the absolute intake of the respective beverages. For the consumption of a "standard drink" (500 mL) of carbonated mineral water, the intake of formaldehyde, acetaldehyde and acrolein can be up to 48, 16, and 0.13 μg, respectively.[31] Lachenmeier and Sohnius[49] showed that the most problematic group seems to be fortified wine, which has the highest concentration of acetaldehyde in a 90 mL standard drink, with 1000 μg *per* standard drink, approximately. Regarding formaldehyde concentrations, the average amount in a "standard drink" (50 mL) of different distilled beverages can reach 12.8, 23.5, and 1.7 μg for cachaça, rum, and vodka, respectively.[50]

The total concentration of carbonyl compounds in the craft beer samples can be expressed as the sum of the individual concentrations of free-CC and the individual concentrations of bound-CC. In this way, the total concentration of carbonyl compounds in craft beer samples ranged from 1.487 to 4.838 mg L^{-1} (Figure 3). The proportion of carbonyl compounds in the bound form represented from 4 (sample 1) to 76% (sample 7) of the total concentration of these substances in the beverage. As can be observed, all craft beers samples showed significant concentrations of carbonyl compounds in the bound form, highlighting the samples 5, 7 and 12, which have the fraction of bound-carbonyl compounds greater than the free-carbonyl compounds.

Bound-carbonyl compounds have a negative influence on the quality of craft beer, as they can significantly interfere with the flavor and aroma of the beverage. According to Trueba *et al.*,[26,51] the low volatility of these bound species is one of the factors that hinder their elimination during the heating of the must and the action of the yeasts in the fermentation stage, being carried to the final product. These compounds, bound to intermediates such as imines, bisulfite, cysteine, proteins, and others, are considered the most relevant for the formation of unpleasant flavors in beer.

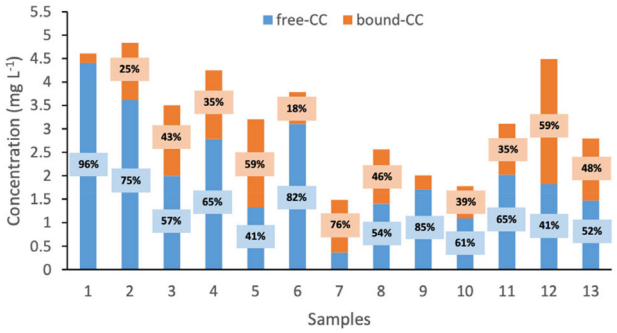

Figure 3. Distribution of carbonyl compounds concentrations for free and bound forms in craft beer samples (CC = carbonyl compounds), according to data in Table 3.

In Brazil, beer quality standards are regulated by the Ministry of Agriculture, Livestock and Supply (MAPA) (Normative Instruction No. 65, December 10, 2019)[1] and by the National Health Surveillance Agency (ANVISA) (RDC No. 65, November 29, 2011).[52] However, to date, no reference to free- and/or bound-carbonyl compounds in beers has been observed in current legislation. Due to the lack of a specific legislation that regulates the concentration of carbonyl compounds in beers, an assessment of the risk of exposure was carried out for the consumption of craft beers containing the compounds identified and quantified in our work. Thus, Table 5 shows the results obtained for the determination of the estimated daily intake (EDI) and the margin of exposure (MOE), related to carbonyl compounds ($CC_{free+bound}$) present in craft beers.

Acrolein had the highest EDI values (21.5 (men (M) and 12.4 (women (W) μg kg^{-1} bw day^{-1}), followed by propionaldehyde (16.9 (M) and 9.69 (W) μg kg^{-1} bw day^{-1}) and acetaldehyde (13.4 (M) and 7.71 (W) μg kg^{-1} bw day^{-1}). For compounds that were identified at concentrations lower than the LOQ in craft beer samples, the EDI was not calculated. In work related to the assessment of toxicity to consumption of wines containing carbonyl compounds, acetaldehyde and acrolein were also among the compounds with the highest EDI values.[21]

Although there are other methods to assess the health risks associated with alcohol consumption, the margin of exposure (MOE) method is recommended to compare the risks of different components present in alcoholic beverages. The MOE compares exposure levels to toxicological thresholds, which are derived from dose-response assessments for carcinogens and non-carcinogens.[38] In view of this, a fact that draws a lot of attention was that the high concentrations of acrolein identified in the analyzed craft beers resulted in MOE < 10000 values in all samples, with the exception of sample 10, considering a moderate consumption for women (Table 5). This could result in public health problems since MOE < 10000 values indicate possible problems for consumers' health. Thus, the formation of acrolein during craft beers production needs to be strictly monitored. Additionally, more rigorous studies related to risk exposure and toxicity to the consumption of craft beers containing acrolein and other carbonyl compounds need to be developed. Exposure of men (daily consumption of 300 mL of wine) and women (200 mL *per* day) to acrolein could also pose a risk to the health of wine consumers, since MOE values were lower than 10000 in more than 50% of the samples analyzed in that study.[21]

Exposure to acetaldehyde could pose risk on the consumer health only for men, since 5 craft beer samples showed MOE < 10000. On the other hand, exposure to formaldehyde could not represent a risk to the health of consumers through the consumption of craft beers analyzed in our study (Table 5).

Hernandes *et al.*[35] assessed the risk of exposure to free carbonyl compounds and other unwanted substances through beer consumption. The highest values found for EDI were for furfural (12.6 (M) and 5.2 (W) μg kg^{-1} bw day^{-1}), followed by furfuryl alcohol (0.1 (M) and 0.06 (W) μg kg^{-1} bw day^{-1}), acrolein (0.03 (M) and 0.02 (W) μg kg^{-1} bw day^{-1}), acetaldehyde (0.01 (M) and 0.02 (W) μg kg^{-1} bw day^{-1}) and formaldehyde (0.03 (M) and 0.008 (W) μg kg^{-1} bw day^{-1}). The authors verified that acrolein could represent a problem for the health of male consumers, since the calculated MOE values were lower than 10000 in some analyzed beer samples. Other compounds analyzed, such as formaldehyde, acetaldehyde and ethyl carbamate, could not represent a risk to the health of consumers of beers evaluated in that study. It is noteworthy that the EDI values found by these authors may have been lower than those obtained in our study, since in our work the concentration of free and bound fractions of carbonyl compounds was evaluated in craft beers.

Conclusions

The method showed good analytical quality for the analysis of free- and bound-carbonyl compounds in craft beers, since it was possible to identify and quantify these

Table 5. Exposure risk assessment through estimated daily intake levels (EDI) and margin of exposure (MOE) for men (M) and for women (W) calculated for toxic compounds ($CC_{free+bound}$) found in craft beers

$CC_{free+bound}$[a]		Craft beer samples												
		1	2	3	4	5	6	7	8	9	10	11	12	13
		EDI / (μg kg^{-1} body weight day^{-1})												
Formaldehyde	M	0.24	0.12	0.21	0.62	0.14	0.10	–	0.09	–	–	0.14	0.73	–
	W	0.14	0.07	0.12	0.36	0.08	0.06	–	0.05	–	–	0.08	0.42	–
Acetaldehyde	M	4.65	0.23	1.32	0.96	13.4	2.98	–	7.94	2.77	8.49	8.85	2.33	10.1
	W	2.67	0.13	0.76	0.55	7.71	1.71	–	4.56	1.59	4.87	5.08	1.34	5.80
Acrolein	M	21.5	21.3	10.4	15.0	0.09	19.1	–		10.5	0.05	9.79	13.5	6.34
	W	12.4	12.2	5.98	8.63	0.05	11.0	–		6.04	0.03	5.62	7.73	3.64
Propionaldehyde	M	–	–	–	–	8.79	–	2.70	6.88	–	4.59	–	16.9	–
	W	–	–	–	–	5.04	–	1.55	3.95	–	2.63	–	9.69	–
Crotonaldehyde	M	–	4.74	–	1.48	–	–	–	2.48	1.10	–	–	–	–
	W	–	2.72	–	0.85	–	–	–	1.42	0.63	–	–	–	–
Methacrolein	M	–	0.95	–	–	–	–	8.36	–	–	–	–	–	–
	W	–	0.55	–	–	–	–	4.80	–	–	–	–	–	–
Butyraldehyde	M	–	–	–	–	0.19	–	–	–	–	–	–	–	–
	W	–	–	–	–	0.11	–	–	–	–	–	–	–	–
Benzaldehyde	M	–	–	9.86	9.61	1.18	3.78	–	–	–	–	–	–	–
	W	–	–	5.66	5.51	0.68	2.17	–	–	–	–	–	–	–
Valeraldehyde + Cyclohexanone	M	0.09	–	–	–	–	–	–	–	–	–	–	–	–
	W	0.05	–	–	–	–	–	–	–	–	–	–	–	–
Hexaldehyde	M	–	–	0.85	2.71	–	1.23	–	–	–	–	0.15	–	–
	W	–	–	0.49	1.56	–	0.70	–	–	–	–	0.09	–	–
Heptanal	M	7.73	8.48	3.31	0.75	–	0.95	–	–	0.54	–	–	–	–
	W	4.44	4.86	1.90	0.43	–	0.54	–	–	0.31	–	–	–	–
Octanal	M	–	0.14	–	–	–	–	–	–	–	0.08	4.18	–	4.20
	W	–	0.08	–	–	–	–	–	–	–	0.05	2.40	–	2.41
Nonanal	M	–	–	–	0.41	–	–	–	1.65	–	–	–	–	0.13
	W	–	–	–	0.24	–	–	–	0.95	–	–	–	–	0.08
Decanal	M	–	–	0.10	–	–	–	–	0.12	–	0.17	–	–	0.21
	W	–	–	0.06	–	–	–	–	0.07	–	0.10	–	–	0.12
		MOE[b]												
Formaldehyde	M	115098	238808	131265	44919	198211	268616	–	296302	–	–	195332	38535	–
	W	200530	416064	228698	78260	345333	467998	–	516234	–	–	340318	67137	–
Acetaldehyde	M	12039	240639	42568	58556	**4168**	18781	–	**7056**	20221	**6599**	**6328**	24065	**5538**
	W	20975	419255	74165	102019	**7261**	32722	–	12294	35230	11497	11026	41928	**9648**
Acrolein	M	**16.71**	**16.89**	**34.58**	**23.95**	**4005**	**18.84**	–	–	**34.20**	**6633**	**36.78**	**26.73**	**56.75**
	W	**29.12**	**29.43**	**60.24**	**41.72**	**6978**	**32.82**	–	–	**59.59**	11556	**64.08**	**46.56**	**98.88**

[a]For the EDI calculations, the sum of the concentrations of the free and bound fractions of each carbonyl compound, individually, were considered. [b]MOE values in bold represent a risk to consumer health (MOE < 10000).

analytes at low concentrations and with good precision.

The concentrations obtained for the carbonyl compounds in the craft beer samples confirmed the presence of these species in the free form and bound to other substances in the beverage. The total concentration of carbonyl compounds presented a proportion of up to 76% of the concentration for carbonyl compounds in the bound form.

In this way, this study can contribute significantly to encourage the deepening of knowledge about the formation of carbonyl compounds in craft beers, mainly in their respective bound forms, improving the quality of the final product. In addition, this work can be an incentive for greater rigor in the legislation for the identity and quality standards of beers, related to the presence of carbonyl compounds in the beverage.

According to the exposure risk assessment, the consumption of craft beers containing free- and bound-CC (especially acrolein) could pose a risk to the health of frequent consumers.

Supplementary Information

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

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Author Contributions

Marinice Santiago dos Santos Acácio was responsible for formal analysis, investigation, validation, writing original draft; Eliete Costa Alves for formal analysis, validation; Jailson B. de Andrade for conceptualization, resources, writing-review and editing; Jeancarlo Pereira dos Anjos for conceptualization, project administration, writing-review and editing.

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