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INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
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Avaliação do efeito do processamento da cerveja nos níveis de compostos tóxicos e de voláteis relacionados ao aroma a partir da incorporação de uma camada extra de polidimetilsiloxano a uma fibra comercial de microextração em fase sólida

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2018

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Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia de Alimentos como um dos requisitos para obtenção do grau de Mestre em Ciência e Tecnologia de Alimentos.

Orientador: Prof.ª Dra. Juliane Elisa Welke
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RESUMO

Na microextração em fase sólida no modo *headspace* (HS-SPME), técnica amplamente utilizada na análise de bebidas alcoólicas, incluindo cerveja, o etanol (composto majoritário nestas matrizes) causa o deslocamento de compostos minoritários, interferindo na performance desta técnica. O objetivo deste trabalho foi avaliar os níveis de voláteis relacionados ao aroma e de compostos tóxicos [incluindo acetaldeído, acroleína, carbamato de etila (CE), formaldeído, furfural e álcool furfurílico] nas etapas de elaboração da cerveja (mosturação, fervura, fermentação, maturação e pasteurização), através da adição de camada extra de polidimetilsiloxano (PDMS) à uma fibra comercial divinilbenzeno/Carboxen®/polidimetilsiloxano (DVB/Car/PDMS). A fibra revestida com uma camada adicional de PDMS apresentou capacidade extratora superior à fibra comercial, uma vez que extraiu um número maior de compostos (61 *versus* 45) e obteve-se área cromatográfica total 20% superior. O teor de etanol das soluções modelo (0, 4, 8 e 12%) não influenciou significativamente na quantidade de analitos extraída quando a fibra revestida foi utilizada, entretanto, o efeito do etanol foi observado em extrações realizadas pela fibra não modificada. O método apresentou linearidade, sensibilidade, repetibilidade e precisão intermediária adequadas. A mosturação destacou-se em relação às demais etapas pelos maiores teores de álcoois superiores. A fervura foi caracterizada pelos maiores níveis de produtos da reação de Maillard, enquanto que a fermentação, maturação e pasteurização foram discriminadas pela presença majoritária de ésteres. Além disso, alguns terpenos foram incorporados ao mosto durante a fervura ou fermentação. A fibra revestida com PDMS foi utilizada na quantificação simultânea de compostos tóxicos durante a elaboração de cerveja *ale* e *lager*. Acetaldeído, acroleína, formaldeído e álcool furfurílico foram encontrados em todos os estágios da elaboração de ambos tipos de cerveja, enquanto CE e furfural não foram detectados (níveis <LOD: 0,1 e 0,01 μg L^{-1}, respectivamente). A fervura e a fermentação parecem ser etapas importantes na formação destes compostos, enquanto a maturação e a pasteurização reduzem seus níveis nas cervejas *ale* e *lager*. Além disso, a matéria-prima, a levedura e as condições de fermentação influenciam a formação e redução destes compostos durante a elaboração de cervejas. Entre as 30 amostras de cervejas *ale* e *lager* comercialmente disponíveis e avaliadas neste estudo, o acetaldeído foi encontrado em uma cerveja *ale* (1,8 μg L^{-1}) e duas *lager* (1,3 e 2,5 μg L^{-1}). Acroleína foi detectada em uma cerveja *ale* (4,1 μg L^{-1}) e 8 *lager* (2,5-5,4 μg L^{-1}). Formaldeído estava presente em níveis inferiores ao LOQ (1,0 μg L^{-1}) em todas as amostras *ale* e superior ao LOQ em uma amostra *lager* (2,6 μg L^{-1}). Furfural foi o composto tóxico

quantificado em níveis mais elevados, detectado em 3 amostras *ale* e 14 *lager*, em níveis variando de 417,7 a 4264,3 µg L^{-1} e 1,15 a 2403,4 µg L^{-1}, respectivamente. O álcool furfurílico foi detectado em todas as cervejas *ale* e *lager*, variando de 4,2 a 20,7 µg L^{-1} e de 5,8 a 30,9 µg L^{-1}, respectivamente. Apenas a acroleína foi encontrada em níveis que podem representar risco para a saúde (MOE < 10.000) em uma amostra *ale* e em três amostras *lager*.

Palavras-chave: Cerveja**;** HS-SPME; PDMS; aroma; compostos carbonílicos.

ABSTRACT

In solid phase microextraction in headspace mode (HS-SPME), a technic widely used for the analysis of alcoholic beverages, including beer, ethanol (the major compound in these matrices) causes the displacement of minority compounds, interfering in the performance of this technic. The objective of this work was to evaluate the volatile levels related to aroma and toxic compounds [including acetaldehyde, acrolein, ethyl carbamate (EC), formaldehyde, furfural and furfuryl alcohol] in brewing stages (mashing, boiling, fermentation, maturation and pasteurization) by the addition of an extra layer of polydimethylsiloxane (PDMS) to a divinylbenzene/Carboxen®/polydimethylsiloxane (DVB/Car/PDMS) commercial fiber. The PDMS-overcoated fiber presented higher extractive capacity than the commercial fiber, since it extracted a greater number of compounds (61 versus 45) and obtained a total chromatographic area of 20% higher. The ethanol content of the model solutions (0, 4, 8 and 12%) did not significantly influence the quantity of analytes extracted when PDMS-overcoated fiber was employed, however, the ethanol effect was observed in extractions performed by the unmodified fiber. The method presented adequate linearity, sensitivity, repeatability and intermediate accuracy. Mashing was highlighted in relation to the other stages due to higher levels of higher alcohols. Boiling was characterized by the higher product levels of the Maillard reaction, while fermentation, maturation and pasteurization were discriminated by the presence of esters. In addition, some terpenes were incorporated into the wort during boiling or fermentation. The PDMS-overcoated fiber was employed in the simultaneous quantification of toxic compounds during ale and lager brewing. Acetaldehyde, acrolein, formaldehyde and furfuryl alcohol were found at all stages of brewing both types of beer, while EC and furfural were not detected (levels <LOD: 0.1 and 0.01 μg L^{-1}, respectively). Boiling and fermentation appear to be important steps in the formation of these compounds, while maturation and pasteurization reduce their levels in ale and lager beers. In addition, the raw material, yeast and fermentation conditions influence the formation and reduction of these compounds during brewing. Among the 30 samples of ale and lager commercially available and evaluated in this study, acetaldehyde was found in ale (1.8 μg L^{-1}) and two lager (1.3 and 2.5 μg L^{-1}). Acrolein was detected in one beer ale (4.1 μg L^{-1}) and 8 lager (2.5-5.4 μg L^{-1}). Formaldehyde was present at levels lower than LOQ (1.0 μg L^{-1}) in all ale samples and higher than LOQ in one lager sample (2.6 μg L^{-1}). Furfural was the toxic compound quantified at higher levels, detected in 3 ale and 14 lager samples, at levels varying from 417.7 to 4264.3 μg L^{-1} and 1.15 to 2403.4 μg L^{-1}, respectively. Furfuryl alcohol was detected in all ale and lager beers, ranging from 4.2 to

20.7 μg L^{-1} and from 5.8 to 30.9 μg L^{-1}, respectively. Only acrolein was found at levels which may represent a health risk (MOE <10,000) in 12.5 and 13.6% of ale and lager samples, respectively.

Key-words: Brewing; HS-SPME; PDMS; aroma; carbonyl compounds.

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ADI	Ingestão diária aceitável, do inglês: *acceptable daily intake*
ANVISA	Agência Nacional de Vigilância Sanitária
BMD	Dose de referência toxicológica, do inglês: *benchmark dose*
BMD10	Dose que causou aumento de 10% na incidência de tumores
BMDL10	Limite inferior do intervalo de confiança da dose que causou aumento de 10% na incidência de tumores, do inglês: *benchmark dose lower confidence limit*
CAR	Carboxen®
CE	Carbamato de etila
CERVBRASIL	Associação Brasileira da Indústria da Cerveja
CT	Concentração aceitável
CW	Carbowax (polietilenoglicol)
DI-SPME	Microextração em fase sólida no modo imersão direta, do inglês: *direct immersion solid phase microextraction*
DVB	Divinilbenzeno
DVB/Car/PDMS	Divinilbenzeno/Carboxen®/polidimetilsiloxano
EBC	Convenção Européia de Cervejarias, do inglês: *European Brewery Convention*
EPA	Agência de Proteção Ambiental dos Estados Unidos, do inglês: *Environmental Protection Agency*
FAO	Organização das Nações Unidas para Agricultura e Alimentação, do inglês: *Food and Agriculture Organization of the United Nations*
GC	Cromatografia gasosa, do inglês: *gas chromatography*
GC/MS	Cromatografia gasosa acoplada à espectrometria de massas, do inglês: *gas chromatography with mass spectrometric detection*
GC/MS-SIM	Cromatografia gasosa acoplada a espectrometria de massa no modo de monitoramento seletivo de íons, do inglês: *gas chromatography with mass spectrometric detection in selected-ion monitoring mode*
HS-SPME	Microextração em fase sólida no modo *headspace,* do inglês: *headspace solid phase microextraction*
HS-SPME-GC/MS	Microextração em fase sólida no modo *headspace* combinada com cromatografia gasosa acoplada à espectrometria de massas, do inglês: *headspace solid phase microextraction and gas chromatography with mass spectrometric detection*
IARC	Agência Internacional de Pesquisa sobre o Câncer, do inglês: *International Agency for Research on Cancer*
IDA	Ingestão Diária Aceitável
IDE	Ingestão Diária Estimada
IPCS	Programa Internacional de Segurança Química, do inglês: *International Programme on Chemical Safety*
IS	Padrão interno, do inglês: *internal standard*
JECFA	Comitê de Especialistas em Aditivos Alimentares da Organização das Nações Unidas para Agricultura e Alimentação e pela Organização Mundial da Saúde, do inglês: *Joint FAO/WHO Experts Committee on Food Additives*
LOD	Limite de detecção, do inglês: *limit of detection*
LOQ	Limite de quantificação, do inglês: *limit of quantification*

MAPA	Ministério de Agricultura, Pecuária e Abastecimento
MOE	Margem de exposição, do inglês: *margin of exposure*
NOEL	Nível de efeito não observado, do inglês: *no observed effect level*
PA	Poliacilato
PC	Peso corpóreo
PDMS	Polidimetilsiloxano
PDMS/DVB	Polidimetilsiloxano/divinilbenzeno
PMTDI	Ingestão diária tolerável máxima provisória, do inglês: *provisional maximun tolerable daily intake*
PTMI	Ingestão tolerável mensal provisória, do inglês: *provisional tolerable monthly Intaike*
PTWI	Ingestão tolerável semanal provisória, do inglês: *provisional tolerable weekly intake*
RI	Índice de retenção, do inglês: *retention index*
SEBRAE	Serviço Brasileiro de Apoio às Micro e Pequenas Empresas
SIM	Monitoramento de íons selecionados, do inglês: *selected ion monitoring*
SPME	Microextração em fase sólida, do inglês: *solid phase microextraction*
WHO	Organização Mundial da Saúde, do inglês: *World Health Organization*

1. INTRODUÇÃO

A cerveja é uma bebida popular e difundida mundialmente. Em 2016, segundo dados da Associação Brasileira da Indústria da Cerveja (CERVBRASIL, 2016), o setor da cerveja produziu cerca de 14 bilhões de litros, com perspectiva de expansão. De acordo com o Ministério de Agricultura, Pecuária e Abastecimento (MAPA), o ano de 2017 foi de grande consolidação do mercado cervejeiro nacional, sendo que o total de cervejarias legalmente registradas e instaladas no país chegou a 679. Neste período, o estado do Rio Grande do Sul ultrapassou São Paulo e se tornou o estado com mais cervejarias registradas, chegando a marca de 142 estabelecimentos. Este crescimento se deve principalmente às novas tendências de consumo, com destaque para as cervejas artesanais, nas quais o consumidor busca experimentar novos sabores e aromas (MAPA, 2018).

A preparação artesanal tem como foco a qualidade dos ingredientes, resultando na produção de diferentes tipos de cerveja com compostos de aroma e sabor variados (AQUILANI et al., 2015). Além disso, apesar do efeito tóxico do etanol (IARC, 1988; LOCONTE et al., 2018), alguns estudos têm relatado benefícios que o consumo moderado de cerveja pode proporcionar ao consumidor devido à presença de compostos benéficos, como os polifenóis (ARRANZ et al., 2012; NOGUEIRA et al., 2017; SILVA et al., 2017)

Além da presença de compostos com propriedades funcionais, a qualidade da cerveja também está relacionada aos voláteis presentes na bebida, uma vez que estes influenciam no aroma e sabor. Estes compostos de aroma podem ser provenientes tanto da matéria-prima utilizada (RICHTER et al., 2017), quanto dos produtos da fermentação alcoólica (OCVIRK; MLINARIČ; KOŠIR, 2018).

A microextracão em fase sólida (SPME, do inglês, *solid phase micro extraction*) tem sido amplamente empregada na extração de compostos voláteis presentes em alimentos e bebidas alcoolicas, como por exemplo, as cervejas (ANDRÉS-IGLESIAS et al., 2016; CASTRO; ROSS, 2015; DA SILVA et al., 2015; RIU-AUMATELL et al., 2014). A SPME se baseia na absorção e/ou adsorção de analitos no revestimento de fibras extratoras, contudo está sujeita a algumas limitações que prejudicam a sua performance. Extrações feitas em matrizes complexas podem ser afetadas pelos constituintes da amostra. No modo *headspace* (HS), os compostos em elevadas concentrações e que possuam alta afinidade pelo revestimento da fibra podem provocar o deslocamento de compostos com menor afinidade pela fase extratora interferindo

no equilíbrio da extração, quando esta fase consiste em um polímero sólido (PAWLISZYN, 2009; RISTICEVIC et al., 2010). Além disso, compostos majoritários podem ser solubilizados na matriz da fase extratora, quando esta for um líquido, de forma a alterar as propriedades físico-químicas do material extrator. No modo de imersão direta (DI, do inglês, *direct immersion*), componentes da matriz, como por exemplo açúcares, ácidos graxos e pigmentos podem causar a incrustação da fase extratora, o que leva não apenas à diminuição da eficiência da extração, mas também reduz a vida útil do recobrimento polimérico da fibra (SOUZA-SILVA; PAWLISZYN, 2012). Estas limitações da DI-SPME motivaram a adição de uma camada adicional de polidimetilsiloxano (PDMS) em fibras comerciais, especialmente a fibra de PDMS/divinilbenzeno (PDMS/DVB) e DVB/Carboxen®/PDMS (DVB/Car/PDMS) (SOUZA-SILVA et al., 2016; SOUZA-SILVA; PAWLISZYN, 2012, 2015). Por ser um revestimento líquido e não poroso, o PDMS sofre menos o efeito de incrustação dos componentes da matriz, quando comparado aos revestimentos sólidos (PAWLISZYN, 2009). Além disso, por ser apolar, o PDMS pode barrar a sorção excessiva de compostos altamente polares, deixando livres os sítios ativos dos demais revestimentos para a extração de compostos minoritários.

Poucos estudos têm se dedicado à quantificação dos compostos identificados na cerveja, sendo que a avaliação quantitativa é importante para determinar quais compostos influenciam no aroma/sabor (CASTRO; ROSS, 2015; MOREIRA et al., 2013). Além disso, o etanol, composto presente em maior concentração em bebidas alcoólicas, tem sido descrito na literatura como um importante interferente para a extração de compostos minoritários através da HS-SPME (CONNER et al., 1998; HARTMANN; MCNAIR; ZOECKLEIN, 2002; PÉREZ-OLIVERO et al., 2014; RODRÍGUEZ-BENCOMO et al., 2002; WHITON; ZOECKLEIN, 2000).

Durante o processamento da cerveja, além dos compostos desejáveis, compostos tóxicos podem estar presentes, os quais comprometem a qualidade desta bebida, bem como a segurança dos consumidores (BARTH, 2013). Em bebidas fermentadas, a presença de compostos carbonílicos é comum, visto que são compostos provenientes da contaminação ambiental dos cereais durante o cultivo, formados durante o metabolismo normal de leveduras ou através de reações que envolvem os compostos produzidos durante a fermentação. A Agência Internacional de Pesquisa sobre o Câncer (IARC, do inglês, *International Agency for Research on Cancer*) classificou, por exemplo, o acetaldeído ingerido especificamente através das bebidas alcoólicas como carcinogênico (grupo 1, evidências suficientes do potencial

carcinogênico em humanos). Semelhantemente, o formaldeído também se encontra no grupo 1, enquanto que o carbamato de etila está classificado como provavelmente carcinogênico (grupo 2A, evidências suficientes da carcinogenicidade em animais) e álcool furfurílico é considerado um possível carcinogênico para humanos (grupo 2B, evidências insuficientes da carcinogenicidade em animais). A IARC reconhece que ainda necessita de mais estudos para classificar a acroleína quanto à sua carcinogenicidade (grupo 3). Segundo informação desta Agência Internacional, a ocorrência de câncer crescerá mais de 75% até 2030 e estima-se que aproximadamente 60% dos casos desta doença estarão vinculados à dieta da população (IARC, 2016). Esta estimativa da IARC também se constitui em uma das motivações deste estudo, visto que existe a necessidade de prever estratégias para reduzir a exposição aos compostos tóxicos através da dieta, de maneira a evitar danos à saúde do consumidor, bem como evitar que o sistema público de saúde seja onerado.

Não há legislação, no Brasil ou em outros países, que estabeleça a concentração máxima dos compostos anteriormente citados em cerveja. A necessidade de estabelecimento de normas que regulamentem os limites máximos permitidos destes compostos em bebidas alcoólicas, incluindo a cerveja, torna esta área de pesquisa ainda mais relevante no que diz respeito à geração de dados sobre os níveis destes compostos, bem como no que tange à avaliação do risco de exposição através do consumo de bebidas alcóolicas. Além dos dados relativos à quantificação de compostos potencialmente tóxicos produzidos durante a elaboração de cerveja serem escassos, até o momento, não há estudos dedicados a avaliar o risco da exposição a estes compostos através do consumo de cerveja.

Considerando que a cerveja é a bebida alcoólica mais consumida e cuja produção têm aumentado no Brasil; existe a possibilidade de exposição aos compostos tóxicos em níveis superiores aos indicados como seguros à saúde humana; o aroma é um dos principais atributos relacionados à qualidade da cerveja; e o etanol pode prejudicar a extração de compostos da cerveja, verifica-se a necessidade de desenvolver um método analítico baseado na técnica de HS-SPME, que comporte uma camada adicional de PDMS aderida a uma fibra comercial (DVB/Car/PDMS) para avaliar os compostos relacionados ao aroma e os compostos tóxicos presentes nessa bebida.

2. OBJETIVOS

2.1. Objetivo geral

Avaliar o efeito das etapas de elaboração da cerveja nos níveis de compostos tóxicos e de voláteis relacionados ao aroma através da adição de uma camada extra de PDMS à uma fibra comercial de SPME.

2.2. Objetivos específicos

- Avaliar a eficiência de uma fibra comercial de SPME na qual uma camada extra de PDMS foi adicionada para minimizar o efeito do etanol na extração de compostos voláteis;
- Verificar o efeito de 5 etapas da produção de cerveja artesanal (mosturação, fervura, fermentação, maturação e pasteurização) nos níveis de compostos voláteis que conferem aroma e de compostos carbonílicos e álcool furfurílico que apresentam potencial tóxico;
- Investigar se a exposição aos compostos carbonílicos e álcool furfurílico presentes nas amostras avaliadas representa risco para a saúde do consumidor.

3. REVISÃO BIBLIOGRÁFICA

3.1. Cerveja – dados de produção e consumo

Segundo a legislação brasileira, cerveja é definida como a bebida obtida pela fermentação alcoólica do mosto cervejeiro oriundo do malte de cevada e de água potável, por ação da levedura, com adição de lúpulo (BRASIL, 2009). Esta bebida é o resultado da transformação de açúcares do malte em etanol, gás carbônico e centenas de outros compostos relacionados a aroma e sabor (PRIEST; STEWART, 2006).

Dados da Organização das Nações Unidas para Agricultura e Alimentação (FAO, do inglês: *Food and Agriculture Organization of the United Nations*) mostram que a cerveja é a quinta bebida mais consumida no mundo, cujo consumo situa-se atrás apenas do chá, bebidas gaseificadas, leite e café (FAOSTAT, 2018). A cerveja é considerada uma bebida popular e seu consumo é atrativo devido as suas propriedades organolépticas e baixo custo, que pode ser menor do que o de outros tipos de bebidas alcoólicas como, por exemplo, o vinho (AQUILANI et al., 2015; BAMFORTH; RUSSELL; STEWART, 2009).

O brasileiro consome em média 62 litros de cerveja por ano, ocupando a 17ª posição no *ranking* mundial, que tem a República Tcheca em 1º lugar, com 143 litros per capita. O Brasil é o terceiro produtor mundial desta bebida (14 bilhões de litros por ano), atrás apenas dos Estados Unidos e China e superando Rússia e Alemanha. Cabe salientar que as cervejas artesanais têm conquistado cada vez mais espaço no mercado brasileiro, pois enquanto o mercado de cervejas produzidas em larga escala cresce à taxa média de 5% ao ano, o de cervejas artesanais cresce pelo menos 20% anualmente (SICOBE, 2016).

Na última década, verificou-se o crescimento exponencial no número de cervejarias registradas junto ao MAPA (Figura 1). Apenas entre 8 de abril e 17 de maio de 2016, o número de cervejarias registradas aumentou de 320 para 397 (MAPA, 2016). Em 2017, esse número apresentou um crescimento de 71%, passando para 679 estabelecimentos. A distribuição geográfica das cervejarias permanece concentrada na região Sul e Sudeste, com 287 e 279 registros, respectivamente. O estado do Rio Grande do Sul é o que apresenta o maior número de cervejarias registradas (142 estabelecimentos), seguido por São Paulo (124), Minas Gerais (87), Santa Catarina (78), Paraná (67) e Rio de Janeiro (57) (MAPA, 2018).

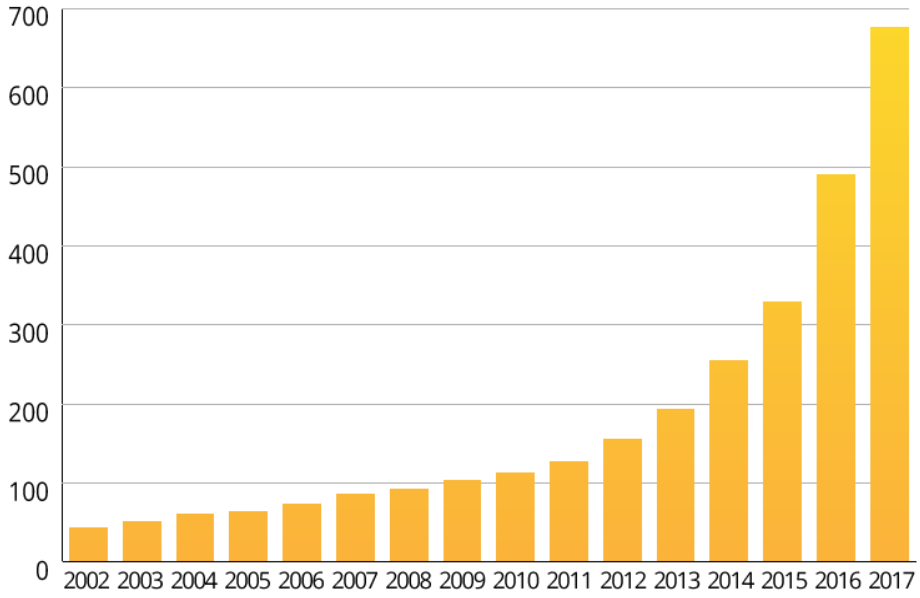


Figura 1. Total de cervejarias registradas por ano no Brasil.
Fonte: (MARCUSSO; MULLER, 2018)

O aumento do número de cervejarias deve-se especialmente ao crescente consumo de cervejas artesanais produzidas em microcervejarias. Cabe destacar que se enquadram como microcervejarias, os estabelecimentos que possuem produção anual de até 10 milhões de litros (BRASIL, 2015). Segundo o Serviço Brasileiro de Apoio às Micro e Pequenas Empresas (SEBRAE), a matéria-prima é o grande diferencial das cervejas artesanais e também um grande desafio, pois as grandes indústrias absorvem a cevada nacional, sendo que cerca de 90% da matéria-prima (malte) utilizado na produção das cervejas artesanais é importada (SEBRAE, 2016). Aquilani et al. (2015) verificaram que a cerveja artesanal é percebida pelos consumidores como sendo de qualidade superior à cerveja produzida em larga escala.

3.2. Classificação da cerveja

Há várias formas de se classificar uma cerveja. A Tabela 1 mostra a classificação da cerveja de acordo com características do processamento e do produto acabado, de acordo o Decreto n° 6871 de 2009 do governo brasileiro. Este decreto regulamenta a Lei n° 8.918, de 14 de julho de 1994, que dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas (BRASIL, 2009). Esta classificação se aplica tanto às cervejas produzidas em larga escala quanto às cervejas artesanais e se baseia nos seguintes

critérios: extrato primitivo (quantidade de substâncias presentes no mosto que deu origem à cerveja), cor, teor alcoólico, fermentação e proporção de malte. Tais parâmetros são importantes tanto para o produtor da cerveja quanto para o consumidor, uma vez que auxiliam no controle e na padronização da bebida.

Tabela 1. Classificação das cervejas de acordo com o Decreto n° 6871 de 2009 (BRASIL, 2009).

Extrato primitivo	
Leve	Acima de 5,0% até 10,5% em massa
Comum	Acima 10,5% até 12,5% em massa
Extra	Acima de 12,5% até 14,0% em massa
Forte	Acima de 14,0% em massa
Cor	
Clara	Menos de 20 unidades EBC (*European Brewery Convention*)
Escura	20 ou mais unidades EBC
Teor alcoólico	
Sem álcool	Menos de 0,5% em volume de etanol
Alcoólica	Igual ou maior do que 0,5% em volume de etanol
Fermentação	
Alta fermentação (12-15 °C)	
Baixa fermentação (5-10 °C)	
Proporção de malte de cevada	
Cerveja de puro malte	100% em peso de malte de cevada
Cerveja	pelo menos 50% em peso de malte de cevada
Cerveja de ... (nome do vegetal predominante)	Quando a cerveja possuir proporção de malte de cevada entre 20 e 50% em peso, além de outro tipo de cereal maltado. Ex: trigo

Fonte: Adaptado de (BRASIL, 2009)

As leveduras *Saccharomyces cerevisiae* e *S. pastorianus*, são utilizadas para a elaboração de cervejas de alta fermentação (chamadas de *Ale*) e baixa fermentação (nomeadas como *Lager*) (ESSLINGER, 2009). Nas cervejas de alta fermentação, as leveduras *S. cerevisiae* permanecem flutuando no topo do tanque de fermentação durante o processo, que ocorre em temperaturas entre 15 e 25 °C, por período de 3 a 6 dias. Como resultado obtém-se uma bebida mais encorpada, com sabores e aromas mais perceptíveis. Nas cervejas de baixa fermentação, o processo ocorre por um período maior (variando de 5 a 10 dias) em temperaturas entre 7 e 14 °C. Nessa condição as leveduras *S. pastorianus* ficam depositadas no fundo do tanque de

fermentação, resultando em cervejas leves e de coloração clara (BUGLASS, 2011; BUIATTI, 2009).

Dentro da definição de cerveja, diferentes estilos da bebida podem ser encontrados. Além do tipo de fermentação, o método de produção e a variedade do malte, do lúpulo e de outros ingredientes que possam ser empregados na elaboração da cerveja irão conferir características próprias à cada estilo da bebida. As principais diferenças entre os estilos de cerveja se referem quanto à cor, ao aroma e ao teor alcoólico da bebida (PREEDY, 2009; PRIEST; STEWART, 2006). Dentre os principais estilos de cervejas disponíveis, pode-se destacar:

- *Pilsener (pilsen ou pils)* - é a mais conhecida e consumida no mundo. Tem sabor delicado e leve, é clara (o malte não é torrado e não se empregam aditivos escuros como o caramelo) e de teor alcoólico entre 3,0 e 5,0 %.
- *Bock* – tem sabor mais pronunciado e encorpado e é geralmente de cor escura por usar malte torrado e caramelado. É originária da cidade de Einbeck, na Alemanha. Tem teor alcoólico mais elevado frente à Pilsener (4,0 – 6,0%).
- *Malzbier* – cerveja escura e doce, de graduação alcoólica entre 3,0 e 4,5 %. Na Alemanha, país de origem, é hoje tratada como bebida energética. Após a filtração, são adicionados caramelo e xarope de açúcar, responsáveis pela coloração escura e pelo sabor adocicado, respectivamente.
- *Munchner Dunkel* – cerveja escura/avermelhada (a coloração é proveniente do malte tostado), produzida originalmente em Munique, de onde vem o seu nome. Era a única cerveja da região da Baviera (Alemanha) antes da chegada das tecnologias que tornaram possível a criação de cervejas claras.
- *Bitter* - cerveja de cor de cobre a ouro, com amargor médio, caráter frutado, corpo leve a médio e doçura de malte residual baixa a média.
- *Pale Ale* – cerveja cuja cor varia do dourado profundo ao cobre. O lúpulo de variedade americana é usado para produzir característico amargor, sabor e aroma. O sabor e o aroma de ésteres frutados são de moderados a fortes.
- *Stout* - originária da Irlanda, ela é feita com malte torrado, o que explica sua cor escura e possui um sabor que associa o amargo do lúpulo ao adocicado do malte. É elaborada com extrato primitivo de 15 % e possui teores de etanol de 4,0 a 6,0%.

- *Porter* - é uma cerveja mais suave que a Stout e também é elaborada com malte torrado, entretanto contém de 1,0 a 2,0 % a menos de etanol.
- *Weiss* - é feita à base de trigo, mas pode conter milho e até mesmo frutas. É característica do sul da Alemanha (Baviera). São cervejas claras, bastante refrescantes e de graduação alcoólica na faixa de 5,0 a 6,0%. São opacas porque normalmente não são filtradas após a fermentação e a maturação. Produzem, em geral, uma espuma densa e persistente (PREEDY, 2009; PRIEST; STEWART, 2006).

3.3. Processamento de cerveja

As etapas da produção de cerveja estão representadas esquematicamente na Figura 2 e incluem: moagem do malte; brasagem (que é dividida em mosturação e fervura), filtração, fermentação, maturação, envase e pasteurização.

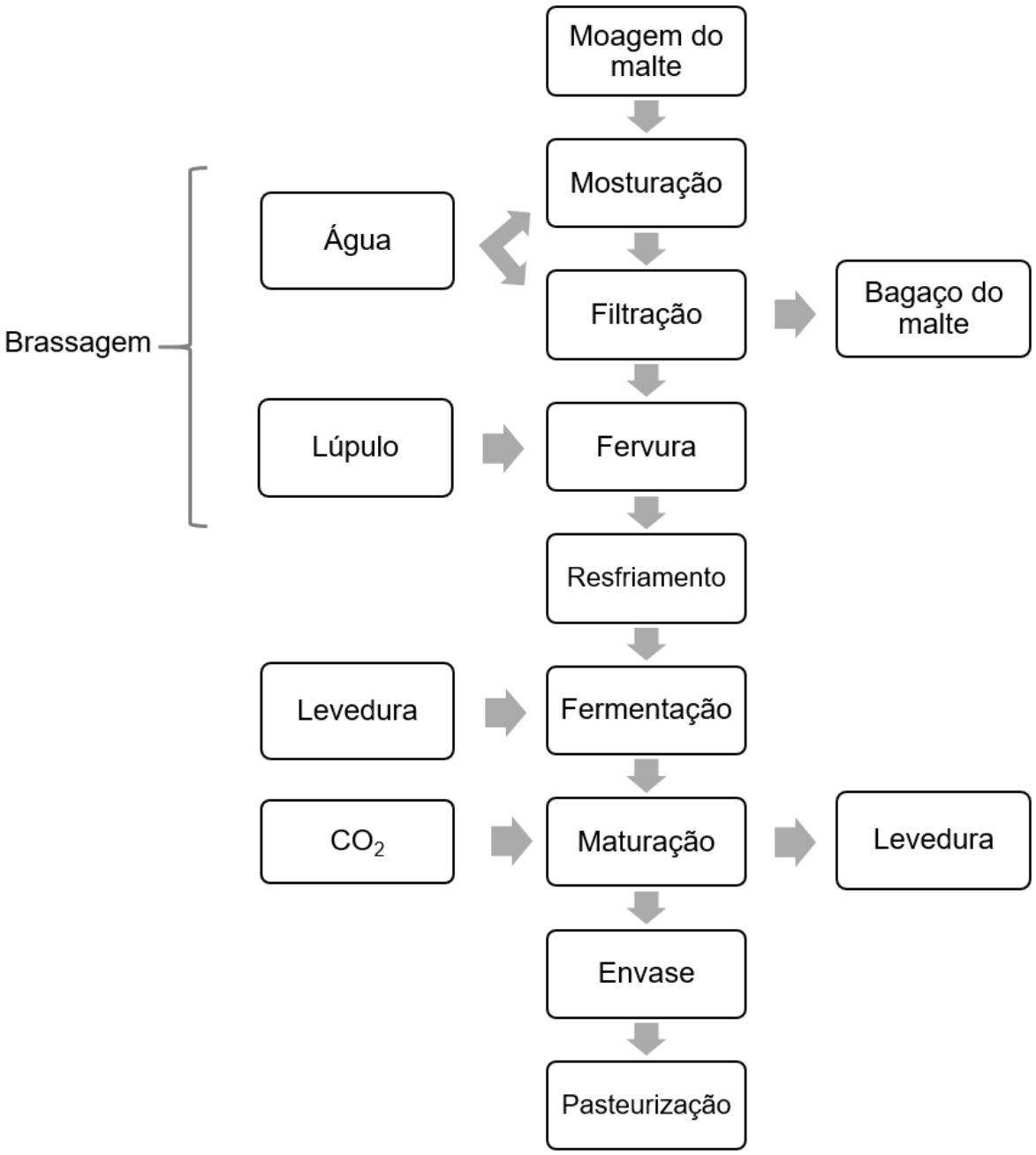


Figura 2. Etapas de elaboração da cerveja.

Fonte: Autoral

(i) O malte utilizado na elaboração de cervejas artesanais deve ser moído para promover o aumento da superfície de contato entre o grão e a água no preparo do mosto. A **moagem** ocorre até que o malte fique descascado, com a parte do amido exposta, para facilitar as próximas etapas da produção;

(ii) A primeira etapa da **brasagem** é chamada de **mosturação** (fase de preparo do mosto), que consiste em misturar o malte moído com água a 68 °C, promovendo a hidratação do grão e a ativação enzimática. O objetivo principal desta etapa é a transformação do amido em açúcares fermentáveis, como maltose e glicose.

(iii) Posteriormente, ocorre a **filtração** para separar a fase sólida (bagaço) e líquida (mosto).

(iv) A **fervura** é a etapa seguinte, que é realizada para promover a esterilização do mosto contra micro-organismos que possam competir com a levedura e causar sabores e aromas indesejáveis e também para provocar a coagulação de proteínas e taninos, que possam interferir na estabilidade da bebida. Além disso, o aquecimento promove a concentração de açúcares e remoção de voláteis indesejáveis por evaporação. Nesta etapa, o lúpulo é adicionado com a finalidade de conferir amargor e aroma à cerveja;

(v) Depois do **resfriamento**, o mosto segue para a **fermentação**, na qual leveduras são adicionadas ao líquido para conversão de parte dos açúcares em álcool e gás carbônico.

(vi) A etapa seguinte é a **maturação**, que é realizada a baixas temperaturas para que ocorra a decantação de impurezas e neutralização de compostos indesejáveis, contribuindo para a clarificação da cerveja e melhoria do seu sabor. Neste período, CO_2 é inserido nos tanques.

(vii) Por fim, o material decantado é retirado, o **envase** é realizado em garrafas, que passam por pasteurização, ou em barris (AQUARONE; ALMEIDA LIMA; BORZANI, 1983; BAMFORTH, 2007; BUGLASS, 2011; PRIEST; STEWART, 2006).

Uma vez que as etapas de produção da cerveja são as mesmas para todos os tipos desta bebida, o diferencial entre um produto e outro consiste, basicamente, na matéria prima empregada (tipo, origem, quantidade). Nesse sentido, além das matérias-primas obrigatórias, definidas no Decreto nº 6871 de 2009 (BRASIL, 2009), outros ingredientes podem ser utilizados na elaboração da cerveja, como frutas, ervas e especiarias, que podem conferir sabor, aroma e características específicas à bebida (SEBRAE, 2016).

3.4. Compostos voláteis relacionados ao aroma de cervejas

A qualidade de bebidas alcoólicas, em geral, é influenciada por atributos de aroma, considerados críticos para a aceitação global da bebida pelos consumidores (PLUTOWSKA; WARDENCKI, 2008). O aroma é determinado por compostos voláteis percebidos pelo olfato e a sua percepção é resultado de múltiplas interações entre compostos químicos e receptores

sensoriais (FISK, 2015). A intensidade de uma sensação olfativa não depende apenas da concentração dessas substâncias na fase líquida, mas também da sua volatilidade e de seu limiar de percepção olfativo (a concentração mínima na qual uma substância odorífera é detectada) (MEILGAARD; CIVILLE; CARR, 1999).

A composição do aroma da cerveja depende da qualidade e tipo das matérias-primas, bem como das condições de processamento (PLUTOWSKA; WARDENCKI, 2008). Centenas de voláteis têm sido detectados em cervejas (Tabela 2), incluindo compostos pertencentes a classes dos ésteres, álcoois, cetonas, aldeídos, hidrocarbonetos, ácidos, compostos contendo furano, compostos aromáticos, heterocíclicos e compostos que contém enxofre (ALVIM et al., 2017; ANDRÉS-IGLESIAS et al., 2016a; CASTRO; ROSS, 2015; CASTRO; ROSS; VIXIE, 2015; DA SILVA et al., 2015; DONG et al., 2015; HE et al., 2018; MOREIRA et al., 2013; NEŠPOR et al., 2018; OCVIRK; MLINARIČ; KOŠIR, 2018; PLUTOWSKA; WARDENCKI, 2008; RIU-AUMATELL et al., 2014; SAISON et al., 2009a). Estes estudos têm sido focados principalmente em cervejas produzidas em larga escala (Tabela 2).

Castro et al. (2015), por exemplo, identificaram 105 compostos voláteis, entre ésteres, álcoois, terpenos, aldeídos, hidrocarbonetos, ácidos e cetonas em cervejas (sem especificar o tipo de cerveja) comercializadas nos Estados Unidos. Riu-Aumatell et al. (2014), no estudo de cervejas com diferentes graduações alcoólicas comercializadas na Espanha identificaram 59 compostos voláteis. Ésteres, álcoois e ácidos estavam presentes em maiores quantidades em cervejas com graduação alcoólica entre 4,5% e 5,5%. As cervejas livres de álcool (menos de 1% de etanol) e com baixo teor alcoólico (menos de 3,0% de etanol) foram caracterizadas pela presença majoritária de compostos derivados da torrefação do malte, incluindo pirazinas e furanos, e compostos voláteis derivados do óleo essencial do lúpulo, como por exemplo, os terpenos. Alvim et al. (2017), identificaram 109 compostos, entre álcoois, ésteres, cetonas, aldeídos, ácidos e terpenos, em diferentes cervejas *lager* e *ale* brasileiras. A análise dos componentes principais (PCA) mostrou que as cervejas possuem perfil volátil semelhante, com predominância de ésteres e álcoois superiores. Além disso, não foram observados compostos presentes exclusivamente em cervejas *ale* ou *lager*.

Tabela 2. Método de extração, separação e detecção de compostos voláteis de cervejas com respectivo número de classes químicas e de compostos identificados nessa bebida e parâmetros de validação dos métodos empregados.

Procedência e tipo das amostras	Tipo de produção	Extração[h]	Separação e detecção[i]	Número de compostos e classes químicas identificadas		Concentração detectada e validação do método				Referência
						Conc.[j] (mg L^{-1})	LOD[L] (mg L^{-1})	LOQ[m] (mg L^{-1})	Recup.[n] (%)	
Brasil	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois Ácidos Aldeídos Cetonas Terpenos Hidrocarbonetos	24 12 9 8 4 38 4	NQ	-	-	-	Alvim et al., 2017
Slováquia	Larga escala	HS-SPME	GC/MS	Álcoois Ésteres Outros	9 9 33	NQ	-	-	-	Ocvirk; Mlinarič; Košir, 2018
China	Escala Laboratorial	HS-SPME	GC/MS	Ésteres Álcoois Aldeídos	4 4 1	0,4-14,4 20,1-80,9 9,4	NI	NI	NI	He et al., 2018
República Tcheca	NI	HS-SPME	GC/MS	Ésteres Ácidos Álcoois Terpenos	10 3 5 1	0,05-22,01 5,76-13,01 6,01-24,23 0,02-0,06	0,0003-0,1190 0,0058-0,0425 0,0345-0,2595 0,0018	0,008-0,5778 0,0515-0,2293 0,0903-1,2379 0,0048	91,0-104,9 86,5-102,0 89,5-95,6 89,7	Nešpor et al., 2018
Espanha [a]	Larga escala	HS-SPME	GC/MS	Aldeído Cetona	8 2	0,05–229,0 7,17-252,76	NI	NI	NI	Andrés-Iglesias et al., 2016
República Tcheca [a]	Larga escala	HS-SPME	GC/MS	Aldeído Cetona	8 2	0,11-174,39 2,25-345,64	NI	NI	NI	Andrés-Iglesias et al., 2016
Estados Unidos [a]	Larga escala	HS-SPME	GC/MS	Éster Hidrocarboneto Aldeído Aromático	2 1 1	NQ	0,17 0,21 0,23	NI	85,62-96,28 97,41 83,24	Castro; Ross, 2015
Estados Unidos [a]	Larga escala	SBSE	GC/FID	Éster Hidrocarboneto Aldeído Aromático	2 1 1	NQ	0,07-0,09 0,11 0,08	NI	90,07-102,18 99,91 98,9	Castro; Ross, 2015
Estados Unidos –	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois	9 5	NQ	-	-	-	Castro; Ross; Vixie, 2015

Cerveja tipo *Ale*[b]				Ácidos Hidrocarbonetos Aldeídos Alicíclicos Aromáticos Cetonas	3 3 1 7 5 1				
Estados Unidos - Cerveja tipo *Lager*[c]	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois Ácidos Hidrocabonetos Aldeídos Alicíclicos Aromáticos Cetonas Compostos de enxofre Compostos heterocíclicos	11 7 3 2 1 13 8 2 1 2	NQ	-	-	-	Castro; Ross; Vixie, 2015
Estados Unidos – Cerveja de Trigo [d]	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois Ácidos Hidrocarbonetos Compostos alicíclicos Compostos aromáticos Compostos heterocíclicos	14 3 2 3 5 3 1	NQ	-	-	-	Castro; Ross; Vixie, 2015
Estados Unidos – Cerveja tipo *IPA*[e]	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois Ácidos Hidrocarbonetos Compostos alicíclicos Compostos aromáticos	18 7 3 8 11 3	NQ	-	-	-	Castro; Ross; Vixie, 2015
Estados Unidos – Cerveja tipo *Stout*[f]	Larga escala	HS-SPME	GC/MS	Ésteres Álcoois Ácidos Hidrocarbonetos Aldeídos Compostos alicíclicos Compostos aromáticos Compostos heterocíclicos	12 4 3 6 1 6 3 1	NQ	-	-	-	Castro; Ross; Vixie, 2015
Portugal – Cerveja tipo *Lager* [c]	NI	HS-SPME	GC/MS	Alcanos Alcenos Aldeídos de Strecker	8 4 6	0,083-10,6 0,010-4,61 0,592-25,6	0,003-0,054 0,009-0,510 0,029-0,059	0,011-0,164 0,010-1,55 0,087-0,180	NI	Moreira et al., 2013

				Dialdeídos	3	2,12-38,4	0,020-0,046	0,061-0,140	
				Cetonas	5	0,022-0,931	0,009-0,025	0,026-0,076	
				Furanos	3	1,68-2109	1,54-3,44	4,58-10,4	
Portugal - Cerveja tipo 100% malte [g]	Larga escala	HS-SPME	GC/MS	Ésteres alifáticos	27				
				Álcoois	8				
				Aldeídos	8				
				Cetonas	6				
				Ácidos	6				
				Éteres	4	NQ	-	-	-
				Hidrocarbonetos	11				
				Compostos de enxofre	1				
				Compostos alicíclicos	8				
				Compostos aromáticos	16				
				Compostos hetrocíclicos	7				
Portugal – Cerveja de trigo [d]	Larga escala	HS-SPME	GC/MS	Ésteres alifáticos	21				
				Alcóis	10				
				Aldeídos	8				
				Cetonas	7				
				Ácidos	6	NQ	-	-	-
				Éteres	3				
				Hidrocarbonetos	15				
				Compostos alicíclicos	8				
				Compostos aromáticos	17				
				Compostos hetrocíclicos	8				
Portugal – Cerveja de trigo [d]	Larga escala	HS-SPME	GC/MS	Ésteres alifáticos	23				
				Alcóis	11				
				Aldeídos	11				
				Cetonas	5				
				Ácidos	5				
				Éteres	3	NQ	-	-	-
				Hidrocarbonetos	13				
				Compostos sulfúricos	2				
				Compostos alicíclicos	7				
				Compostos aromáticos	18				
				Compostos hetrocíclicos	26				
Portugal – Cerveja de trigo sem álcool [d]	Larga escala	HS-SPME	GC/MS	Ésteres alifáticos	16				
				Álcoois	7				
				Aldeídos	11	NQ	-	-	-
				Cetonas	7				
				Ácidos	9				
				Éteres	1				

				Hidrocarbonetos	13					
				Compostos alicíclicos	3					
				Compostos aromáticos	13					
				Compostos hetrocíclicos	7					
Portugal – Cerveja tipo 100% malte sem álcool [g]	Larga escala	HS-SPME	GC/MS	Ésteres	12	NQ	-	-	-	Pinho; Ferreira; Santos, 2006
				Álcoois	6					
				Aldeídos	6					
				Cetonas	5					
				Ácidos	10					
				Éteres	1					
				Hidrocarbonetos	5					
				Compostos alicíclicos	1					
				Compostos aromáticos	9					
				Compostos hetrocíclicos	7					

Fonte: Autoral

Notas:

[a] Tipo de cerveja não informado pelos autores; [b] Cerveja de alta fermentação; [c] Cerveja de baixa fermentação; [d] Cerveja de alta fermentação à base de trigo, de coloração clara e teor alcoólico médio; [e] Cerveja de alta fermentação, com maior adição de lúpulo e teor alcoólico, de sabor amargo e aromática; [f] Cerveja de coloração escura, geralmente de baixa fermentação dotada de forte sabor de chocolate, café e malte torrado, com pouca carbonatação e teor alcoólico elevado; [g] Cerveja de baixa fermentação sem adição de adjuntos substituíntes ao malte, possuem sabor e amargor um pouco mais acentuados, com leves notas de lúpulo; [h] Métodos de extração: HS-SPME: microextração em fase sólida no modo headspace; SBSE: extração sortiva em barra de agitação; [i] Métodos de separação e detecção: GC/MS: cromatografia gasosa acoplada à espectrometria de massas; GC-FID: cromatografia gasosa com detector por ionização de chama; [j] Concentração, NQ: Não quantificado; [L] Limite de Detecção; [m] Limite de Quantificação; [n] Recuperação; NI: não informado pelos autores.

3.4.1. Compostos do malte

A influência da matéria-prima na qualidade da cerveja é importante, uma vez que seus constituintes atuam como precursores da formação de compostos voláteis. O aroma e sabor dos produtos de malte derivam da composição química da cevada e do modo como é processada durante a malteação (transformação do grão de cereal em malte) (BARTH, 2013).

A malteação pode ser dividida em três etapas consecutivas: maceração, germinação e secagem. A maceração tem por finalidade fornecer aos grãos a umidade necessária para estimular a germinação do embrião, aumentando o teor de água de 13-15% para 43-46%. Durante a maceração, e principalmente durante a germinação, os grãos de cevada produzem enzimas hidrolíticas (β-glucanases e amilases) que levam a modificações na estrutura do grão, tornando-o mais macio e solúvel em água (MAYOLLE et al., 2012). A hidrólise do amido é necessária para que a fermentação alcoólica ocorra a partir de açúcares fermentáveis, como glicose e maltose. As condições de temperatura, umidade e aeração são controladas e o processo é interrompido tão logo o grão tenha iniciado o desenvolvimento de uma nova planta. O grão de cereal germinado é denominado de malte verde. Na etapa seguinte, o malte verde é seco (ou torrado) para reduzir o teor de água para 4,0–5,0%, o que interrompe as reações bioquímicas (PRIEST; STEWART, 2006). Dong et al. (2013) identificaram 47 compostos voláteis no malte seco, incluindo aldeídos, cetonas, álcoois, ácidos e furanos. Os níveis de 2-metil propanal (aroma de solvente), 3-metil butanal (aroma de malte), 2-metil butanal (aroma de coco) e 2-nonenal (aroma de gordura, pepino e papel) aumentaram na torrefação do malte, enquanto a concentração de hexanal (aroma de grama e gordura) e 2-hexenal (aroma verde e maçã) diminuíram nesta etapa.

O grau de coloração do malte adquirido durante a secagem se correlaciona positivamente com a formação de quantidades crescentes de produtos de reação de Maillard (HUGHES, 2009). Esta reação ocorre entre açúcares e aminoácidos livres em temperaturas superiores a 60 °C e leva à formação de compostos derivados do furano, como furfural e álcool furfurílico (VANDERHAEGEN et al., 2004). Além disso, durante a reação de Maillard, o oxigênio dos anéis de furano pode ser substituído por enxofre ou nitrogênio, levando à formação dos tiofenos e pirróis, respectivamente. Tais compostos apresentam aroma característico de cebola, sendo assim, são indesejados em cervejas (HUGHES, 2009). Além disso, a torrefação do malte, em temperatura superior a 200 °C, leva à degradação térmica do ácido ferúlico a 4-vinilguaiacol

(aroma característico de queimado ou cravo) (COGHE et al., 2004). Assim, ao mesmo tempo em que a torrefação é responsável pelo aumento de aroma indesejáveis, ela é crucial para o desenvolvimento de aromas adocicados e torrados, que são de grande interesse em alguns estilos de cerveja, como por exemplo Bock e Stout.

3.4.2. Compostos do lúpulo

O lúpulo utilizado na fabricação de cerveja é a flor seca da planta fêmea de uma trepadeira pertencente ao gênero *Humulus*, com ocorrência natural em zonas temperadas da Europa, dos Estados Unidos e da China. A presença do lúpulo é essencial para as características organolépticas da cerveja (aroma), estabilidade do sabor e retenção da espuma (BUGLASS, 2011). O lúpulo é adicionado ao mosto durante a fervura e transfere a este, compostos como os terpenos e α-ácidos. Enquanto os terpenos conferem à cerveja o aroma típico de lúpulo, os α-ácidos isomerizados são responsáveis pelo amargor (BAMFORTH, 2007; OBERHOLSTER; TITUS, 2016).

Na cerveja, o aroma de lúpulo é influenciado pela origem (solo e clima), cultivar, e por biotransformações que ocorrem durante a fermentação (KISHIMOTO et al., 2006; PRAET et al., 2012). A biotransformação de terpenos é dependente da sua concentração no lúpulo e da atividade metabólica da cepa de levedura utilizada. De acordo com King e Dickison (2003), tanto as leveduras *S. cerevisiae* e *S. pastorianus* (usadas para produzir cervejas *ale* e *lager*, respectivamente) podem transformar linalol (aroma floral) e nerol (aroma fresco, verde) em α-terpineol (aroma de pêssego); e geraniol (aroma floral) em citronelol (aroma frutado e floral). Entretanto, apenas as leveduras *lager* têm a capacidade de transformar geraniol e citronelol nos seus respectivos ésteres terpenóides; acetato de geranilo e acetato de citronelilo (ambos de aroma frutado e floral) (KING; DICKINSON, 2003).

3.4.3. Compostos provenientes da fermentação

Durante a fermentação, as leveduras produzem compostos relacionados ao aroma e sabor da cerveja. O teor desses compostos depende das condições do processamento (BAMFORTH; RUSSELL; STEWART, 2009), sendo que as principais classes de produtos da fermentação incluem: álcoois, ésteres, ácidos, aldeídos e cetonas.

O etanol produzido pelas leveduras durante a fermentação é o composto volátil majoritário das cerveja alcoólicas (AQUARONE; ALMEIDA LIMA; BORZANI, 1983). Este composto está presente nas cervejas alcoólicas em níveis pelo menos duas ordens de grandeza maiores do que qualquer outro álcool. Além disso, o etanol pode influenciar na percepção do aroma de outros componentes da bebida (HUGHES, 2009). Além do etanol, outros álcoois podem ser formados pelas vias metabólicas das leveduras, mostradas na Figura 3. Na produção de álcoois superiores (álcoois com massa molecular superior à do etanol) pela via catabólica de Ehrlich, aminoácidos sofrem uma desaminação, com transferência do grupamento amino para os α-cetoácidos. Em seguida, o α-cetoácido é descarboxilado ao seu respectivo aldeído e reduzido a álcool (Figura 3). Pela via anabólica, α-cetoácidos são formados quando carboidratos são catabolizados em aminoácidos. Após descarboxilação e redução, os α-cetoácidos são transformados nos álcoois superiores correspondentes (PIRES et al., 2014). Os álcoois superiores mais importantes na composição do aroma de bebidas fermentadas, incluindo a cerveja, são: álcool isoamílico (aroma alcoólico, banana), propílico (aroma alcoólico), isobutílico (solvente) e o 2-fenil-etanol (aroma doce, rosa). Além de contribuírem para o aroma da cerveja, os álcoois superiores também são precursores de ésteres (SAERENS et al., 2008).

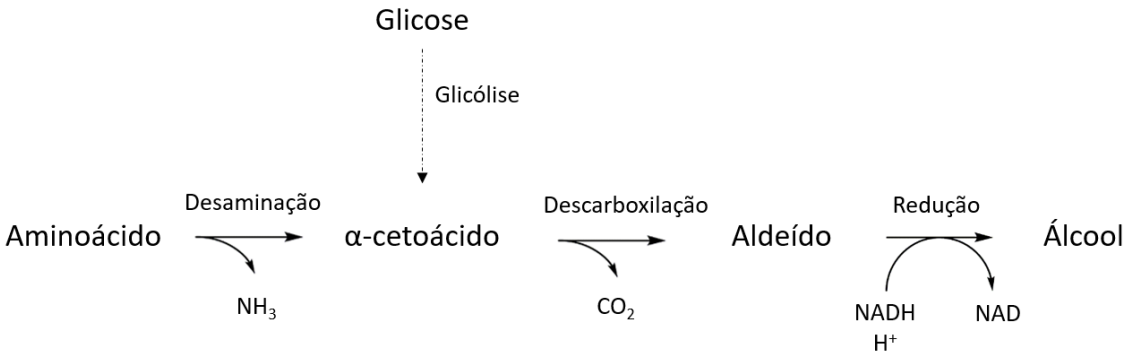


Figura 3. Vias anabólicas e catabólicas de formação de aldeídos e álcoois superiores e etanol. Adaptado de: Pires et al. (2014).

Os ésteres são formados pela condensação enzimática de ácidos orgânicos e álcoois. A contribuição deste grupo para o sabor e aroma de bebidas é grande, uma vez que são caracterizados, principalmente, pelo odor de frutas, com destaque para acetato de isoamila (aroma de banana), acetato de isobutila (aroma frutado), fenil acetato de etila (rosas e aroma de

mel), hexanoato de etilo (aroma de maçã doce) e octanoato de etila (aroma de maçã azeda) (VERSTREPEN et al., 2003).

Os ácidos podem ser derivados do mosto e podem ser produzidos durante a fermentação como resultado do metabolismo da levedura. Ácidos orgânicos de cadeia curta (< C_6) são produzidos por leveduras a partir de aminoácidos. Os ácidos graxos de cadeia média (C_6-C_{12}) resultam do anabolismo de ácidos graxos de cadeia longa sob condições anaeróbicas e/ou são liberados pelo mecanismo de autólise celular. Os ácidos graxos de cadeia longa (> C_{12}) da cerveja se originam principalmente do mosto (BRÁNYIK et al., 2008).

Os ácidos podem contribuir tanto positiva quanto negativamente no aroma de cervejas. Os ácidos graxos de cadeia média, como os ácidos hexanoico, octanoico e decanoico são descritos por suas características de aroma rançoso (PINHO; FERREIRA; SANTOS, 2006), enquanto que para o ácido nonanoico foi atribuído aroma floral, frutado e doce (NICOLLI et al., 2018).

Os aldeídos podem ser formados durante a preparação do mosto, a partir da reação de Maillard e de oxidação lipídica, ou a partir de vias metabólicas da síntese de etanol, durante a fermentação, como produto intermediário (BAERT et al., 2012). O acetaldeído (aroma de maçã verde, frutado) é o principal aldeído presente na cerveja devido à sua importância como intermediário na formação de etanol e acetato (PRIEST; STEWART, 2006). Pode-se destacar ainda outros aldeídos com importância para o aroma, como por exemplo (*E*)-2-nonenal (papelão, pepino), hexanal (enjoado), fenilacetaldeído (floral) e benzaldeído (amêndoa).

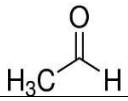
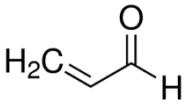
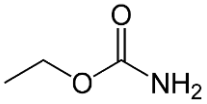
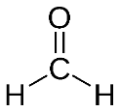
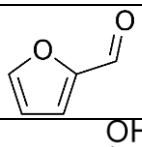
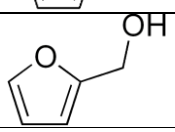
As cetonas são formadas através da descarboxilação oxidativa não enzimática de ácidos intermediários na via de biossíntese da valina e da isoleucina (ROSSI et al., 2014). Apesar das concentrações de cetonas serem geralmente muito baixas em cerveja fresca, estes compostos tem uma contribuição importante e principalmente indesejada para o perfil de aroma por causa de seus descritores sensoriais (ANDRÉS-IGLESIAS et al., 2016a). O diacetil (2,3-butanodiona) e a 2,3-pentanodiona, também chamadas de dicetonas vicinais, são consideradas as cetonas mais importantes em cervejas e apresentam aroma amanteigado, rançoso e mofado (KROGERUS; GIBSON, 2013).

3.5. Compostos tóxicos encontrados em cerveja

Na elaboração da cerveja, além dos voláteis relacionados ao aroma, outros compostos também podem ser formados, incluindo os compostos carbonílicos (acetaldeído, formaldeído, carbamato de etila, furfural e acroleína) e o álcool furfurílico que têm sido descritos como tóxicos (CARRILLO; BRAVO; ZUFALL, 2011; GONÇALVES et al., 2010; HU; WANG, 2015; LACHENMEIER et al., 2010; LI et al., 2009; MOREIRA et al., 2013; SAISON et al., 2008, 2009a; TIAN, 2010; VANDERHAEGEN et al., 2003; ZHAO et al., 2015).

A Tabela 3 apresenta a fórmula molecular e estrutural, bem como a massa molecular dos compostos carbonílicos e álcool furfurílico. A toxicidade destes compostos pode ser atribuída a sua natureza eletrofílica, e consequente possibilidade de interagir com os sítios nucleofílicos do DNA, o que caracteriza a genotoxicidade (BELAND et al., 2005; CHURCHWELL et al., 2015; COSTA et al., 2015; DING et al., 2012; JANZOWSKI et al., 2003; LACHENMEIER; MONAKHOVA, 2011; NOMURA et al., 1996; WANG et al., 2009, 2012).

Tabela 3. Informações estruturais dos compostos carbonílicos e do álcool furfurílico.

Composto	Fórmula molecular	Fórmula estrutural	MM (g/mol)[a]
Acetaldeído	C_2H_4O		44,05
Acroleína	C_3H_4		56,06
Carbamato de etila	$C_3H_7NO_2$		89,08
Formaldeído	CH_2O		30,03
Furfural	$C_5H_4O_2$		96,07
Álcool furfurílico	$C_5H_6O_2$		98,10

[a] Massa molecular

A Tabela 4 apresenta os níveis detectados de compostos tóxicos em cervejas de diferentes estilos e procedências, as técnicas de extração, de separação e de detecção, bem como os parâmetros de validação dos métodos analíticos empregados. Não foram encontrados na literatura, estudos que avaliaram estes compostos tóxicos em cervejas artesanais e/ou em cervejas brasileiras. Estudos têm sido conduzidos com cervejas provenientes da China, Venezuela e países da Europa (CARRILLO; BRAVO; ZUFALL, 2011; GONÇALVES et al., 2010; MOREIRA et al., 2013; RIU-AUMATELL et al., 2014; SAISON et al., 2009b; VANDERHAEGEN et al., 2003), sendo o furfural o composto mais avaliado em cervejas. Além disso, os estudos desenvolvidos até o momento são voltados para a avalição do efeito do envelhecimento de cervejas sobre os níveis de compostos carbonílicos e derivados do furano (ANDRÉS-IGLESIAS et al., 2016a; GONÇALVES et al., 2010; MIKYŠKA et al., 2011; MOREIRA et al., 2013). A HS-SPME e a cromatografia gasosa acoplada ao detector espectrométrico de massas (GC/MS) foram as técnicas de extração e quantificação mais empregadas, respectivamente. É importante registrar que nenhum estudo desenvolvido até o momento objetivou determinar simultaneamente os compostos com potencial genotóxico que podem ser encontrados na cerveja, incluindo os carbonílicos, furfural e álcool furfurílico, embora determinações de alguns destes compostos, individualmente, em cerveja já tenham sido reportadas (DENG et al., 2016; KÄCHELE et al., 2014; KUCHARCZYK; TUSZYNSKI, 2016; LI et al., 2017, 2009; LIU et al., 2018; TIAN, 2010; TSAI; KAO, 2012; ZHAO et al., 2015).

Tabela 4. Compostos tóxicos detectados em cervejas comercializadas em diversos locais, técnicas de extração e análise empregadas, parâmetros de validação de métodos analíticos e suas faixas de níveis de concentração detectados.

Tipo de cerveja	Tipo de produção	Procedência das amostras	Técnica de extração[f]	Método de separação e detecção[g]	Parâmetros de validação			Níveis detectados (µg L^{-1})	Referência
					LOD[i] (µg L^{-1})	LOQ[J] (µg L^{-1})	Recup[L] (%)		
Furfural									
NI	Larga escala	China	SPE	HPLC	5	NI	99.7–100.1	10 - 90	Li; Yang; Yang, 2009
Várias marcas [a,b]	Larga escala	Portugal	GDME	HPLC–UV	1,5	4,9	NI	NQ	Gonçalves et al., 2010
Pilsner [b]	NI	Bélgica	HS-SPME	CG/MS	2,791	9,304	NI	20,9 – 282,2	Saison et al., 2008
Amber [c]								62,43 – 346,0	
Blond [c]								43,53 – 522,76	
Pilsner [b]	Larga escala	Venezuela	SPME	GC/MS	1,60	5,20	97-102	25,7 – 59,3	Carrillo; Bravo; Zufall, 2011
NI	Larga escala	Espanha	HS-SPME	CG/MS	NI	NI	NI	0,87 ± 0,73	Riu-aumatell et al., 2014
Baixo teor de álcool [d]								2,04 ± 0,97	
Sem álcool [e]								3,53 ± 1,41	
Ale [c]	NI	Bélgica	P&T	GC/MS	NI	NI	NI	48 - 2535	Vanderhaegen et al., 2003
Álcool furfurílico									
NI	Larga escala	Espanha	HS-SPME	CG/MS	NI	NI	NI	0,95 ± 0,17	Riu-Aumatell et al., 2014
Baixo teor de álcool [d]								0,78 ± 0,45	
Sem álcool [e]								1,16 ± 0,20	
Ale[c]	NI	Bélgica	P&T	GC/MS	NI	NI	NI	2342 - 4321	Vanderhaegen et al., 2003
Acetaldeído									
Várias marcas [a,b]	Larga escala	Portugal	GDME	HPLC–UV	12,3	41	NI	NQ	Gonçalves et al., 2010
Ale[c]	NI	Bélgica	P&T	GC/MS	NI	NI	NI	1052-3961	Vanderhaegen et al., 2003
Acroleína									
Lager[b]	NI	Portugal	HS-SPME	GC/MS	0,510	1,55	NI	0,976 – 4,61	Moreira et al., 2013
Lager[b]	NI	Bélgica	HS-SPME	GC/MS	0,24	0,81	NI	1,37 ± 0,13	Saison et al., 2009
Carbamato de etila									

Lager[b]	NI	China	SPE	GC/MS	NI	NI	NI	2 - 3	Wu et al., 2012
Formaldeído									
Lager[b]	Larga escala	China	SD	HPLC	< 3	NI	NI	0,062 - 0,453	Hu; Wang, 2015
NI	Larga escala	China	SPE	HPLC-DAD	0,016	0,042	91,3-104,3	NQ	Zhao et al., 2015

Fonte: Autoral

Notas:

[a] Marcas: Pilsner Urquell, Bud-Weiser Budvar, Staropramen, Stella Artois, Zatec Xantho E Heineken; [b] Cerveja de baixa fermentação; [c] Cerveja de alta fermentação; [d] Cerveja de baixo teor alcoólico (<3% etanol); [e] Cerveja sem álcool (<1% etanol); [f] Método de extração: SPE: Extração em fase sólida; GDME: Microextração por difusão gasosa; HS-SPME: Microextração em fase sólida no modo *headspace*; P&T: Extração e concentração por purga e armadilha; SD: Destilação por arraste de vapor; [g] Separação e detecção: HPLC: Cromatografia líquida de alta eficiência; HPLC–UV: Cromatografia líquida de alta eficiência com detector ultravioleta; CG/MS: Cromatografia gasosa acoplada à espectrometria de massas; HPLC-DAD: Cromatografia líquida de alta eficiência acoplada a um detector de diodos;[h] Derivatizante: PFBHA: O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride; DNPH: 2,4-Dinitrophenylhydrazine; EAHC: Ethoxyamine hydrochloride; ND: Não derivatizado; NI: Não informado pelos autores; NQ: Não quantificado; [i] Limite de detecção; [J] Limite de quantificação; [L] Recuperação.

3.5.1. Acetaldeído

Em bebidas alcóolicas, este aldeído pode ser produzido durante a fermentação a partir da descarboxilação do ácido pirúvico, proveniente da glicólise. A oxidação do etanol e de compostos fenólicos por ação das leveduras também pode levar à formação do acetaldeído (PAIANO et al., 2014).

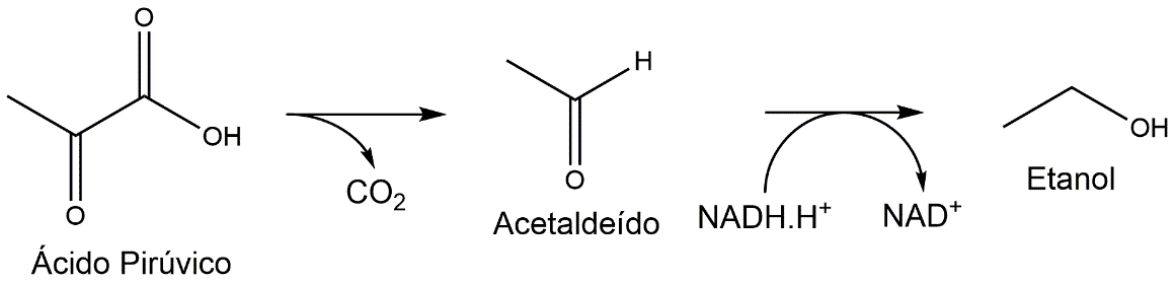


Figura 4. Via metabólica da fermentação alcoólica a partir do ácido pirúvico com formação de acetaldeído e etanol.
Fonte: Adaptado de Azevêdo et al. (2007)

O consumo de etanol resulta na produção imediata de pequenas quantidades de acetaldeído na cavidade oral devido aos microrganismos presentes na saliva. Além disso, etanol pode ser oxidado a acetaldeído no fígado através da enzima álcool desidrogenase (ADH) (LACHENMEIER; KANTERES; REHM, 2009) e no cérebro, através de vias que envolvem a catalase, o citocromo CYP2E1 e a ADH (HERNÁNDEZ; LÓPEZ-SÁNCHEZ; RENDÓN-RAMÍREZ, 2016).

O acetaldeído presente nas bebidas alcoólicas pode ocasionar sintomas relacionados à dores de cabeça, queda da pressão sanguínea e náuseas (LACHENMEIER; KANTERES; REHM, 2009). Seitz e Stickel (2010), ao revisarem estudos sobre os mecanismos de mutagenicidade e carcinogenicidade do acetaldeído, relataram que a exposição a este composto pode aumentar o risco de câncer no trato aerodigestivo superior (cavidade oral, faringe, laringe e esôfago), fígado, intestino grosso e mama (SEITZ; STICKEL, 2010).

Na indústria de bebidas, é importante conhecer as concentrações de acetaldeído, pois este tem papel central na manifestação da intoxicação alcoólica. Não há legislação nacional, nem internacional que estabeleça limites máximos para o acetaldeído em

cerveja. Contudo, para bebidas destiladas no Brasil, admite-se valores máximos de aldeídos totais, expressos em quantidade de acetaldeído em cada 100 mL de etanol anidro: aguardente 30 mg, conhaque 40 mg, graspa 80 mg, destilado simples de uva 40 mg e pisco 200 mg (BRASIL, 2005, 2010).

3.5.2. Acroleína

A acroleína, também chamada de aldeído acrílico ou 2-propenal, é produzida a partir do glicerol, conforme ilustrado na Figura 5. O glicerol, um dos principais compostos resultantes da fermentação alcóolica pela ação das leveduras, é transformado a 3-hidróxipropanal (3-HPA) pela enzima glicerol desidratase; em seguida ocorre a redução a 1-3-propanodiol ou conversão à acroleína (AZEVÊDO et al., 2007).

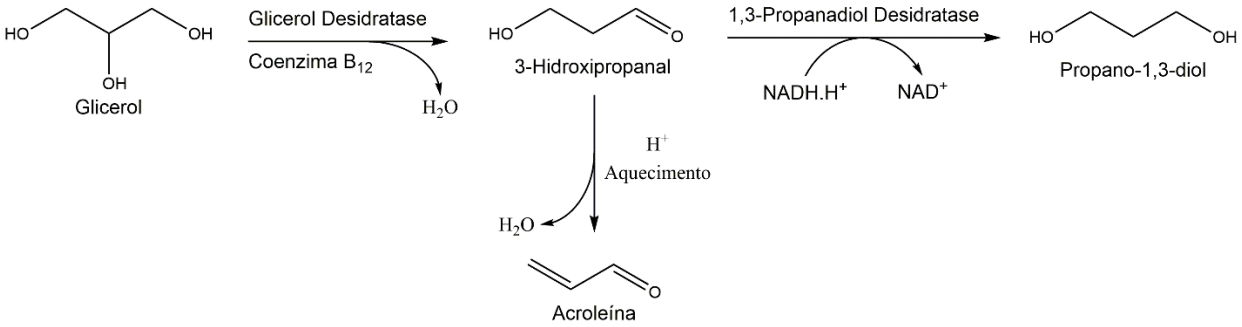


Figura 5. Via metabólica de obtenção de acroleína a partir do glicerol.
Fonte: Azevêdo et al. (2007)

A exposição à acroleína por via oral pode causar mal-estar abdominal, diarreia e vômito (FAROON et al., 2008). Este aldeído, assim como os demais compostos carbonílicos, pode formar adutos com o DNA, RNA e proteínas devido ao seu perfil eletrofílico. Além disso, a exposição à acroleína pode ter relação com a ocorrência do Mal de Alzheimer (NAN; ARSENEAULT; RAMASSAMY, 2010), diabetes (FEROE; ATTANASIO; SCINICARIELLO, 2016) e potencializar o risco de incidência de doenças cardiovasculares (DEJARNETT et al., 2014).

3.5.3. Carbamato de etila (CE)

O CE, também chamado de uretano, é o éster etílico do ácido carbâmico. A formação deste éster ocorre através da reação entre o etanol e ureia ou outros compostos nitrogenados, tais como resíduos de aminoácidos. Entretanto, a via mais comum de formação em bebidas fermentadas ocorre a partir da ureia. A levedura *S. cerevisiae* produz arginase, enzima capaz de catabolizar a arginina do malte, resultando na formação da ureia (EFSA, 2007).

Cui et al. (2016) verificaram que no fígado, o CE pode ser oxidado pelas enzimas do citocromo P450, transformando-o em epóxido de vinil carbamato, o que caracteriza a genotoxicidade deste éster. A forma biotransformada do CE é altamente reativa e eletrofílica, o que potencializa a possibilidade de formação de adutos com DNA e consequente carcinogênese (LAJOVIC et al., 2015)

Segundo pesquisas do o Comitê de Especialistas em Aditivos Alimentares da Organização das Nações Unidas para Agricultura e Alimentação e Organização Mundial da Saúde (JECFA, do inglês: *Joint FAO/WHO Experts Committee on Food Additives*), a população mundial expõe-se diariamente ao CE através do consumo de produtos como pão, produtos lácteos fermentados e molho de soja em um nível de até 15 mg kg^{-1}, enquanto que, quando ocorre a ingestão de bebidas alcóolicas somadas ao consumo desses outros alimentos, a exposição diária pode chegar a 80 mg kg^{-1} de PC (JECFA, 2016).

3.5.4. Formaldeído

O formaldeído, conhecido também por metanal, formol e aldeído fórmico, é considerado a forma mais simples de um aldeído (CH_2O). Esse composto forma-se a partir da foto-oxidação do metano (Figura 6) e está naturalmente presente no ambiente urbano, pois é liberado em processos de combustão, incluindo escapamentos automotivos e atividades industriais. Incêndios florestais e erupções vulcânicas também estão relacionados à emissão de formaldeído para o ambiente. Além disso, a fumaça de cigarro, o consumo de bebidas alcóolicas e o uso de fluidos de embalsamento em laboratórios de patologia são considerados fontes de exposição ao formaldeído (WHO, 2002).

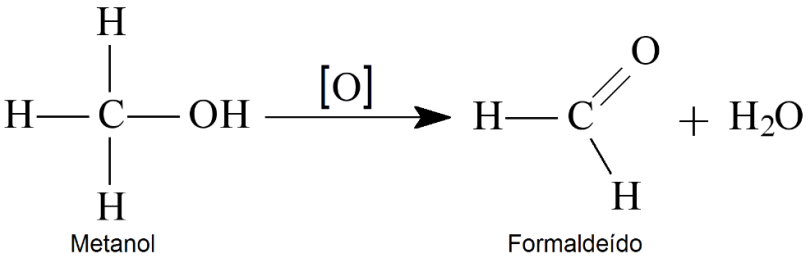


Figura 6. Reação de formação do formaldeído via oxidação do metanol.

Fonte: autoral (2018)

O formaldeído presente nas bebidas alcóolicas, pode ser formado durante a fermentação alcóolica através da oxidação do metanol, que se origina da hidrólise da pectina (IPCS, 2002). Este composto, assim como os demais compostos carbonílicos que podem ser encontrados na cerveja, tem capacidade em formar adutos com o DNA, RNA e proteínas (SZENDE; TYIHÁK, 2010; ZERIN et al., 2015). Estudos relacionaram a exposição ao formaldeído por via oral com à ocorrência de úlceras no trato gastrintestinal e a sua inalação pode causar alterações respiratórias como irritação da mucosa bucal, olhos e nariz (NAYA; NAKANISHI, 2005; NORLIANA et al., 2009). Arkeman (2008) associou a ingestão do formaldeído à necrose das camadas superficiais da mucosa gástrica e ocorrência de úlceras em fêmeas adultas de camundongos da espécie Sprague-Dawley, as quais foram expostas ao formaldeído através da água por um período de 12 semanas (ARKEMAN; ARKEMAN, 2008).

Em 2002, o Programa Nacional de Segurança Química (IPCS, do inglês, *International Programme on Chemical Safety*) da Organização Mundial da Saúde definiu um limite de concentração tolerável de 2600 μg L^{-1} para a presença de formaldeído em alimentos e bebidas alcóolicas. Este limite foi baseado no nível de efeito não observado (NOEL, do inglês: *no observed effect level*) de 260.000 μg L^{-1}, que foi estabelecido, considerando-se a ocorrência de danos histopatológicos em ratos expostos por via oral ao formaldeído (IPCS, 2002).

3.5.5. Furfural

O furfural é um aldeído heterocíclico e aromático, também denominado 2- furano carboxialdeído, furaldeído, 2-furanaldeído, fural e furfuraldeído. Resulta da desidratação

de pentoses e é produzido durante a reação Maillard. Sua formação está correlacionada com a cor da cerveja, sendo que quanto maior for a concentração do furfural, mais escura é a cerveja (VANDERHAEGEN et al., 2007).

Em relação à toxicidade, Lake et al. (2001) associaram a exposição oral diária ao furfural via gavagem (administração de composto através de um tubo até o estômago) à necrose e inflamação crônica do fígado, além da ocorrência de adenoma e carcinoma hepatocelulares, em ratos e camundongos.

No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) não reconhece o uso do furfural e do álcool furfurílico como aditivos alimentares. Além disso, não há legislação que estabeleça níveis máximos destes compostos em cervejas. Apenas para aguardente composta são previstos valores máximos do somatório de furfural e hidróxi metil furfural de 5 mg em cada 100 mL de álcool anidro (MAPA, 2010).

3.5.6. Álcool furfurílico

O álcool furfurílico é também conhecido por furfuril álcool, 2-furilmetanol e 2-furancarbinol. Este álcool ocorre em alimentos processados termicamente, como resultado da redução enzimática ou química do furfural. O principal mecanismo de formação do álcool furfurílico em cervejas é através da degradação térmica da maltose via reações de Maillard, durante a secagem e torrefação do malte e durante a fervura do mosto (VANDERHAEGEN et al., 2004). Em condições ácidas, o álcool furfurílico se polimeriza resultando na coloração marrom dos alimentos (SWASTI; MURKOVIC, 2012).

A exposição por inalação ao álcool furfurílico pode provocar dor de cabeça, náuseas e irritação na boca e no estômago (CDC, 2018). O efeito mutagênico desse álcool foi primeiramente relatado por Stich (1981), quando um aumento do número de aberrações cromossômicas e trocas entre cromátides irmãs foi observado em culturas de células dos ovários de hamster. O mecanismo postulado de carcinogenicidade do composto se dá através da ativação por sulfotransferases, resultando na formação de um aduto de 2-metilfuranil-DNA, conforme verificado em ratos submetidos a doses de 400 mg kg^{-1} de peso corpóreo via injeção intraperitoneal (SACHSE et al., 2014, 2016).

Em cervejas, Riu-Aumatell et al. (2004) encontraram níveis de álcool furfurílico entre 0,78 e 1,16 μg L^{-1} e Vanderhaegen et al. (2004) demostraram o efeito da reação de Maillard durante o armazenamento desta bebida ao verificar um aumento de 93% nos níveis de álcool furfurílico nas cervejas após esta etapa. Embora em 2017 a IARC tenha classificado o álcool furfurílico como possível carcinogênico para humanos, cuja monografia está em fase de preparação, a Autoridade Europeia para a Segurança dos Alimentos considera o consumo do álcool furfurílico e de derivados furanos como seguro quando usados como flavorizantes em alimentos (EFSA, 2016).

3.6. Avaliação do risco relacionado à exposição a compostos tóxicos

A avaliação do risco causado pela exposição humana às substâncias tóxicas presentes na dieta é amplamente reconhecida como um processo fundamental no desenvolvimento de padrões alimentares seguros. Esta ferramenta é importante para o processo de tomada de decisão sobre questões relacionadas à segurança dos alimentos, através da qual pode-se identificar um problema potencial, avaliar a probabilidade da sua ocorrência, estimar o seu impacto e sugerir medidas para solucioná-lo (DUBUGRAS; PÉREZ-GUTIÉRREZ, 2008; LIU et al., 2013).

A avaliação da exposição aos compostos tóxicos ingeridos através do consumo de cerveja pode ser feita através do cálculo da ingestão diária estimada (IDE) segundo a equação 1:

$$IDE(\mu g\ kg^{-1}\ PC\ dia^{-1}) = \frac{concentração\ do\ composto\ (\mu g\ mL^{-1}) \times consumo\ de\ cerveja\ (mL\ dia^{-1})}{peso\ do\ indivíduo\ (kg)}$$

Equação 1

A determinação experimental da concentração de um composto tóxico é o primeiro passo para avaliar o risco da exposição relacionado ao consumo da cerveja. Como dados de consumo da cerveja, pode-se considerar o consumo moderado dessa bebida que têm sido associado na literatura à efeitos benéficos, como sendo de até 1 dose de 300 mL de cerveja (~13,5 g de etanol) para mulheres e duas doses para homens por dia (POLI et al., 2013). Cabe ressaltar que o conceito de consumo moderado é relativo e discutido entre

os pesquisadores, variando de um copo (250 mL) até uma garrafa por dia (600 mL) (ARRANZ et al., 2012; NARDINI et al., 2006). O peso corpóreo médio de 60 kg pode ser utilizado para expressar os resultados em μg kg $^{-1}$ de PC.

A caracterização do risco relacionado à exposição aos compostos genotóxicos é feita através do cálculo da margem de exposição (MOE, do inglês: *margin of exposure*) segundo a equação 2:

$$MOE = \frac{referência\ toxicológica}{exposição} = \frac{BMDL10\ (\mu g\ kg^{-1}\ pc\ dia^{-1})}{IDE\ (\mu g\ kg^{-1}\ pc\ dia^{-1})}$$

Equação 2

Para fins de cálculo da MOE, é adotado que a exposição ao composto genotóxico equivale à sua IDE (equação 1). A dose que causou um aumento de 10% na incidência de um efeito tóxico (BMD10, do inglês, *benchmark dose lower confidence limit*) relacionado à exposição oral de animais a um determinado composto tem sido usada na literatura como referência toxicológica para calcular a MOE (Tabela 5).

Tabela 5. Valores de BMDL10 e respectivos efeitos tóxicos observados após exposição oral a alguns compostos genotóxicos que podem ser encontrados na cerveja.

Composto	**BMDL10 (mg kg^{-1} de PC dia^{-1})**	**Efeito tóxico**	**Referência**
Formaldeído	28	Gastrite crônica em ratos	Monakhova; Jendral; Lachenmeier, 2012
Acetaldeído	56	Tumores em ratos (mamários, estomacais, intestinais, testiculares)	Lachenmeier; Kanteres; Rehm, 2009
Acroleína	0,36	Hiperplasia epitelial escamosa em estomago de ratos	ATSDR, 2007
Carbamato de etila	0,25	Neoplasia alveolar e bronquiolar em ratos machos e fêmeas	Schlatter; Dinovi; Setzer, 2010

A MOE pode ser usada para classificar substâncias genotóxicas de acordo com o risco, indicando o nível de preocupação para estabelecimento de prioridades de ações para os gerenciadores de risco e para subsidiar os indivíduos a tomar decisões pessoais de estilo de vida. Um valor de MOE igual ou maior que 10.000 tem sido proposto como uma indicação de uma situação de risco pouco preocupante sob a ótica de saúde pública (BENFORD et al., 2010; BOOBIS et al., 2013).

A caracterização do risco da exposição humana a substâncias não genotóxicas é realizada pela comparação entre a IDE (calculada de acordo com a equação 1) e o parâmetro de ingestão segura determinado pelo JECFA. Estima-se risco quando a IDE ultrapassa esse parâmetro (BOOBIS et al., 2013; JARDIM; CALDAS, 2009).

Dentre os parâmetros de ingestão crônica segura, para as substâncias que não são genotóxicas, estão a ingestão diária aceitável (ADI, do inglês: *acceptable daily intake*), a ingestão diária tolerável máxima provisória (PMTDI, do inglês, *provisional maximum tolerable daily intake*), a ingestão tolerável semanal provisória (PTWI, do inglês, *provisional tolerable weekly intake*) e a ingestão tolerável mensal provisória (PTMI do inglês,: *provisional tolerable monthly intake*). Esses parâmetros representam a quantidade de uma substância que pode ser consumida diariamente (ADI e PMTDI), semanalmente (PTWI) ou mensalmente (PTMI) ao longo da vida, sem que ocorram efeitos adversos à saúde. Para compostos intencionalmente adicionados nos alimentos (aditivos, resíduos de pesticidas e de medicamentos de uso veterinário), aplica-se a ADI. Os parâmetros de ingestão tolerável provisória (PMTDI, PTWI e PTMI) são definidos para os contaminantes, sendo que a PMTDI é definida para substâncias que não se acumulam no organismo. A PTWI e PTMI são usadas para compostos que tem potencial de acumulação no organismo, sendo que para aquelas substâncias que têm vida longa no organismo utiliza-se a PTMI (JARDIM; CALDAS, 2009).

O JECFA estabeleceu uma ADI de 0,5 mg kg^{-1} de PC para compostos que contém furano na sua estrutura como furfural, álcool furfurílico, acetato de furfurila, propionato de furfurila, pentanoato de furfurila, octanoato de furfurila, 3-metil butanoato de furfurila, 2-furoato de metila, 2-furoato de propila, 2-furoato de amila, 2-furoato de hexila e 2-furoato de octila, e sugeriu que o composto não era motivo de preocupação nos níveis

atuais de ingestão, quando usado como aromatizante (WHO, 2001). A legislação brasileira não estabelece limites máximos para a presença de álcool furfurílico e furfural em alimentos e bebidas. Entretanto, a IARC classificou recentemente o álcool furfurílico como possível carcinogênico para humanos, cuja monografia está em fase de preparação (IARC, 2017), o que resultará possivelmente em mudança no parâmetro de ingestão segura estabelecido para este composto pelo JECFA.

3.7. Microextração em fase sólida

Técnicas apropriadas de preparação de amostras devem ser suficientemente rápidas, de fácil manipulação, baixo custo e compatíveis com instrumentos analíticos diversos. A microextração em fase sólida (SPME, do inglês: *solid phase microextraction*) tem sido aplicada com sucesso à análise de voláteis relacionados ao aroma, bem como na determinação de contaminantes em diversos tipos de amostras (SOUZA-SILVA; GIONFRIDDO; PAWLISZYN, 2015). Esta técnica se baseia no estabelecimento de um equilíbrio entre o analito e um revestimento de fase extratora, que pode ser um polímero, um sorvente sólido ou a combinação de ambos, que se encontra sobre uma fibra de sílica fundida. A SPME combina a extração e a concentração dos analitos em uma única etapa, dispensa o uso de solventes e requer uma pequena quantidade de amostra, diminuindo o tempo de extração e reduzindo custos, além de ser considerada ambientalmente correta (PAWLISZYN, 2009).

O procedimento de extração e dessorção da amostra por SPME é mostrado Figura 7. Semelhantemente a uma seringa, uma fibra SPME consiste em uma haste de sílica fundida ligada a um êmbolo de aço inoxidável. A ponta da haste de sílica é revestida com um polímero e é protegida dentro de uma agulha oca. Quando o êmbolo é pressionado, a haste e o respectivo polímero são expostos e a amostra é coletada por absorção e/ou adsorção, dependendo do tipo de revestimento. Após um tempo de exposição adequado, a fibra é retraída para dentro do êmbolo metálico e em seguida é inserida no injetor do cromatógrafo, quando o êmbolo é novamente pressionado para expor o polímero contendo os analitos. Uma vez no injetor aquecido, os compostos extraídos são dessorvidos termicamente e arrastados pela fase móvel do cromatógrafo para a coluna de separação (ORMSBY, 2005; RISTICEVIC et al., 2010)

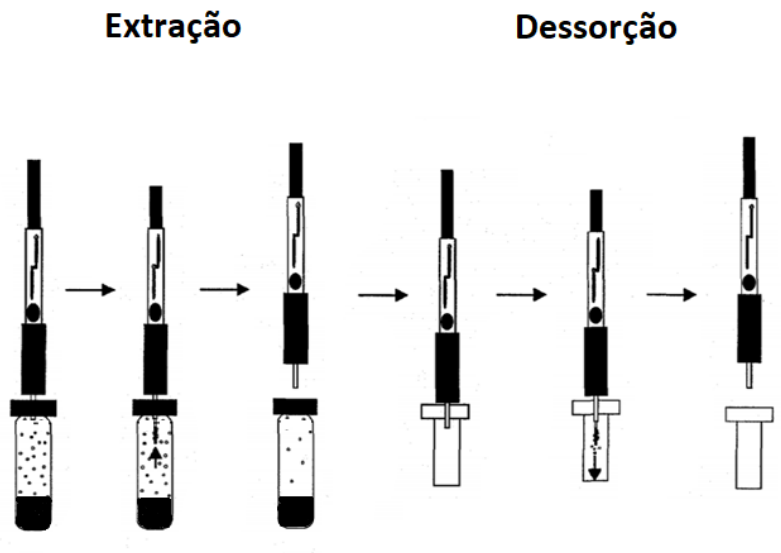


Figura 7. Etapas da extração por HS-SPME e dessorção térmica no cromatógrafo gasoso.
Adaptado de: Ormsby, (2005)

A extração dos constituintes da matriz da amostra via SPME pode ser realizada por imersão direta (DI-SPME, do inglês, *direct immersion solid phase microextraction)* ou por *headspace* (HS-SPME, do inglês, *headspace solid phase microextraction*). Na DI-SPME a fibra é diretamente imersa na amostra líquida e na HS-SPME a fibra é exposta sob a amostra. Para a análise de compostos voláteis em uma amostra em matriz complexa, a HS-SPME é o modo de amostragem mais adequado, uma vez que a fibra é colocada na atmosfera que se encontra acima da amostra líquida ou sólida e não está em contato com a amostra. Por outro lado, no modo de amostragem DI-SPME, a fibra é inserida diretamente na amostra fazendo com que a sua vida útil diminua (INAMADDIN; MOHAMMAD, 2014; MIN et al., 2015). A HS-SPME tem sido a técnica escolhida por vários pesquisadores para a extração de compostos voláteis relacionados ao aroma de cerveja, conforme mostrado na Tabela 2. Diversos tipos de recobrimentos extratores, de diferentes espessuras, polaridades e tamanho de poro, estão disponíveis comercialmente, mostrando grande seletividade para diferentes analitos. Os revestimentos de fibra mais utilizados para análise de alimentos são polidimetilsiloxano (PDMS), poliacilato (PA), Carboxen® (CAR), divinilbenzeno (DVB) e Carbowax (CW; polietilenoglicol). A escolha do revestimento de fibra depende principalmente da natureza dos analitos (GÓRECKI; YU; PAWLISZYN, 1999). Uma opção apropriada para extrair analitos com

diferentes características físico-químicas são misturas de revestimento (BALASUBRAMANIAN; PANIGRAHI, 2011). DVB/Car/PDMS tem sido o recobrimento mais utilizado na análise de compostos relacionados ao aroma de cerveja (ANDRÉS-IGLESIAS et al., 2016b; GONÇALVES et al., 2014; RODRIGUES; CALDEIRA; CâMARA, 2008; SVOBODA et al., 2011).

Existe uma diferença substancial entre o desempenho de revestimentos líquidos e sólidos. Nos revestimentos líquidos, os analitos se dispersam pela fase extratora, na qual as moléculas são solvatadas pelas moléculas de revestimento. O coeficiente de difusão no revestimento líquido permite que as moléculas penetrem em todo o volume da fase, dentro de um tempo razoável de extração. Os sorventes sólidos têm uma estrutura cristalina vítrea ou bem definida, que reduz substancialmente os coeficientes de difusão dentro da estrutura. Em tempos reduzidos de extração, a sorção ocorre apenas na superfície porosa do revestimento (PAWLISZYN, 2009). Durante a extração de matrizes complexas, quando um dos analitos está presente em concentrações muito superiores aos demais, os compostos com baixa afinidade pela fase extratora sólida são frequentemente deslocados por analitos que apresentam maior afinidade pela mesma. Isso ocorre porque apenas uma área de superfície limitada é disponível para adsorção. Se esta área estiver ocupada, a competição ocorre, interferindo no equilíbrio entre analitos e fase extratora (PAWLISZYN, 2009; RISTICEVIC et al., 2010).

Segundo Risticevic e Pawliszyn (2013), extrações em matrizes complexas com DVB/Car/PDMS estão sujeitas ao efeito de deslocamento dos analitos com uma menor afinidade pelo revestimento. O etanol, composto presente em maior concentração em bebidas alcoólicas, tem sido descrito na literatura como uma importante interferência para a extração de compostos minoritários, especialmente durante o procedimento HS-SPME (CONNER et al., 1998; HARTMANN; MCNAIR; ZOECKLEIN, 2002; PÉREZ-OLIVERO et al., 2014; RODRÍGUEZ-BENCOMO et al., 2002; WHITON; ZOECKLEIN, 2000).

Para monitorar mudanças no tipo e quantidade de compostos aromáticos produzidos durante a fermentação alcoólica de mosto de uvas, Zhang et al. (2011) avaliaram o impacto do etanol sorvido na fibra DVB/Car/PDMS. Nesse caso, influência do etanol é,

provavelmente, devida a dois aspectos: inchamento da fibra por absorção e disputa pelos sítios de adsorção. Padrões analíticos de álcoois, ésteres, ácidos e monoterpenos foram usados para preparar sete conjuntos de soluções modelo de vinho (que é uma solução que visa simular a matriz da amostra analisada, contendo em quantidades correspondentes, os principais componentes do vinho, 7 g L^{-1} de ácido tartárico e pH 3,3) contendo diferentes quantidades de etanol (2, 4, 6, 8, 10, 12 e 14%) e 4-metil-2-pentanol foi usado como padrão interno. Os resultados mostraram que a eficiência de extração do HS-SPME foi afetada pelo etanol. Quanto maior a concentração de etanol na solução, menores eram as quantidades de compostos extraídos pela fibra. Além disso, o uso do padrão interno não foi suficiente para evitar o efeito do etanol na extração. Os autores também sugerem que é possível minimizar o efeito do etanol na HS-SPME, usando-se curvas de calibração contendo a quantidade de etanol correspondente à encontrada na amostra (ZHANG et al., 2011).

Limitações relacionadas à realização de SPME no modo de imersão direta (DI) em matrizes complexas motivaram o desenvolvimento de revestimento de fibra modificada através da implementação de uma camada PDMS adicional em fibras comerciais (SOUZA-SILVA et al., 2016; SOUZA-SILVA; PAWLISZYN, 2012, 2015). Uma desvantagem do DI-SPME está relacionada com a ligação de componentes da matriz, principalmente açúcares, aminoácidos e compostos coloridos, na superfície do revestimento polimérico. Essas macromoléculas podem causar a incrustação da fase extratora que leva não apenas à diminuição da eficiência da extração, mas também reduz a vida útil da fibra (SOUZA-SILVA; PAWLISZYN, 2012). Por ser um revestimento líquido não poroso, o PDMS sofre menos o efeito de incrustação dos componentes da matriz quando comparado aos revestimentos sólidos (PAWLISZYN, 2009).

Souza-Silva e Pawliszyn (2012) acrescentaram uma camada PDMS externa a uma fibra comercial PDMS/DVB para extrair pesticidas em uvas, o que não alterou os parâmetros cinéticos nem termodinâmicos de extração desses compostos hidrofóbicos quando comparados aos parâmetros do revestimento original (SOUZA-SILVA; PAWLISZYN, 2012). Além disso, a fibra revestida com PDMS apresentou uma taxa de absorção mais lenta apenas para compostos mais polares (pertencentes a classe dos pesticidas, produtos químicos industriais e produtos farmacêuticos com coeficiente de

partição inferior a 3) extraídos da água (SOUZA-SILVA et al., 2017) ou de polpa de uvas (SOUZA-SILVA; PAWLISZYN, 2015). O número de extrações aumentou de 60 para 100 quando uma fibra revestida com PDMS foi usada, melhorando significativamente a vida útil da mesma, quando a extração de pesticidas em suco de uva foi realizada (SOUZA-SILVA et al., 2016). Além disso, o revestimento modificado permitiu a obtenção de coeficientes de variação inferiores a 20 % entre réplicas de extração, mesmo na presença de pigmentos (antocianinas) e alto teor de açúcar (cerca de 20 %) encontrados no suco de uva (SOUZA-SILVA et al., 2016).

Gionfriddo et al. (2015) compararam o desempenho de fibras com revestimento extra de PDMS (PDMS-PDMS/DVB e PDMS-DVB/Car/PDMS) as suas análogas comerciais não modificadas (PDMS/DVB e DVB/Car/PDMS) na extração de analitos de diferentes hidrofobicidades (log P entre 1,3 e 4,4), pesos moleculares (variando entre 78,1 e 136,2 g mol^{-1}) e classes químicas (entre álcoois, cetonas, ésteres, terpenos e compostos aromáticos). Verificaram que a camada extra de PDMS possibilitou a redução dos fenômenos de deslocamento de analitos em comparação com os revestimentos comerciais (GIONFRIDDO; SOUZA-SILVA; PAWLISZYN, 2015). Esses resultados abrem uma nova possibilidade para a aplicação de revestimentos modificados por PDMS para análise de matrizes complexas por HS-SPME.

3.8. Cromatografia gasosa acoplada à espectrometria de massas

O princípio básico da cromatografia é a partição dos analitos entre duas fases: móvel e estacionária. Diferentes constituintes da amostra são fisicamente separados com base em suas volatilidades e afinidade em relação à fase estacionária. A taxa de distribuição de um analito depende de sua interação com as moléculas nas duas fases. Poucas interações ocorrem entre os analitos e a fase móvel, que é um gás inerte. Compostos com baixa volatilidade e forte interação com a fase estacionária são retidos na coluna cromatográfica e se movem ao longo da mesma, enquanto que compostos com pouca afinidade se movem mais rapidamente, separando-se ao longo da corrida cromatográfica (HEFTMANN, 2004).

A cromatografia gasosa acoplada à espectrometria de massas (GC/MS) tem sido o método analítico empregado para a determinação de compostos relacionados ao aroma e

compostos tóxicos de cerveja, conforme mostrado nas Tabelas 2 e 4, respectivamente. Após a separação dos analitos na coluna cromatográfica, estes compostos são bombardeados por elétrons dentro do espectrômetro de massas sob condições de vácuo, o que resulta na ionização das moléculas. Os íons formados são instáveis e rapidamente se dividem em fragmentos menores à medida que as ligações químicas são quebradas, em um processo chamado de fragmentação. As massas dos íons e dos fragmentos são então determinadas à medida que os íons são acelerados para fora da fonte. Os íons são separados de acordo com a razão entre a massa (m) e a carga (z) em um campo eletrostático e são subsequentemente medidos com o detector, gerando um espectro de massas (GAUGLITZ; MOORE, 2014; SKOOG; WEST; HOLLER, 2008).

Um composto desconhecido pode ser identificado por comparação de seu espectro de massas ao espectro de massas de uma substância de referência, além da comparação do tempo de retenção do composto presente na amostra com aquela obtido na análise do padrão analítico (HANSEN; PEDERSEN-BJERGAARD, 2015; MELLON, 2003). Quando os padrões analíticos não estão disponíveis, a identificação pode ser feita através da comparação do espectro de massas do composto desconhecido com os espectros de uma biblioteca aliada à determinação dos índices de retenção, calculados a partir da injeção de uma série homóloga de hidrocarbonetos lineares. O índice de retenção de um componente é um número, obtido por interpolação, que relaciona o tempo de retenção do componente em estudo com o tempo de retenção de dois padrões (geralmente hidrocarbonetos) eluídos antes e após o pico do composto de interesse. Dessa forma, a determinação do índice de retenção fornece informação sobre o comportamento de retenção do composto que, ao ser comparada com dados existentes na literatura, pode ser usada para a identificação tentativa de compostos (RUBIOLO et al., 2010).

4. ARTIGOS CIENTÍFICOS

Os materiais e métodos usados nesta dissertação, bem como os resultados obtidos, foram descritos nos artigos científicos apresentados a seguir. No **artigo I**, a adição de uma camada extra de PDMS a uma fibra de SPME comercial de DVB/Car/PDMS foi avaliada com o objetivo de minimizar o efeito de deslocamento do etanol sobre compostos voláteis minoritários verificados em 5 etapas da elaboração de cerveja *lager*.

No **artigo II**, a fibra revestida com PDMS foi aplicada para a quantificação simultânea de compostos carbonílicos e derivados de furano potencialmente tóxicos nas etapas de elaboração de cervejas *ale* e *lager* e em cervejas comerciais. Além disso, a avaliação do risco da exposição aos compostos tóxicos através do consumo das cervejas em estudo foi verificada. Os parâmetros de validação dos respectivos métodos de HS-SPME-GC/MS usados na quantificação dos voláteis e dos compostos tóxicos foram apresentados em ambos artigos.

Artigo I - Matrix-compatible solid phase microextraction coating improves quantitative analysis of volatile profile throughout brewing stages

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Abstract

Ethanol is the major matrix constituent of beer and has been reported as an important interferant during headspace solid phase microextraction (HS-SPME) of minor compounds due to its displacement effect. The addition of a thin hydrophobic and nonporous polydimethylsiloxane (PDMS) layer on a commercial divinylbenzene/Carboxen/PDMS (DVB/Car/PDMS) fiber, which is the most used fiber for beer profiling due to its potential for extracting compounds of different polarities and sizes, was evaluated to minimize the displacement effect caused by ethanol in the quantitative determination of volatile profile of five stages of brewing. The extractive capacity of the PDMS-overcoated fiber was superior to the commercial analogous fiber, since the modified version extracted a greater number of compounds (61 versus 45) and allowed to obtain 20 % more of total chromatographic area than the commercial fiber. The ethanol content of model solutions (0, 4, 8 and 12%) did not result in significant differences in responses neither to polar nor to medium polar or nonpolar analytes when PDMS-overcoated fiber was used. On the other hand, a displacement effect was observed when polar compounds were extracted by the commercial fiber. There was no need to prepare different analytical curves with distinct ethanol levels close to those found in each brewing stage, when PDMS-overcoated fiber, which turn the analytical method simpler, less laborious and time consuming. It showed adequate linearity, sensitivity, repeatability and intermediate precision. A heat map displayed the quantitative differences in the volatile profile of each stage of brewing. Mashing stood out in relation to the others steps

by the highest levels of higher alcohols, such as 2-octenol, 2-heptanol, 1-octen-3-ol, 2-ethyl-1-hexanol, 1-nonanol, 2,3-butanediol and 2-octanol. Boiling was characterized by the highest levels of Maillard reaction products (2-furanmethanol, 2,3-dihydro-benzofuran and tetramethyl pyrazine) and γ-nonalactone, while fermentation, maturation and pasteurization were discriminated by a major presence of esters. Terpenes ~~are~~ were incorporated to the wort during boiling (β-myrcene, linalool, α-humulene, and α-cadinol) or fermentation (τ-cadinol, carvone, nerolidol, farnesol and nerol) and the concentration of these compounds remained similar throughout the subsequent brewing steps.

Keywords: PDMS-overcoated fiber; polydimethylsiloxane, DVB/Car/PDMS, beer, HS-SPME, aroma, flavor, lager, volatile compounds.

1. Introduction

Solid phase microextraction (SPME) is based on the absorption and/or adsorption of analytes in the fiber coating. Several types of coatings with different thicknesses, polarities and pore sizes are commercially available showing great selectivity for different analytes. Some examples of coatings used on the SPME fibers include polydimethylsiloxane (PDMS), polyacrylate (PA), Carboxen® (Car), divinylbenzene (DVB) and carbowax (CW; polyethylene glycol). The choice of fiber coating depends mainly on the nature of the analytes. Mixed coatings are an appropriate option to extract analytes of different physicochemical characteristics (Balasubramanian & Panigrahi, 2011). The mixed-mode coating, DVB/Car/PDMS, is the most used coating to extract a broad range of volatile compounds from complex matrices, such as food and beverages, due to the combination of two different types of adsorption particles, DVB and Car, being held together by PDMS, which yields excellent extraction efficiency, covering analytes in the range from C_2 to C_{20} and molecular weight from 40 to 275 u (Xu et al., 2016). However, SPME of complex matrices may present limitations in both direct immersion (DI) or headspace (HS) modes. In the case of DI mode, the drawback is related to the attachment of matrix components, mainly sugars, amino acids, color compounds and others, onto the coating surface. These macromolecules may cause the fouling of the extraction phase that leads out not only the decrease of extraction efficiency, but also a reduced fiber lifetime (Souza-Silva & Pawliszyn, 2012). This limitation has motivated

the modification of adsorptive SPME fibers through the implementation of an additional PDMS layer onto a commercial fiber (PDMS/DVB) (Souza-Silva, Gionfriddo, Alam, & Pawliszyn, 2017; Souza-Silva, Gionfriddo, Shirey, Sidisky, & Pawliszyn, 2016; Souza-Silva & Pawliszyn, 2012, 2015).

Promising results have been reported in the literature on the use of a thin extra layer of PDMS onto the commercial PDMS-DVB fiber. Since PDMS is a nonporous liquid coating with hydrophobic character, it suffers less from the fouling effect of matrix components, such as sugars, when compared to other coatings (Souza-Silva et al., 2017). The resulting PDMS-PDMS/DVB fiber could be used for 130 extractions of triazole pesticides from grapes, while the commercial fiber had a loss of performance of 80% by the twentieth extraction. Furthermore, the modified fiber did not alter the kinetic and thermodynamic parameters, evaluated through extraction time and rate of mass transfer, respectively (Souza-Silva & Pawliszyn, 2012). The increased fiber lifetime was also verified when the extra PDMS coating was used to extract compounds from various classes, including pesticides, industrial chemicals, and pharmaceuticals, from grape juice (100 extractions were performed with PDMS-overcoated fiber, while the number of extractions was 60 when the commercial fiber was used) (Souza-Silva et al., 2016). The extra layer of PDMS also improved retention capacity for hydrophobic compounds (partition coefficient octanol/water lower than 3) extracted from water, resulting in the reduction of inter-analyte displacement phenomena, which occurred when the commercial analogous fiber was used (Gionfriddo, Souza-Silva, & Pawliszyn, 2015; Souza-Silva et al., 2017). Furthermore, the modified coating also allowed to obtain relative standard deviation (RSD) lower than 20% between extraction replicates of these compounds, even in the presence of pigments (anthocyanins) and high level of sugar (around 20%) found in grape juice, while an RSD of 50-60% was verified in the use of the original fiber (Souza-Silva et al., 2016).

Regarding HS-SPME, a noteworthy drawback may be related to the analyses of alcoholic beverages, such as beer, in which ethanol is the major matrix constituent. In such cases, alcohol has been reported as an important interference in the performance of the extraction due to its displacement effect affecting the extraction of compounds present in minor levels in alcoholic beverages, whenever solid coatings were employed (Conner, Birkmyre, Paterson, & Piggott, 1998; Hartmann, McNair, & Zoecklein, 2002; Pérez-

Olivero, Pérez-Pont, Conde, & Pérez-Trujillo, 2014; Rodríguez-Bencomo, Conde, Rodríguez-Delgado, García-Montelongo, & Pérez-Trujillo, 2002; Whiton & Zoecklein, 2000; Zhang, Pan, Yan, & Duan, 2011).

Minor volatile compounds from the raw materials and produced during fermentation are closely related to the quality and consequently acceptability of beer by the consumers (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014) . Hundreds of compounds, including esters, alcohols, terpenes, aldehydes, ketones and furan derivatives, have been detected in beers (Castro, Ross, & Vixie, 2015; Riu-Aumatell et al., 2014). Understanding the aroma profile of beer is important for the brewing industry, assisting both in the choice of raw material and yeasts, as well as in controlling the quality of the product (Castro et al., 2015).

The use of internal standard (IS) has been the most simple and routine tool to minimize matrix effect in alcoholic beverages, especially in studies focused on wines (Hartmann et al., 2002; Pérez-Olivero et al., 2014; Rodríguez-Bencomo et al., 2012; Zhang et al., 2011) and beers (Nešpor, Karabín, Hanko, & Dostálek, 2018; Ocvirk, Mlinarič, & Košir, 2018). In wine analysis, Rodríguez-Bencomo et al. (2012) selected five internal standards as suitable to minimize the ethanol effect in the quantification of esters found in wines with ethanol levels ranging from 9 to 15%. Similar approach was adopted in the quantification of lactones of wines with the same ethanol percentage, in which the internal standards capable of reducing the ethanol effect were selected (Pérez-Olivero et al., 2014). Conversely, the use of IS alone has shown to be insufficient to minimize the effect of ethanol in the HS-SPME of methoxypyrazines found in wines (5-20% ethanol), since the normalized area (ratio between the areas of the analyte and the IS) decreased with an increase in ethanol concentration (Hartmann et al., 2002). Similarly, concentrations of alcohols, esters, acids and monoterpenes monitored along alcoholic fermentation of wine decreased while ethanol content increased (Zhang et al., 2011). This limitation has been overcome through the preparation of seven calibration curves containing the following ethanol levels of 2, 4, 6, 8, 10, 12 and 14% to mimic the ethanol content found in the samples obtained in different fermentation stages (Zhang et al., 2011). Therefore, the alternatives found to minimize the displacement effect of ethanol in the HS-SPME of volatile compounds has been described especially for wine analysis and include the evaluation of IS (Pérez-Olivero et al., 2014; Rodríguez-Bencomo et al.,

2012) and calibration curves containing the corresponding concentration of ethanol present in the wines (Zhang et al., 2011). In beer analysis, the absence of the compound in the samples under study has been the only criterion for the selection of the IS (Kishimoto, Noba, Yako, Kobayashi, & Watanabe, 2018; Nešpor et al., 2018; Ocvirk et al., 2018), without any previous evaluation of a several possible appropriate IS for each analyte in order to minimize the ethanol effect. In another approach, the evaluation of monoterpenes in wort and beer, Sharp, Steensels and Shellhammer (2017) was carried out through several analytical curves in a model solution of wort (pH 5.0, McIlvaine buffer) and beer (5% v/v ethanol in pH 4.2 citrate buffer) aiming to minimize the matrix effect in the quantification of these compounds. However, this strategy may be laborious and costly.

In this study, a matrix-compatible SPME fiber, comprising a thin PDMS layer added onto a commercial DVB/Car/PDMS fiber was evaluated as a mean to minimize the effect of ethanol in the acquisition of the aroma profile of beer samples obtained throughout the stages of brewing. The effect of the PDMS outer layer on the extraction efficiency toward compounds with a diverse range of polarities, molecular weight, chemical class and aroma contribution was studied using HS-SPME. In addition, the volatile profile of five stages of brewing, including mashing, boiling, fermentation, maturation and pasteurization, was quantitatively evaluated.

2. Materials and methods

2.1. Samples

Samples were provided by a microbrewery from Porto Alegre, Rio Grande do Sul, Brazil. Lager beer was evaluated in this study as it is the most popular type of beer with the largest scale of production and consumption in the world (Cabras & Higgins, 2018). A sample of beer from the lot produced in September 2016 was used to evaluate the effect of the PDMS outer layer on the extraction efficiency. Samples collected after (1) mashing, (2) boiling, (3) fermentation, (4) maturation and (5) pasteurization, as indicated in the scheme of Figure S1 of Supplementary Material, were produced in November 2016. The sample obtained in the last stage of production (step 5, Figure S1) corresponds to beer ready for commercialization and consumption.

Malt (200 kg) was milled (Monster Mill 3 roller, Fayetteville, USA) and transferred to the mashing and filtration vat (stainless steel 304, 1080 L, Gehause, Bento Gonçalves, Brazil). The mashing was carried out by the addition of water at 73 °C and mechanical stirring. Temperature was maintained around 66ºC until total conversion of starch into smaller sugars, such as maltose and maltodextrose. Their presence was verified through the use of a refractometer (Akso, model RHB32, São Leopoldo, Brazil). The filtration vat is composed of a false bottom, where the bagasse is retained, forming a filter medium for the wort. The wort was transferred to the boiling vat (stainless steel 304, 1680 L, Gehause), where it boiled for 90 minutes to decrease the levels of off-flavors (especially dimethylsufide). During boiling, hop (2 kg) was gradually added to promote bitterness, flavor and aroma. After boiling, *Whirlpool* process was performed, which consists of producing a swirl through mechanical agitation (15 minutes)**,** grouping the suspended hop particles and coagulated proteins. After decantation, the wort was pumped through plate heat exchangers (Indupropil, Chiller, São Paulo, Brazil) to the fermentation tank (AGM, 2400 L, Garibaldi, Brazil). Lyophilized *Saccharomyces cerevisiae* yeast (W-34/70, 500 g, Fermentis, Marcq-en-Baroeul, France) was added after hydration and the fermentation occurred at 12 °C for approximately 7 days. The fermentation was monitored daily by the °Brix measurement in a refractometer and was considered finished when the readings remained constant for 3 days, which is equivalent to a density of 1.008 and ethanol 4.5%. After this stage, yeast decantation was done at 5 °C for 3 days. For decantation of the remaining particles, a clarifier (Spindasol, AEB Group, Bento Gonçalves, Brazil) was added and the beer was kept at 0 °C for 5 to 8 days (maturation). During this period, CO_2 (White Martins, Triunfo, Brazil) was added, followed by bottling (600 mL) and pasteurization (62°C for 2 min).

2.2. Analytical reagents, supplies and solutions

Chemical standards (decanoic acid, 1-dodecanol, 1-hexanol, 2,3-butanediol, 2-furanmethanol, 2-phenyl ethyl alcohol, 2-octanone, isoamyl alcohol, acetic acid, benzaldehyde, diethyl succinate, ethyl decanoate, ethyl dodecanoate, ethyl hexanoate, ethyl octanoate, furfural, hexanoic acid, isoamyl acetate, isoamyl alcohol, linalool, methyl hexadecanoate, nerol, nonanoic acid, octanoic acid and phenylethyl acetate) were purchased from Sigma (St. Louis, USA). Individual stock solutions (10,000 mg L^{-1}) of

each compound were prepared in double distilled ethanol. A mix model solution containing all the analytical standards was prepared according to CAMPILLO et al. (2009) using tartaric acid (6 g L^{-1}, Synth, São Paulo, Brazil) in ultrapure water (Millipore purification system, Bedford, MA, USA) and pH adjusted to 4.7 with sodium hydroxide (Nuclear, São Paulo, Brazil). This value corresponds to the mean pH value of the samples evaluated after each stage of brewing: mashing (4.7), boiling (4.4), fermentation (4.4), maturation (4.8) and pasteurization (4.5).

A mix solution containing the following IS: 1,4-cineole (5.0 mg L^{-1}), 2-methyl pentanoic acid (250.0 mg L^{-1}), 3-octanol (12.5 mg L^{-1}), dodecane (5.0 mg L^{-1}), methyl nonanoate (2.5 mg L^{-1}) and phenyl acetate (2.5 mg L^{-1}) was prepared in ethanol (Sigma). Ten microliters of this mix solution were added to 1 mL of the model solution used in analytical curves and beer samples before HS-SPME. IS were chosen having in mind that their chemical class and/or structure should be similar to the volatile compounds evaluated in beer. In addition, former tests were performed to verify their absence in beer samples (data not shown). The characteristics of the compounds used as IS, including chemical class, molecular weight, molecular and structural formula are shown in Table S1.

Sylgard® 184 (PDMS prepolymer and curing agent) was purchased from Dow Corning (Midland, MI, USA) and used to overcoat the commercial fiber. Commercial SPME fibers (DVB/Car/PDMS) of 1 cm were purchased from Supelco (Bellefonte, PA, USA) and conditioned according to the manufacturer's recommendation prior to its first use.

Sodium chloride (NaCl) of analytical grade was purchased from Nuclear (São Paulo, SP, Brazil) and oven dried at 110 °C overnight before use. Headspace vials with magnetic screw caps sealed with polytetrafluoroethylene (PTFE)/silicone septa were purchased from Supelco.

2.3. Preparation of PDMS-overcoated fiber and HS-SPME-GC-MS analysis

The PDMS-overcoating was added to commercial DVB/Car/PDMS fiber according to Souza-Silva et al. (2017) with some modifications as follows: the degassing process of the Sylgard® 184 mixture was performed on ultrasound for 30 min. After, the mixture was stand at room temperature for more 30 min before the coating procedure for

polymerization. For the curing process, the coated fibers were placed into an oven at 50 °C for 12 h.

Samples obtained after fermentation, maturation and pasteurization were degassed by sonication for 5 min using an ice bath (Ultronique, model Q3.0/40A) to keep the sample at 4 °C (preventing the loss of volatile compounds). Samples from the other brewing stages (mashing and boiling) were not submitted to any previous step prior HS-SPME, since CO_2 was not present in these samples. A commercial DVB/Car/PDMS fiber and coating and another one of the same film coated with an extra PDMS coating (named PDMS-overcoated fiber) were used in the HS-SPME as described in item 2.5. In addition, four commercial DVB/Car/PDMS fibers were coated with a thin PDMS film to verify inter-fiber reproducibility, which was assessed by means of comparing the relative standard deviation (RSD) obtained from 12 extractions (triplicate experiments for each fiber). Headspace extraction was performed with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) using 1 mL of sample, 30% of NaCl (m/v), 55 °C as extraction temperature, without agitation. A more detailed description of this procedure is given by Welke et al. (2012).

The volatile compounds were evaluated by a Shimadzu gas chromatograph coupled to a mass spectrometric detector QP 2010S (GC/MS) equipped with a DB-WAX (30 m × 0.25 mm × 0.25 µm) column. Oven temperature was kept at 35 °C for 5 min and it was heated up to 140 °C at a rate of 3 °C min^{-1}, reaching a final temperature of 240 °C at 20 °C min^{-1}. Temperature of injector and detector was 240 °C. Helium (analytical purity 99.999%, Linde Gases, Canoas, RS, Brazil) was used as carrier gas with at flow rate of 1 mL min^{-1}. Desorption was made in splitless mode. Electron ionization was performed at 70 eV and the mass range was 45 to 450 in full-scan acquisition mode.

Compounds were positively identified by comparing the mass spectra of analytical standards with the spectra found in samples. For unavailable standards, tentative identification of beer volatile compounds was performed by comparing their experimental retention indices (RI_{exp}) with RI reported in scientific literature (RI_{lit}). For calculation of RI_{exp}, retention data of a series of *n*-alkanes (C_9–C_{24}, Supelco, Bellefonte, PA) obtained under the same chromatographic conditions was employed for the chromatographic analyses of beer volatiles. A compound was considered tentatively identified when experimental and reported RI did not differ by more than 10 units and when similarity

between mass spectrum of each chromatographic peak and spectrum of NIST Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, USA) was at least 80%.

Quantitative data were obtained using the internal standard method after normalization of the peak area of each compound in relation to an IS, as described in section 2.2. Method validation was evaluated through linearity, recovery, intermediate precision, repeatability, limit of quantification (LOQ) and detection (LOD). The lowest, intermediate and highest concentrations of the analytical curve of each compound were used to determine recovery, repeatability and precision parameters. Repeatability was obtained by the coefficient of variation (CV) of four independent assays performed under the same analytical conditions on the same day and intermediate precision was calculated by the CV of independent assays performed under the same analytical conditions in four different days.

Odor threshold (OT), defined as the lower concentration of an odoriferous compound that all members of a sensory panel can recognize, was used to estimate the possible sensory contribution of the volatile compounds to the beer aroma. A volatile compound contributes to aroma when its concentration in beer is above the perception threshold (Grosch, 2001). Odor activity value (OAV) was calculated using the ratio of the concentration of each compound and its odor threshold value, which is available in the scientific literature.

The effect of the addition of an extra PDMS layer to a commercial fiber was evaluated in relation to the extraction time (15, 30, 45, 60, 90 and 120 min) and ethanol content (0, 4, 8 and 12%) through monitoring the chromatographic areas of 12 compounds of different polarities defined according to the partition coefficient (log P). Log P is the logarithmic value defined as the ratio of the concentration of a compound in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. Compounds with log P ranging from -0.2 to 7.4 were evaluated as listed in Table 1. In order to facilitate the discussion of the results, we classified the compounds as follows: polar ($\log P < 2$), medium polar ($2 < \log P < 4$) and nonpolar ($\log P > 4$). Compounds that positively and negatively influence the aroma were chosen as representatives of each of these three classes of polarity, as shown in Table 1. These compounds were added to the model solution prepared as described in item 2.2. Furthermore, the volatile profile of a

lager craft beer was evaluated by the PDMS-overcoated fiber and the commercial analogous to compare the number of volatile compounds and total chromatographic area obtained by both fibers.

2.4. Statistical analysis

The statistical analysis was performed using XLSTAT2017 (Addinsoft, New York, USA) for Microsoft Excel. The analysis of variance (ANOVA), followed by the Tukey's test was used to verify if there was significant difference between the chromatographic area of analytes extracted from matrices with different concentrations of ethanol. Student's t-test was employed to compare chromatographic areas of compounds extracted by the PDMS-overcoated and commercial fibers according to different extraction times.

Fisher ratio was used to determine the features that best described the data of volatile profile in terms of discriminative power between the brewing steps and also to reduce the dimension of the original variables before performing hierarchical clustering analysis (HCA) and heat map. Fisher ratios were calculated according to the approach previously applied by Welke et al. (2014), using Excel software, considering the ratio between the variance of the concentration levels of a compound verified in the different brewing steps and within each step. The compounds with the highest values of Fisher ratios were used in HCA and heat map.

3. Results and discussion

In the first step of this study, the effect of extraction time and alcohol content on the chromatographic areas of twelve representative beer volatile compounds were evaluated, as presented in Table 1, were verified using the original DVB/Car/PDMS fiber and the PDMS-overcoated version of this commercial fiber. These two types of fibers were also used to evaluate the volatile profile of a lager craft beer, in which the number of volatile compounds and total chromatographic area obtained by both fibers were compared. Finally, the volatile compounds were quantitatively monitored along the five steps of lager craft brewing using the PDMS-overcoated fiber.

3.1. Effect of extraction time on fiber performance

Figure 1 shows the chromatographic area of the volatile compounds evaluated using the commercial and PDMS-overcoated fibers during 15, 30, 45, 60, 90 e 120 min of extraction. Differences in chromatographic areas obtained by both fibers for each extraction time are discussed according to the polarity of analytes. The *p*-values obtained with t-test and used to compare both fibers at each extraction time are presented in Table S2.

Polar compounds (log P < 2, Table 1, acetic acid, 2-furanmethanol, isoamyl alcohol and benzaldehyde) present lower affinity to PDMS (nonpolar) than medium polar and nonpolar compounds. In the case of commercial fiber, these compounds bearing low molecular weight and high polarity pass quickly through the large pores of the DVB, which is the first layer of this fiber, to be then adsorbed by the CAR layer (Souza-Silva et al., 2017). However, the amounts of polar compounds extracted by both fibers were not statistically different when extraction times were 45, 60, 90 and 120 min, according to the t test ($p > 0.05$). Differences were found only before reaching equilibrium (15 and 30 min of extraction), when area counts were lower for the extraction with the overcoated fiber (Figure 1).

For medium polar compounds (2 > log P > 4)isoamyl acetate, linalool, nonanoic acid and ethyl octanoate) and nonpolar compounds (log P > 4, decanoic acid, ethyl decanoate, ethyl dodecanoate, methyl hexadecanoate) (Table 1), the additional PDMS overcoat resulted in increased fiber extraction capacity, although isoamyl acetate and linalool (log P 2.3 and 3.0, respectively) were exceptions, as the amount extracted of these compounds by both fibers presented no statistically significant differences (Table S2).

The time to reach extraction equilibrium for both fibers was 60 min for medium and nonpolar compounds, although polar compounds reached equilibrium in 45 min. Having in mind that after reaching equilibrium, the fiber does not accumulate more analytes, 60 min was chosen for further experiments (Pawliszyn, 2012).

3.2. Effect of ethanol concentration on fiber performance

Figure 2 shows the normalized chromatographic areas for the studied volatile compounds extracted by PDMS-overcoated and commercial fibers according to the ethanol levels in the matrix. When a commercial fiber was used, the chromatographic

areas of the polar compounds (log P < 2) were significantly lower ($p < 0.05$) for the beer model solutions containing ethanol in comparison to alcohol-free matrix. The result of ANOVA followed by the Tukey test is shown in Table S3. For example, the responses for acetic acid and 2-furanmethanol (most polar compounds studied, log P = - 0.20 and 0.77, respectively) obtained from model solutions containing 8 or 12% ethanol were significantly lower than those obtained with the solutions containing 4% ethanol. The response of isoamyl alcohol (log P = 1.20), with the 12% ethanol solution was significantly lower than those obtained from the matrices containing 8 and 4% of ethanol. Conversely, benzaldehyde (log P = 1.48) did not present significant differences in the responses obtained when the solutions containing 4, 8 and 12% of ethanol were compared. Ethanol appears to effectively cause the displacement of lower molecular weight (MW < 107 g mol^{-1}) and polar (log P < 2) compounds as could be seen when the commercial fiber was used, including acetic acid, 2-furanmethanol, isoamyl alcohol and benzaldehyde. This phenomenon occurs in the CAR layer, due to its pore size capable of adsorbing compounds of molecular weight lower than 150 g mol^{-1} (Pawliszyn, 2012).

For medium polar and nonpolar compounds, the ethanol content of the model solutions did not result in significant difference in normalized chromatographic areas, when extracted by commercial fiber. Similarly, the ethanol content of model solutions did not result in significant differences in responses neither to polar nor to medium polar or nonpolar analytes when PDMS-overcoated fiber was used. It means that the PDMS-overcoated fiber presents a significant advantage on this regard, since beers without ethanol (< 0.5%) and/or containing different concentrations of ethanol (ranging from 0.5 to greater than 10%; typical concentration in beers: 3.5 - 5% according Preedy (2009), or samples of different brewing stages may be evaluated with the same analytical approach using only one single analytical curve, no matter the ethanol content of the standard solutions of the analytical curve. The current way of circumventing this analytical problem consists in using different analytical curves, each one containing distinct ethanol concentration, procedure that is much more labourious and time consuming. It should be noted that the standard deviation between replicates was higher for commercial fiber than those verified for PDMS-overcoated fiber (Table S3). The water vapor formed during the extraction due to the heating of the sample results in droplets that can remain on the surface of the fiber and consequently interfere in the repeatability of the area of the

compounds extracted by the commercial fiber. These water particles are repelled from the surface in the case of the overcoated fiber, due to the hydrophobic nature of PDMS.

Images of commercial and PDMS-overcoated fibers are shown in Figure S2A and S2B, respectively. The extra PDMS layer presented a uniform and smooth coverage throughout the coating (Figure S2B). A zoom of the irregular junction of the polymeric coating with the commercial fiber metallic portion is shown in Figure S2C. On the contrary, the proper sealing of the upper extremity of the coating and the metal junction was achieved with the PDMS-overcoated fiber (Figure S2D). This difference regardig sealing of the coating and metal junction may have been of paramount importance for the achievement of suitable RSD values, as no porosity related to DVB e CAR particles would have been exposed during extraction. Consequently, displacement of compounds on this part of the fiber coating that may occur with the commercial fiber would not happen with the PDMS-overcoated analogue (Figure S2B). RSD values lower than 13% were obtained in the evaluation of inter-fiber reproducibility for the twelve representative beer volatile compounds shown in Table 1 for the PDMS-overcoated fiber.

3.3. Use of the PDMS-overcoated and commercial fiber to profiling analysis of beer

Table 2 and Table S4 show the volatile profile of a lager beer obtained through HS-SPME-GC/MS using PDMS-overcoated and commercial DVB/Car/PDMS fibers. Compounds were listed according to polarity (polar, medium polar and nonpolar) and in ascending order of log P. The extractive capacity of the PDMS-overcoated fiber was superior to the commercial version, which was verified by the number of extracted compounds (61 and 45, respectively) and by a 20% increase in the total chromatographic area for the PDMS-overcoated fiber compared to the commercial version.

Regarding polar compounds, the extra PDMS layer did not affect the number of extracted analytes, since both fibers provided 18 compounds pertaining to this class. Therefore, the addition of a PDMS layer to a DVB/Car/PDMS fiber has not prevented the absorption and/or adsorption of polar compounds, which have less affinity for PDMS, although the chromatographic areas of these compounds was lower when coated fiber was used in comparison to commercial fiber. (seria interessante especificar a faixa de redução de área cromatográfica para dar noção se é muito ou pouco?)

The best performance of the PDMS-overcoated fiber compared to commercial fiber was verified mainly in relation to nonpolar compounds (18 and 8 extracted compounds, respectively) and medium polar (25 and 19, extracted compounds, respectively). These results proved that medium polar and especially nonpolar compounds have greater affinity ~~with~~ for the extra PDMS layer. Among the compounds exclusively extracted by the PDMS-overcoated fiber (16), 10 compounds (63%) are nonpolar and are listed in increasing order of log P (# refers to the numbers of compounds listed in Tables 3, S4 and S5): 2-undecanol (#46), α-humulene epoxide (#47), ethyl nonanoate (#51), τ-cadinol (#53), β-farnesene (#54), farnesol (#56), methyl hexadecanoate (#58), methyl 9-octadecenoate (#59), ethyl 9-hexadecenoate (60), and ethyl hexadecanoate (#61). The remaining 6 components (37%) are medium polar, including 2-octanone (#25), heptanoic acid (#26), ethyl cinnamate (#27), 2-ethyl-hexanoic acid (#28), carvone (#34), and octyl acetate (#42).

Even though olfactometric analyses are important to accurately define the contribution of each compound to the beer aroma, some considerations can be made, based on literature data as reported in Table S5**,** to show the importance of the evaluation of compounds exclusively extracted by PDMS-overcoated fiber. Ethyl nonanoate (#51, odor described as fruity), methyl hexadecanoate (#58, fruity), methyl 9-octanoate (#59, green/sweet), ethyl 9-hexadecenoate (#60, fruity), ethyl hexadecanoate (#61, fruity) ethyl cinnamate (#27, strawberry/sweet) and octyl acetate (#42, orange blossoms/jasmine) were esters extracted exclusively by PDMS-modified fiber. Esters are the largest group of flavour-active compounds and are highlighted mainly for the fruity, floral and/or sweet notes of aroma and therefore they constitute an important group of compounds with positive influence on beer quality (Ocvirk et al., 2018).

Terpenes including τ-cadinol (#53, herb/woody), β-farnesene (#54, fruity), farnesol (#56, floral), α-humulene epoxide II (#47, herbal) and carvone (#34, minty/caraway) were also extracted only by the PDMS-modified fiber. These compounds are derived from hops and can impart organoleptic properties to beer (Priest & Stewart, 2006). Among the other compounds detected exclusively by the PDMS-overcoated fiber, 2-octanone (#25) presents a negative contribution for sensory perception with an aroma described as earthy (Feng et al., 2015), and 2-undecanol (#46) presents odor characterized as fruity. However, the presence of heptanoic acid (#26) and 2-ethyl-hexanoic acid (#28) may be undesirable,

since both acids may present a sweaty odor (Malfondet, Gourrat, Brunerie, & Le Quéré, 2016).

3.4. Quantitative evaluation of the volatile profile of the stages of brewing

Table 3 shows the performance of the method used for quantification of volatiles present in different brewing stages, using a PDMS-overcoated fiber, covering compounds of different chemical classes and polarities. The calibration curves showed adequate linearity with determination coefficients (r^2) ranging from 0.98 for methyl hexadecanoate to 0.99 for other compounds. Limits of detection and quantification demonstrated the adequate sensitivity of the method. Benzaldehyde and ethyl dodecanoate presented the lowest LOD (0.001 mg L^{-1}) among the evaluated compounds and the lowest LOQ value was found for both octanoic acid, ethyl dodecanoate, ethyl hexanoate and ethyl octanoate (0.05 mg L^{-1}). The relative standard deviation obtained in repeatability and intermediate precision assays were lower than 12.4 and 14.9%, respectively, and recoveries ranged from 92 to 100 %, therefore, demonstrating the efficiency of the proposed method.

Table S5 shows quantitative data for 76 compounds, listed according to chemical classes, that were either positively (18) or tentatively (58) identified throughout the brewing stages, in addition to odor description and experimentally acquired RI, as well as from literature. A total of 23 esters, 20 alcohols, 11 terpenes, 9 acids, 4 phenols, 2 furan compounds, 2 lactones, 2 ketones, 1 pyrazine, 1 aldehyde and 1 sulfur compound were found. In a lager beer produced with extruded corn starch evaluated by He et al. (2018), esters comprised the major class of compounds, followed by alcohols and acids, found, which was used as an adjunct to increase the fermentable sugar content. Riu-Aumatell et al. (2014) have also found esters as majores compounds in beers (the type of beer was not mentioned), followed by acids and alcohols. Conversely, terpenes were present in Brazilian lager and ale beers as major components, followed by esters and alcohols Alvim et al. (2017). Several factors may justify such divergences between the majority classes identified in the study sample and those evaluated by Riu-Aumatell et al. (2014), He et al. (2018) and Alvim et al. (2017), such as differences in the raw material (type of cereal, origin and proportion of malt used in the formulation), yeast strain, process conditions (adjuvants, time, temperature, amount produced), among others.

Fisher ratios were calculated taking into account the levels of the 76 volatile compounds found in the five stages of brewing and are shown in Table S5. Compounds with the highest Fisher ratios are the ones that contributed the most to differentiate the samples. Forty-eight compounds that presented Fisher ratio corresponding to at least 15% of the Fisher ratio value of the most discriminant compound (2-octenol, Fisher ratio: 399) were selected to plot a heat map with hierarchical clustering. This approach has been successfully applied in previous studies, such us for example discrimination of base and sparkling wines (Welke et al., 2014), sparkling wines produced with free and immobilized yeasts (Costa, Nicolli, Welke, Manfroi, & Zini, 2018) and Merlot wines produced following different canopy managements (Nicolli et al., 2018) in previous studies.

The heat map (Figure 3) aids to visualize the differences in the volatile profile of each stage of brewing, where red, orange and yellow colors represent higher, medium and lower levels of volatile compounds, respectively. Clusters related to the grouping of volatiles were designated from 1 to 8 in vertical axis as indicated by the green lines in Figure 3. In addition, the differentiation of the five stages of brewing according to the volatile profile is clear on the horizontal axis and the discussion was based on the compounds found in higher levels in each stage.

The compounds found in highest levels after mashing were grouped in cluster 1. It is important to note that only higher alcohols (those with higher molecular weight than ethanol), such as 2-octenol (#74, 0.18 mg L^{-1}), 2-heptanol (#69, 0.20 mg L^{-1}), 1-octen-3-ol (#71, 0.17 mg L^{-1}), 2-ethyl-1-hexanol (#73, 0.21 mg L^{-1}), 1-nonanol (#75, 0.16 mg L^{-1}), 2,3-butanediol (#1, 2.3 mg L^{-1}) and 2-octanol (#70, 0.16 mg L^{-1}) stood out for the differentiation of this brewing step in relation to the others, since the levels of these compounds decreased after boiling (values were lower than 0.34 mg L^{-1} for 2,3-butanediol and 0.14 mg L^{-1}, that is the LOQ, for other compounds), which is the next stage of brewing (Table S5).

These higher alcohols are formed either by anabolism or catabolism (Ehrlich pathway) of amino acids (Hughes & Baxter, 2001). A decrease of their levels occurred after the boiling step may be due to their volatility and also due to reactions they undergo to form other compounds. For example, oxidation of 2-octanol (#70) results in 2-octanone (#25, which appears in the cluster 7) (Stewart, Russell, & Anstruther, 2018), since was found in higher level (0.38 mg L^{-1}) after the fermentation stage.

Boiling resulted in the highest levels of 2-furanmethanol (#97, 0.28 mg L^{-1}), 2,3-dihydro-benzofuran (#98, 0.36 mg L^{-1}), tetramethyl pyrazine (#10, 0.32 mg L^{-1}), and γ-nonalactone (#95, 0.29 mg L^{-1}), which are in cluster 3. Furans and pyrazines are Maillard reaction products formed during heating, through the reaction between amino acids and reducing sugars, while γ-nonalactone (#95) is an oxidation product of polyunsaturated fatty acids by malt lipoxygenase (Priest & Stewart, 2006). These compounds may come from the processing of cereal malting and are released into the wort in the boiling process (Yu et al., 2014). Drying and/or roasting of the malt, which are the thermal steps of malting, had not been evaluated in this study, since this step is not done in the brewery. Concentrations of these compounds decreased to levels lower than the LOQ of the method (0.25 mg L^{-1} for the furans, the pyrazine and the lactone) throughout brewing. It should be noted that their odors are described as socks/musty/earthy/feet, milk/cream and baked potato/nutty for 2-furanmethanol (#97), 2,3-dihydro-benzofuran (#98) and tetramethyl pyrazine (#10), respectively (Table S5) respectively. They have been considered as defects for the sensorial quality of beer. Similary, γ-nonalactone (#95), although it has an odor described as sweet by Kishimoto et al. (2018) it has also been considered as a negative contribution to beer aroma by the Beer Judge Certification Program (BJCP, 2018).

Compounds that reached their highest levels after both mashing and boiling stages, including four acids [9-decenoic (#85), hexanoic (#83), octanoic (#84), and decanoic (#45)] and two alcohols [2-phenylethyl alcohol (#79) and 1-decanol (48)] are in cluster 2. The levels of 1-decanol found after mashing and boiling (0.18 mg L^{-1} in both stages) decreased in the following stages of brewing (levels lower than 0.14 mg L^{-1} were found after fermentation, maturation and pasteurization) which may decrease the perception of floral notes, since these compounds have this aroma description according to Table S5. The concentration of the four acids and 2-phenylethyl alcohol (#79) found after boiling (0.39, 0.15, 0.17, 0.65 and 0.31 mg L^{-1}, respectively) decreased to levels lower than their respective LOQ [0.05 mg L^{-1} for hexanoic (#83) and octanoic acids (#84); 0.25 mg L^{-1} for 9-decenoic acid (#85), decanoic acid (#45) and phenylethyl alcohol (#79) at the end of brewing stages. On the other hand, the concentration of their respective ethyl esters presented higher levels in the subsequent steps. Ethyl 9-decenoate (#50) was found in higher concentration after fermentation (0.37 mg L^{-1}) (cluster 7), while ethyl decanoate

was found at major levels after maturation (0.14 mg L^{-1}) (cluster 5). Finally, ethyl hexanoate (#30, 2.39 and 2.26 mg L^{-1}), ethyl octanoate (#41, 16.37 and 15.86 mg L^{-1} and β-phenylethyl acetate (#63, 1.58 and 1.63 mg L^{-1}) were highlighted in cluster 6 due to the higher concentration of these compounds both after maturation and pasteurization. Among these compounds, only ethyl hexanoate and octanoate were found in beer at levels higher than their odour thresholds (0.16 and 0.29 mg L^{-1}, respectively) as shown in Table S5. The mechanism of ester formation during the brewing steps is well known and involves the condensation of acids and ethanol. It may result in a positive change in the aroma because the fatty/rancid character of these acids is then replaced to the fruity characteristics of their corresponding esters (Hughes & Baxter, 2001). β-Phenylethyl acetate results from the condensation of acetic acid and 2-phenylethyl alcohol, both the ester and the alcohol presenting a floral contribution to beer aroma (Stewart et al., 2018).

Also with respect to esters, these compounds were the predominant in clusters 5, 6 and 7. The major esters after maturation are grouped in the cluster 5, such as ethyl decanoate (#44, already mentioned above) and other ethyl esters, like dodecanoate (#55, 0.44 mg L^{-1}, sweet, floral, fruity aroma), 9-hexadecenoate (#60, 0.31 mg L^{-1}, odor not described in literature) and octadecenoate (#67, 0.09 mg $L^{-1,}$ odor not described in literature). In addition, isoamyl octanoate (#35, 0.11 mg L^{-1}, fruity odor), β-phenylethyl butyrate (#65, 1.58 mg L^{-1}, rose odor), and methyl 9-octadecenoate (#59, 0.25 mg L^{-1}, odor not described in the literature). Their concentrations decrease after pasteurization, which may lessen the perception of fruity and floral notes described for these compounds according to Table S5. The temperature of the pasteurization may have been responsible for the decrease in the levels of these compounds (Hughes & Baxter, 2001).

The levels of compounds found in similar concentration after maturation and pasteurization are in cluster 6 (their concentrations found in these steps are mentioned between parameters as follows), including four ethyl esters: hexanoate (#30, 2.39 and 2.26 mg L-1), heptanoate (#37, 0.92 and 0.90 mg L-1), octanoate (#41, 16.37 and 15.86 mg L-1) and 4-decenoate (#62, 0.06 and 0.05 mg L-1), diethyl succinate (#8, 0.20 and 0.19 mg L-1), β-phenylethyl acetate (#63, 1.58 and 0.93 mg L-1), acetic acid (#4, 358.96 and 302.20 mg L-1), and 4-ethyl guaiacol (#93, 1.34 and 1.30 mg L-1). Among these compounds, only the odor attributed to acetic acid and 4-ethyl guaiacol are not desired in beer, as these compounds have a contribution described as vinegar, and clove,

respectively and were found at higher levels than their respective odor threshold (130 and 0.12 mg L-1 as specified in Table S5). Both acetic acid and 4-ethyl guaiacol are products microbiological contamination caused by Acetobacter and Bretanomyces species, respectively. Proper practices of sanitizing equipment and utensils used in brewing must be adopted to reduce the occurrence of these contaminants (Cabras & Higgins, 2018; Lentz, 2018).

The compounds found in higher levels after fermentation and whose concentrations decreased in the subsequent brewing stage (maturation) are in cluster 7: isoamyl acetate (#22, 5.60 decreased to 2.51 mg L^{-1}, banana odor), ethyl 9-decenoate (#50, from 0.37 to < 0.05 mg L^{-1}, rose), ethylphenyl propanoate (#64, from 0.48 to 0.27 mg L^{-1}, floral), phenoxyethanol (#77, from 0.32 to 0.25 mg L^{-1}, floral) and 2-octanone (#25, from 0.36 for 0.02 mg L^{-1}, earthy). Among these descriptions of odor, only floral aroma is desirable for lager beer according BJCP (2018). Decantation of the yeast performed trough the addition of ~~the~~ a clarifier may be suggested as the step responsible for a reduction of the levels of these compounds, as they may be adsorbed on yeast cell wall and/or other particles that decant and are eliminated from the process (Stewart et al., 2018).

Finally, cluster 4 and cluster 8 contains only terpenes. Cluster 4 is formed by β-myrcene (#86), linalool (#32), α-humulene (#87), and α-cadinol (#52) (levels around 0.25 mg L^{-1}) released into the wort from the hop added during boiling and that were also detected at similar levels during subsequent steps of the brewing (levels ranging from 0.25 to 0.28 mg L^{-1}). Among these terpenes, only α-humulene was not detected after pasteurization, probably due to the oxidation of the carbon-carbon double bond of this terpene, that results in the formation of α-humulene epoxide (Hughes & Baxter, 2001), which can be seen after pasteurization (cluster 6, Figure 3), 0.25 mg L^{-1} in Table S5). The role of epoxidation reaction in beer flavor is not yet fully understood, although Rettberg et al. (2018) suggested that α-humulene presents spicy odor and the epoxidized analogue has aroma contribution described as hay-like.

Other terpenes [τ-cadinol (#53), carvone (#34), nerolidol (#90), farnesol (#91) and nerol (#89)] have probably been found only after fermentation (cluster 8) because they are released into the wort during boiling in the form of glycosylated compounds and the yeast enzymes added in the fermentation are capable of breaking the glycosidic bond, rendering these free terpenes and analyzable by HS-SPME-GC/MS (Rettberg, Biendl, &

Garbe, 2018). The levels of all these terpenes ranged from 0.25 to 0.29 mg L^{-1} and only the presence of nerolidol (rose, apple, green, citrus), farnesol (floral) and nerol (citrus, rose, fresh) may be positive to sensory characteristics of beer due to their pleasant notes of aroma. However, these compounds were detected in beer at levels below their odor thresholds (1, 2.4 and 0.4 mg L^{-1} nerolidol, farnesol and nerol, respectively, as presented in Table S5).

Twelve compounds found in beer showed OAV > 1, which correspond to 17.9% of total detected compounds (Table S5). Linalool (# 32) showed the highest OAV [250.00, floral aroma (Kishimoto et al., 2018)], followed by ethyl cinnamate [# 27 in table S5, OAV 150.00, acid red fruits and banana aroma (Guyot-Declerck, François, Ritter, Govaerts, & Collin, 2005)], ethyl octanoate [# 41 in table S5, OAV 54.70, fruity aroma (Kishimoto et al., 2018)] and 2-undecanol [# 48 in table S5, OAV = 36.58, fruity aroma (Qian & Wang, 2005)]. Linalool, like other terpenes, is incorporated into the wort after boiling, whereas esters and alcohols are mainly formed after fermentation.

4. Conclusion

The addition of a thin layer of PDMS on a commercial DVB/Car/PDMS fiber ~~shows~~ proved to be a promising alternative in mitigating the ethanol displacement effect on HS-SPME of volatiles from samples taken throughout brewing stages. This modification improved the extraction capacity of the SPME fiber (higher number of extracted compounds and total chromatographic are) and may be a useful tool in quantitative profile analysis of other alcoholic beverages. Moreover, the validation parameters of the method, including linearity, sensitivity, repeatability and intermediate precision disclosed the adequate capacity of PDMS-overcoated fiber in extracting compounds of different polarities and molecular weights.

The use of the overcoated fiber allowed a quantitative evaluation of each compound found in samples of different brewing stages presenting different ethanol concentrations, using only one calibration curve for each component, providing a simpler, less laborious and time consuming analytical method. Fisher ratios and a heat map helped verifying which were the volatiles that presented the highest concentrations in each brewing step. Mashing stood out in relation to the others steps due to the highest levels of higher

alcohols and boiling was characterized by the highest levels of Maillard reaction products. Esters were the major compounds after fermentation, maturation and pasteurization, while terpenes were incorporated to the must during boiling or fermentation

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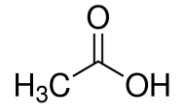
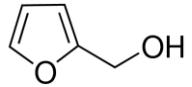
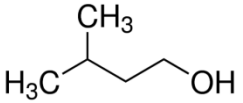
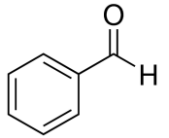
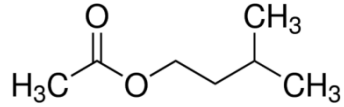
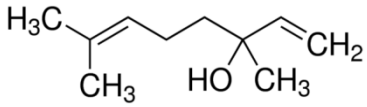
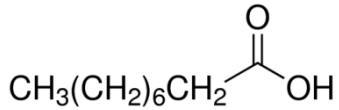
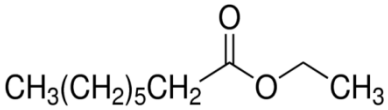
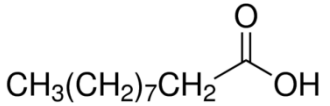
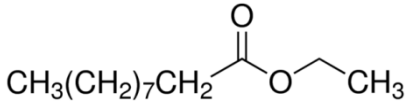
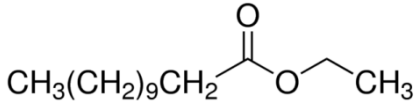
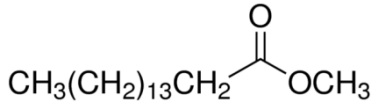
Table 1. Characteristics of the polar, medium polar and nonpolar compounds used in the evaluation of the extraction time and ethanol content of the PDMS-overcoated version of DVB/Car/PDMS and the original fiber.

Compound	CAS[a]	MW ($g\ mol^{-1}$)[b]	log P[c]	Odour description[d]	Aroma contribution[e]	Structural formula
Polar (log P < 2)						
Acetic acid	64-19-7	60.052	-0.20	Acidic, vinegar [1]	Negative	
2-Furanmethanol	98-00-0	98.101	0.77	Burnt, unpleasant, sweaty [2]	Negative	
Isoamyl alcohol	123-51-3	88.150	1.20	Floral [3]	Positive	
Benzaldehyde	100-52-7	106.124	1.48	Nutty [4]	Negative	
Medium polar (2 < log P < 4)						
Isoamyl acetate	123-92-2	130.187	2.30	Solvent [3]	Negative	
Linalool	78-70-6	154.253	3.00	Floral, citrus [3]	Positive	

Nonanoic acid	112-05-0	158.241	3.42	Cheesy [1]	Negative	
Ethyl octanoate	106-32-1	172.268	3.81	Fruity[1]	Positive	
Nonpolar (log P > 4)						
Decanoic acid	334-48-5	172.268	4.10	Rancid, sweaty [3]	Negative	
Ethyl decanoate	110-38-3	200.322	4.90	Fruity [1]	Positive	
Ethyl dodecanoate	106-33-2	228.376	5.71	Fruity [1]	Positive	
Methyl hexadecanoate	112-39-0	270.457	7.40	Fruity [5]	Positive	

[a] CAS: Chemical Abstracts Service; [b] Molecular weight, [c] Octanol-water partition coefficient, [d] Odour described by: [1] Niu et al. (2017); [2] Chin et al. (2011); [3] Kishimoto et al. (2006); [4] Sheibani et al. (2016); [5] Nicolli et al. (2018).

Table 2. Volatile profile of a lager beer (lot produced in September 2016 by a microbrewery from Porto Alegre, Rio Grande do Sul, Brazil) obtained through HS-SPME-GC/MS using PDMS-overcoated and commercial fibers. Compounds were listed according to polarity (polar, medium polar and nonpolar) and in ascending order of log P. Retention indices used in tentative identification are shown in Table S4 of Supplementary Material. Experimental conditions are described in section 2.4.

#	Compound	CAS[a]	t_R(min)[b]	log P[c]	Area ± SD[d]	
	Polar (log P < 2)				**Overcoated**	**Commercial**
1	2,3-Butanediol	513-85-9	29.64	-0.92	$4.0\ 10^5 \pm 1.0\ 10^4$	$5.7\ 10^5 \pm 1.5\ 10^4$
2	3-Methyl-2-cyclopenten-1-one	80-71-7	28.29	-0.43	$4.2\ 10^4 \pm 1.2\ 10^3$	$5.3\ 10^4 \pm 4.2\ 10^3$
3	3-Hydroxy-2-butanone	513-86-0	18.57	-0.36	$6.2\ 10^4 \pm 1.1\ 10^4$	$7.5\ 10^5 \pm 6.2\ 10^4$
4	Acetic acid [e]	64-19-7	26.08	-0.17	$5.4\ 10^5 \pm 1.0\ 10^4$	$6.8\ 10^5 \pm 2.6\ 10^4$
5	3-(Methylthio)-1-propanol	0505-10-2	36.52	0.50	$4.6\ 10^4 \pm 1.9\ 10^3$	$5.6\ 10^4 \pm 4.2\ 10^3$
6	Butanoic acid	107-92-6	33.29	1.07	$3.0\ 10^4 \pm 1.3\ 10^3$	$3.9\ 10^4 \pm 2.0\ 10^3$
7	Isoamyl alcohol	123-51-3	15.42	1.16	$1.9\ 10^7 \pm 6.0\ 10^5$	$2.3\ 10^7 \pm 6.7\ 10^5$
8	Diethyl succinate	123-25-1	35.09	1.20	$2.4\ 10^4 \pm 1.0\ 10^2$	$1.2\ 10^3 \pm 1.2\ 10^2$
9	2-Methyl-2-cyclopenten-1-one	1120-73-6	21.97	1.26	$8.9\ 10^4 \pm 1.8\ 10^3$	$1.9\ 10^4 \pm 3.2\ 10^3$
10	Tetramethyl-pyrazine	1124-11-4	26.68	1.28	$9.8\ 10^4 \pm 3.5\ 10^3$	$1.0\ 10^5 \pm 4.3\ 10^3$
11	2-Hexanone	591-78-6	9.13	1.38	$6.1\ 10^4 \pm 3.5\ 10^3$	$6.4\ 10^4 \pm 3.9\ 10^3$
12	Benzaldehyde [e]	100-52-7	28.65	1.48	$3.5\ 10^4 \pm 2.0\ 10^3$	$5.2\ 10^4 \pm 4.4\ 10^3$
13	3-Hexanone	589-38-8	7.97	1.49	$3.1\ 10^4 \pm 1.9\ 10^1$	$3.4\ 10^4 \pm 1.9\ 10^3$
14	1-Pentanol	71-41-0	17.13	1.51	$4.2\ 10^4 \pm 3.2\ 10^3$	$6.4\ 10^4 \pm 2.7\ 10^4$
15	3-Methyl-cyclopentanol	18729-48-1	21.02	1.56	$2.9\ 10^4 \pm 2.5\ 10^3$	$4.7\ 10^4 \pm 8.9\ 10^3$
16	3-Hexanol	623-37-0	14.82	1.65	$2.4\ 10^4 \pm 3.0\ 10^3$	$4.4\ 10^4 \pm 4.4\ 10^3$
17	2-Hexanol	626-93-7	15.90	1.76	$2.1\ 10^4 \pm 1.2\ 10^3$	$3.4\ 10^4 \pm 6.2\ 10^3$
18	3-Methyl-butanoic acid.	503-74-2	34.86	1.98	$2.6\ 10^5 \pm 5.8\ 10^3$	$2.6\ 10^5 \pm 2.3\ 10^4$
	Area (compounds log P < 2)				**$2.1\ 10^7$**	**$2.6\ 10^7$**
	Number of compounds (log P < 2)				**18**	**18**

	Medium polar (2 < log P < 4)				Overcoated	Commercial
19	1-Hexanol [e]	111-27-3	21.73	2.03	$1.8\ 10^5 \pm 1.1\ 10^4$	$1.4\ 10^5 \pm 1.2\ 10^4$
20	Dihydro-5-pentyl-2(3H)-furanone	104-61-0	43.39	2.08	$4.4\ 10^5 \pm 2.7\ 10^4$	$5.1\ 10^5 \pm 2.9\ 10^4$
21	2-Methoxy-4-vinylphenol	7786-61-0	44.73	2.24	$1.2\ 10^5 \pm 3.4\ 10^3$	$1.7\ 10^5 \pm 9.8\ 10^3$
22	Isoamyl acetate [e]	123-92-2	10.87	2.25	$2.6\ 10^6 \pm 6.5\ 10^4$	$8.0\ 10^5 \pm 7.8\ 10^4$
23	Ethyl phenylacetate	101-97-3	38.97	2.28	$4.6\ 10^4 \pm 2.7\ 10^2$	$4.5\ 10^4 \pm 2.9\ 10^3$
24	2-Phenylethyl acetate	103-45-7	40.02	2.30	$1.4\ 10^7 \pm 4.0\ 10^5$	$1.3\ 10^7 \pm 6.8\ 10^5$
25	2-Octanone	111-13-7	18.39	2.37	$4.2\ 10^4 \pm 5.0\ 10^3$	ND
26	Heptanoic acid	111-14-8	42.62	2.42	$1.3\ 10^5 \pm 1.0\ 10^4$	ND
27	Ethyl cinnamate	103-36-6	44.29	2.62	$9.9\ 10^3 \pm 1.9\ 10^1$	ND
28	2-Ethyl-hexanoic acid	149-57-5	42.59	2.64	$3.3\ 10^5 \pm 2.0\ 10^3$	ND
29	Ethyl hydrocinnamate	2021-28-5	41.60	2.73	$1.9\ 10^4 \pm 7.3\ 10^1$	$2.5\ 10^4 \pm 2.5\ 10^3$
30	Ethyl hexanoate [e]	123-66-0	16.02	2.82	$5.5\ 10^5 \pm 5.3\ 10^4$	$3.8\ 10^5 \pm 6.9\ 10^4$
31	Hexyl acetate	142-92-7	17.89	2.83	$6.9\ 10^4 \pm 4.3\ 10^3$	$5.4\ 10^4 \pm 5.7\ 10^4$
32	Linalool [e]	106-24-1	30.07	2.97	$6.9\ 10^4 \pm 3.3\ 10^3$	$6.3\ 10^4 \pm 5.5\ 10^3$
33	1-Octanol	111-87-5	30.38	3.00	$9.3\ 10^4 \pm 5.6\ 10^2$	$8.6\ 10^4 \pm 7.3\ 10^3$
34	Carvone	2244-16-8	36.80	3.07	$1.3\ 10^5 \pm 3.6\ 10^4$	ND
35	Isoamyl octanoate	106-27-4	34.35	3.25	$2.5\ 10^5 \pm 2.4\ 10^4$	$1.4\ 10^5 \pm 5.9\ 10^4$
36	Nonanal	124-19-6	23.25	3.27	$6.0\ 10^4 \pm 4.6\ 10^2$	$1.1\ 10^4 \pm 1.3\ 10^4$
37	Ethyl heptanoate	106-30-9	20.67	3.32	$3.5\ 10^4 \pm 1.0\ 10^3$	$2.0\ 10^4 \pm 2.3\ 10^3$
38	Nonanoic acid [e]	112-05-0	44.13	3.42	$5.6\ 10^4 \pm 5.4\ 10^3$	$4.0\ 10^4 \pm 2.6\ 10^4$
39	Myrcenol	18479-58-8	32.66	3.47	$2.2\ 10^4 \pm 5.0\ 10^2$	$1.3\ 10^4 \pm 6.0\ 10^2$
40	Nonanoic acid [e]	112-05-0	44.49	3.52	$6.1\ 10^5 \pm 1.5\ 10^4$	$5.7\ 10^5 \pm 2.2\ 10^4$
41	Ethyl octanoate [e]	106-32-1	25.25	3.81	$1.6\ 10^7 \pm 1.5\ 10^6$	$1.4\ 10^7 \pm 2.1\ 10^6$
42	Octyl acetate	112-14-1	26.93	3.84	$1.3\ 10^4 \pm 1.4\ 10^3$	ND
43	Undecylenic acid	112-38-9	45.50	3.86	$6.0\ 10^5 \pm 9.9\ 10^3$	$5.5\ 10^5 \pm 3.8\ 10^5$
	Area (compounds with 2 < log P < 4)				**$3.9\ 10^7$**	**$3.0\ 10^7$**

	Number of compounds (2 < log P < 4)				**25**	**19**
	Nonpolar (log P > 4)				**Overcoated**	**Commercial**
44	Ethyl decanoate	110-38-3	33.61	4.09	$2.4\ 10^7 \pm 9.5\ 10^5$	$1.4\ 10^7 \pm 1.0\ 10^6$
45	Decanoic acid [e]	334-48-5	45.13	4.09	$2.0\ 10^7 \pm 2.9\ 10^5$	$1.5\ 10^7 \pm 3.0\ 10^5$
46	2-Undecanol	1653-30-1	36.76	4.25	$5.2\ 10^4 \pm 4.5\ 10^3$	ND
47	α-Humulene epoxide	19888-34-7	43.49	4.51	$6.5\ 10^4 \pm 4.5\ 10^2$	ND
48	1-Decanol	112-30-1	38.28	4.57	$6.0\ 10^4 \pm 3.7\ 10^3$	$4.6\ 10^4 \pm 7.2\ 10^3$
49	Dodecanoic acid	0143-07-07	46.33	4.60	$9.3\ 10^5 \pm 7.3\ 10^4$	$4.7\ 10^5 \pm 1.4\ 10^5$
50	Ethyl 9-decenoate	67233-91-4	35.59	4.66	$7.8\ 10^5 \pm 3.7\ 10^4$	$9.2\ 10^4 \pm 1.3\ 10^4$
51	Ethyl nonanoate	67233-91-4	29.42	4.66	$2.0\ 10^5 \pm 1.4\ 10^4$	ND
52	α-Cadinol	481-34-5	44.96	4.77	$5.5\ 10^4 \pm 1.0\ 10^3$	$1.6\ 10^4 \pm 4.0\ 10^2$
53	τ-Cadinol	1474790	44.55	4.90	$1.8\ 10^4 \pm 3.7\ 10^2$	ND
54	β-Farnesene	77129-48-7	34.66	5.70	$8.9\ 10^3 \pm 1.9\ 10^2$	ND
55	Ethyl dodecanoate [e]	106-33-2	40.94	5.71	$9.1\ 10^6 \pm 3.4\ 10^5$	$4.9\ 10^6 \pm 5.3\ 10^5$
56	Farnesol	3790-71-4	45.61	5.77	$6.5\ 10^4 \pm 1.8\ 10^3$	ND
57	Isoamyl decanoate	2306-91-4	41.29	6.19	$6.5\ 10^5 \pm 2.9\ 10^4$	$2.1\ 10^5 \pm 4.4\ 10^4$
58	Methyl hexadecanoate [e]	112-39-0	44.83	7.38	$7.0\ 10^5 \pm 2.1\ 10^4$	ND
59	Methyl 9- octadecenoate	112-62-9	46.14	7.45	$3.3\ 10^4 \pm 3.4\ 10^2$	ND
60	Ethyl 9-hexadecenoate	54546-22-4	45.23	7.51	$6.3\ 10^4 \pm 3.4\ 10^3$	ND
61	Ethyl hexadecanoate	628-97-7	45.06	7.74	$4.5\ 10^5 \pm 2.4\ 10^4$	ND
	Area (compounds log P > 4)				**$5.7\ 10^7$**	**$3.5\ 10^7$**
	Number of compounds (log P > 4)				**18**	**8**
	Total area				**$1.1\ 10^8$**	**$9.1\ 10^7$**

a Chemical Abstracts Service; b Retention time (minutes); c Partition coefficient; d Standard deviation; e Positively identified compounds

Table 3. Method validation parameters for quantification of volatiles during brewing using the PDMS-overcoated DVB/Car/PDMS commercial fiber and gas chromatography with mass spectrometric detector. Experimental conditions are described in section 2.4.

Compound	log P	Range (mg L^{-1})	Regression equation	r^2 [a]	LOD (mg L^{-1})[b]	LOQ (mg L^{-1})[c]	Conc. (mg L^{-1})[d]	Prec. (%)[e]	Repe. (%)[f]	Rec. (%)[g]
Polar (log P < 2)										
Acetic acid	-0.17	300-600	y = 0.0055x - 1.5554	0.9946	51.3	300	300	13.0	11.2	98.9
							400	7.0	7.2	100.1
							600	10.6	5.6	97.9
2-Furanmethanol	0.28	0.25-3	y = 0.2236x - 0.0208	0.9959	0.1	0.25	0.25	10.0	6.5	92.3
							2.0	4.7	2.9	100.0
							3.0	9.6	8.6	99.5
Furfural	0.41	0.25-3	y = 11.602x + 1.391	0.9969	0.01	0.25	0.25	2.7	3.4	100.2
							1.5	4.0	2.1	96.9
							3.0	5.0	4.9	97.8
Isoamyl alcohol	1.16	0.25-2.5	y = 2.2713x - 0.4052	0.9958	0.02	0.25	0.25	7.3	8.8	99.0
							1.5	4.4	6.4	100.0
							2.5	7.1	9.0	97.7
Benzaldehyde	1.48	0.25-3	y = 35.717x - 8.8594	0.9912	0.001	0.25	0.25	10.0	1.9	100.4
							1.0	2.6	1.1	96.8
							3.0	4.1	5.6	98.4
Medium polar (2 < log P < 4)										
1-Hexanol	2.03	0.14-3	y = 5.9581x - 0.8322	0.9983	0.06	0.14	0.14	4.6	4.2	99.0
							1.5	10.5	7.0	98.7
							3	8.9	8.9	99.3
Isoamyl acetate	2.25	0.25-2.5	y = 1.5329x - 0.389	0.9902	0.05	0.25	0.25	11.2	11.6	97.4
							1.0	4.1	6.2	98.8

							2.5	14.6	2.8	99.1
Ethyl hexanoate	2.40	0.05-1	y = 7.4923x - 0.4229	0.9904	0.02	0.05	0.05	12.0	9.3	98.1
							0.5	13.5	10.1	99.0
							1.0	10.4	11.3	100.2
Linalool	2.97	0.25-2	y = 39.177x + 30.926	0.9941	0.004	0.25	0.25	10.0	7.2	97.5
							0.5	14.1	10.7	97.8
							2	10.1	9.6	99.6
Octanoic acid	3.05	0.05-1	y = 6.9647x - 0.4841	0.9930	0.004	0.05	0.05	11.7	11.6	99.6
							0.1	10.6	10.0	93.7
							0.75	10.3	9.6	98.4
Nonanoic acid	3.42	0.075-1	y = 11.538x - 1.021	0.9946	0.02	0.75	0.075	11.1	12.4	98.0
							0.75	10.7	8.0	96.5
							1.0	7.4	8.6	100.0
Nerol	3.47	0.25-3	y = 58.144x - 14.483	0.9976	0.003	0.25	0.25	6.5	7.1	99.0
							1.5	11.2	4.7	99.4
							3.0	13.7	6.4	98.8
Ethyl octanoate	3.50	0.05-1	y = 19.129x - 0.3267	0.9937	0.01	0.05	0.05	11.7	7.6	94.1
							0.25	5.2	4.2	91.9
							1.0	4.6	10.1	99.3
Nonpolar (log P > 4)										
Decanoic acid	4.09	0.25-1	y = 11.269x - 2.3067	0.9934	0.04	0.25	0.25	5.0	5.3	97.3
							0.5	13.5	12.7	93.9
							0.75	6.5	6.0	96.8
1-Dodecanol	5.13	0.25-3	y = 0.0907x + 0.0195	0.9980	0.09	0.25	0.25	12.6	2.8	100.0
							1.5	13.5	10.2	98.7
							3.0	12.4	10.8	98.1
Ethyl dodecanoate	5.71	0.05-0.75	y = 183.1x - 10.497	0.9974	0.001	0.05	0.05	10.7	10.7	96.2

							0.1	14.9	8.7	100.0
							0.75	10.9	6.1	99.9
Methyl hexadecanoate	7.38	0.1-1	y = 90.562x - 12.852	0.9768	0.01	0.1	0.1	10.5	6.7	93.9
							0.25	12.9	12.2	90.9
							1.0	14.6	8.4	100.0

[a]r^2: Determination coefficient; [b]LOD: Limit of detection; [c]LOQ: Limit of quantification; [d]Conc.: Concentration corresponding to the lowest and higher concentration of analytical curve of each volatile compound used for determining the precision, repeatability and recovery; [e]Prec.: Intermediate precision: coefficient of variation of four independent assays performed under the same analytical conditions in four different days; [f]Rep.: Repeatability: coefficient of variation of seven independent assays performed under the same analytical conditions on the same day; [g]Rec.: Recovery.

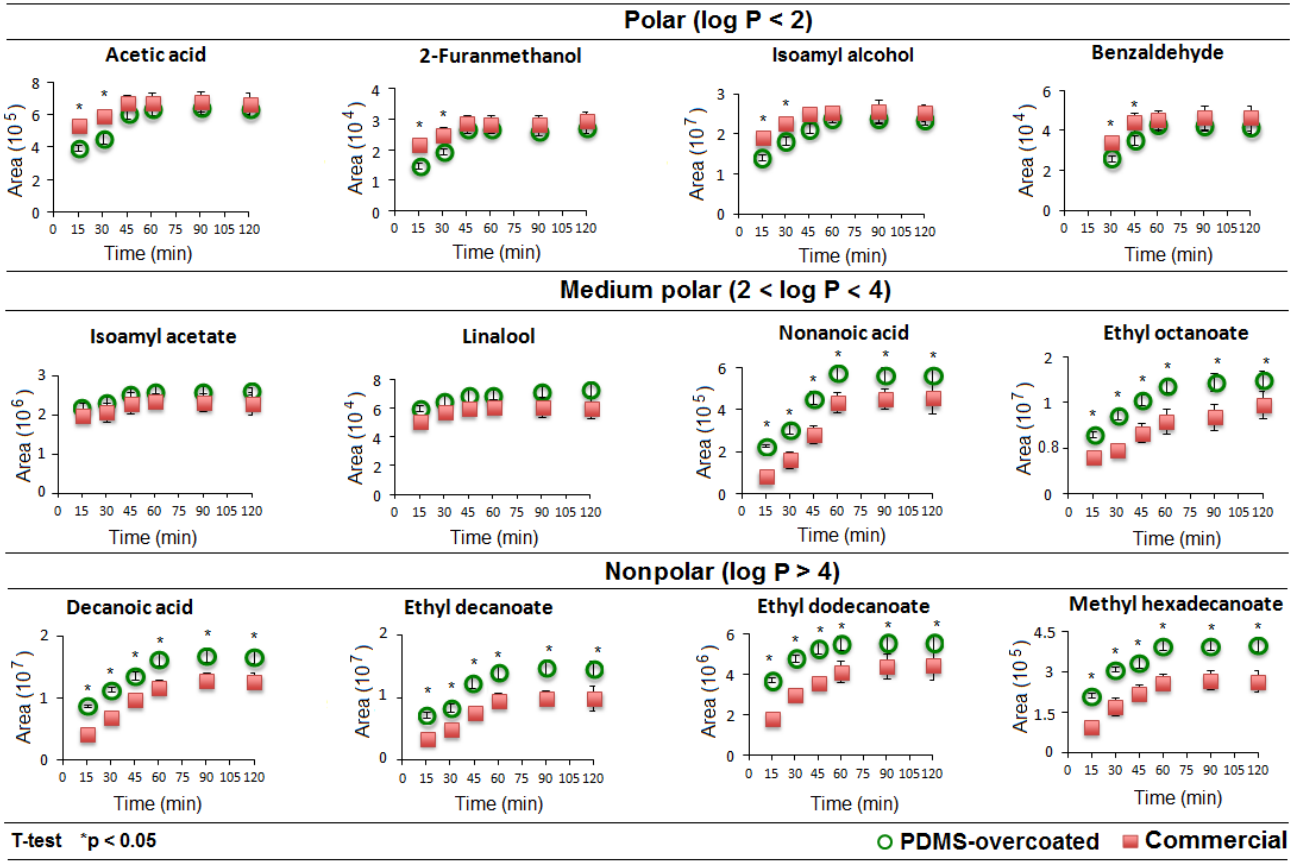


Figure 1. Chromatographic area of compounds extracted by the PDMS-overcoated and commercial fibers according to different extraction times. Experimental conditions are described in section 2.4.

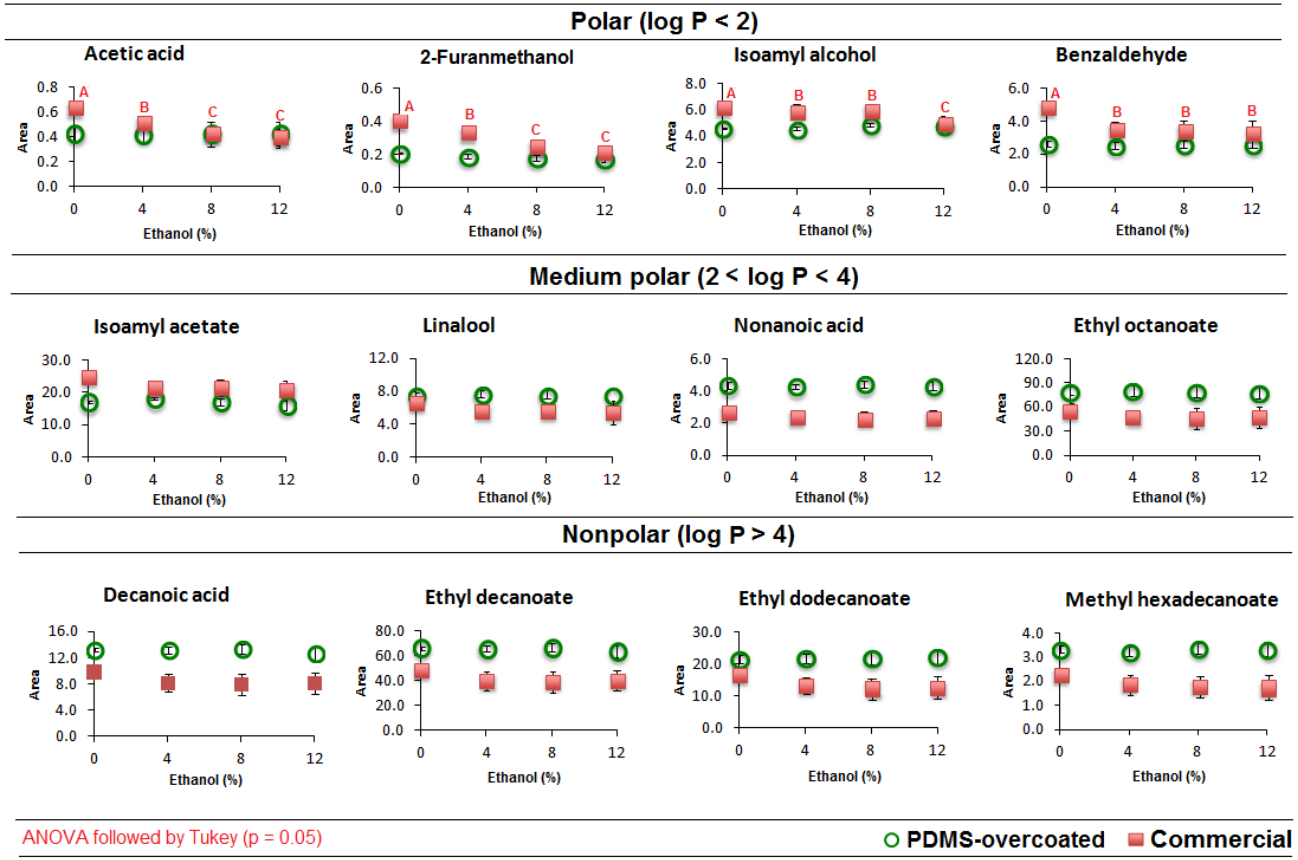


Figure 2. Normalized chromatographic area of compounds extracted by the PDMS-overcoated DVB/Car/PDMS and commercial. Red letters correspond to ANOVA followed by the Tukey test (p = 0.05). Whenever letters are the same for two or more values, it means that no statistically significant differences were found among these values. For the compounds in which letters were not indicated in the figure, there was no significant difference when evaluated in solution containing different concentrations of ethanol.

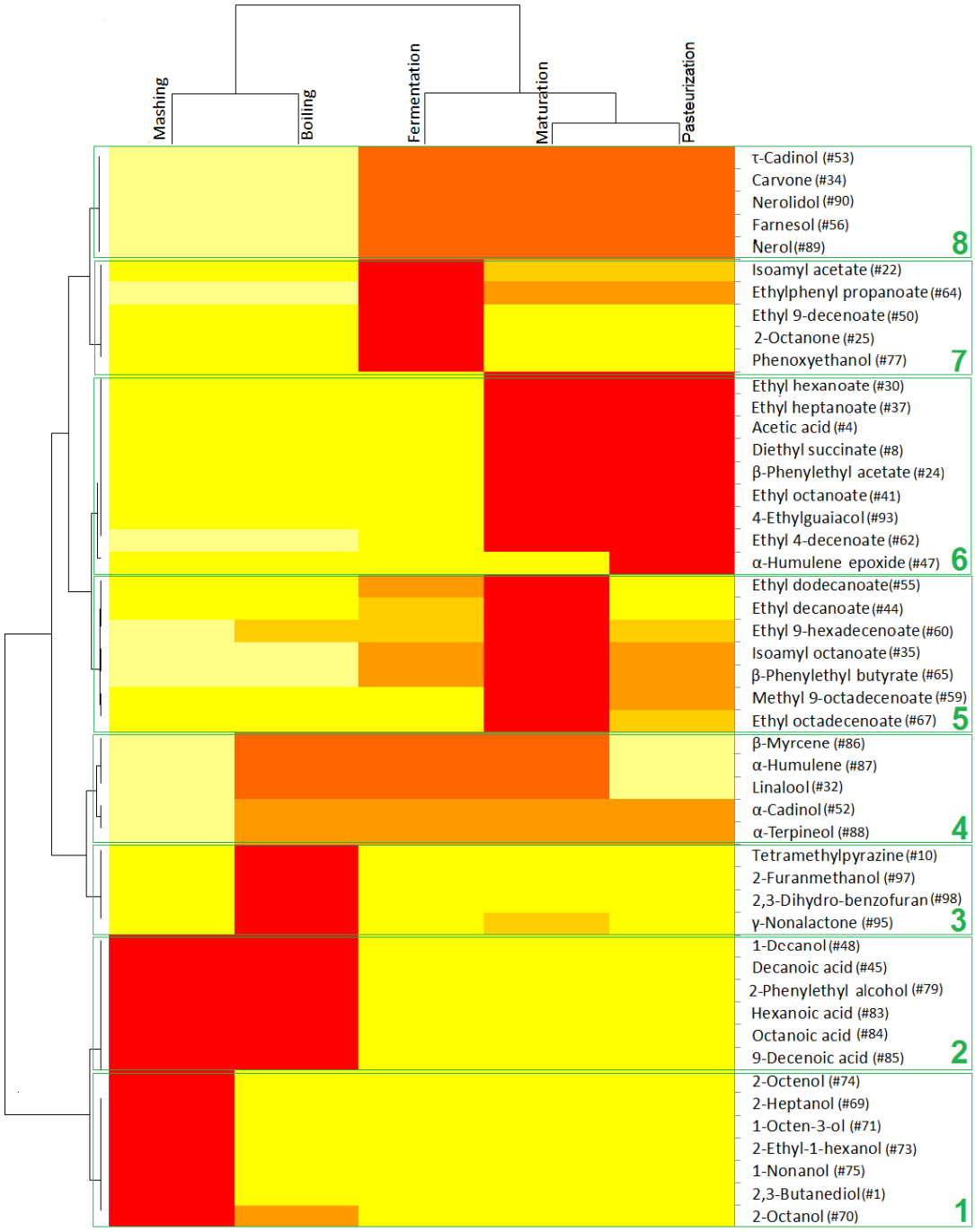


Figure 3. Heat map obtained using quantitative data related to volatile compounds (Table S5) determined throughout the five steps of lager craft brewing (lot produced in November 2016 by a microbrewery from Porto Alegre, Rio Grande do Sul, Brazil). Red, orange and yellow colors represent higher, medium and lower levels of the volatile compounds. Clusters related to the grouping of volatiles were designated from 1 to 8 (vertical axis). Experimental conditions are described in section 2.4.

Supplementary Material

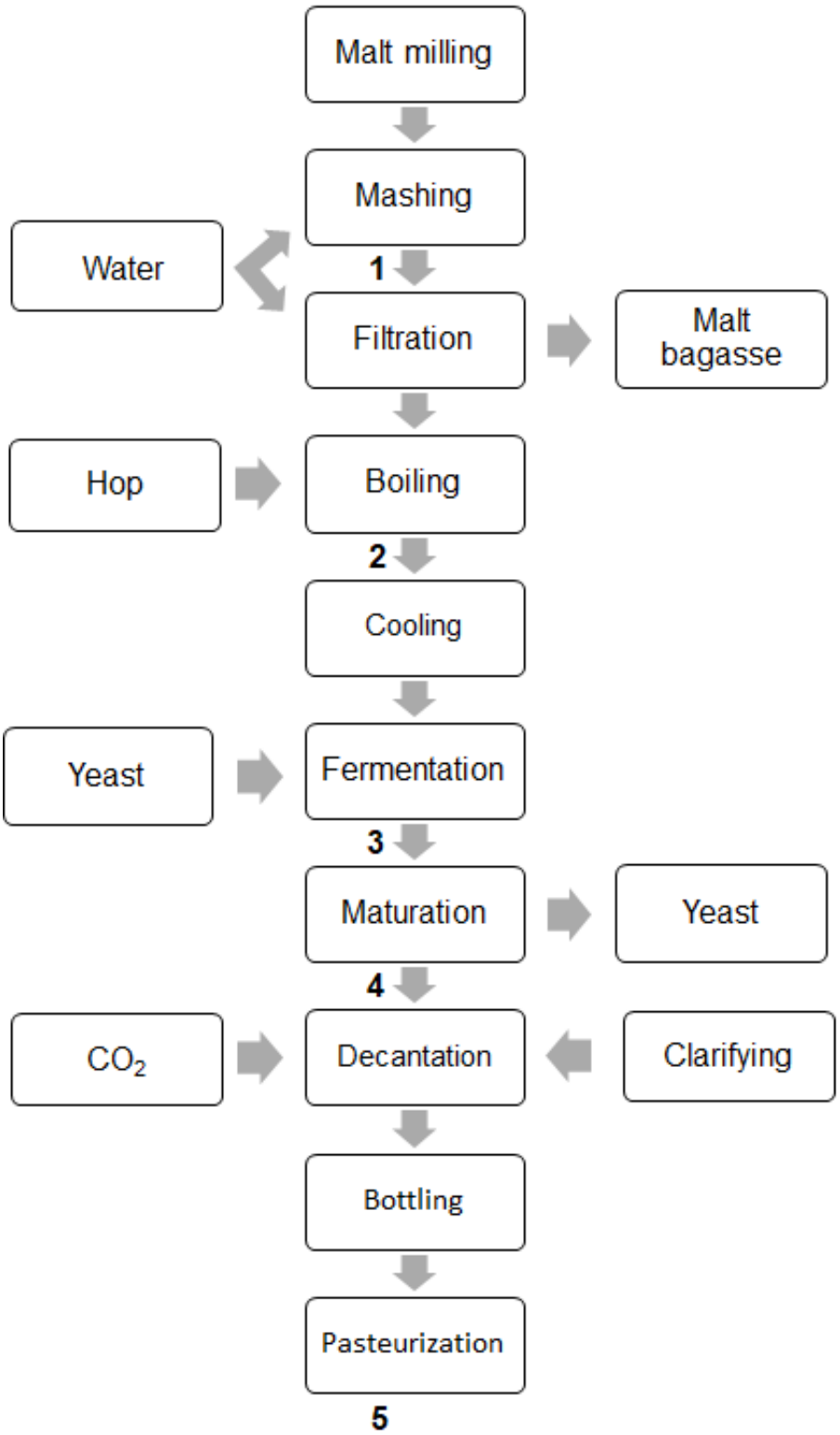


Figure S1. Brewing flow diagram with indication of the points (numbers 1 to 5) at which samples were collected for the analysis of volatile profile, as described in section 2.1.

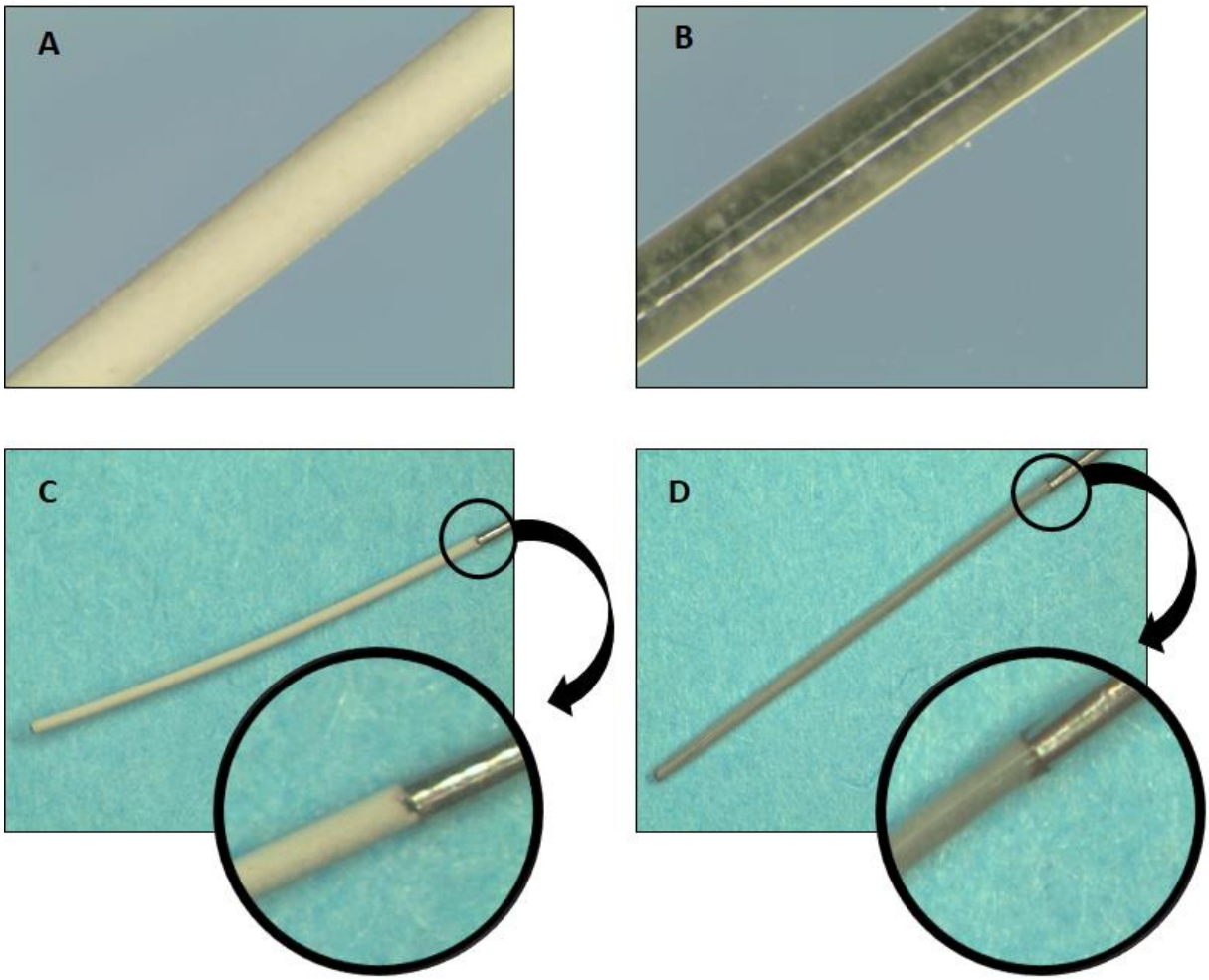
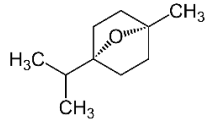
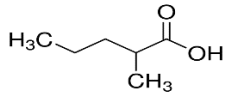
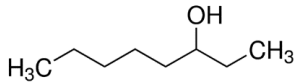
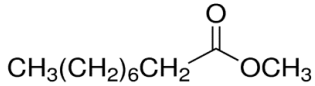
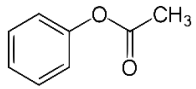


Figure S2. Surface of a commercial DVB/Car/PDMS fiber (A) and a version of this fiber coated with an extra PDMS-layer showing the uniform and smooth coverage of the additional PDMS layer (B). Detail of the upper extremity of the commercial fiber (C) and PDMS-overcoated analogue with emphasis on the proper sealing of the coating in the metal junction of PDMS-overcoated fiber (D).

Table S1. Characteristics of the compounds used as internal standards.

Internal standard	CAS[a]	Chemical class	MW (g/mol)[b]	Molecular formula	Structural formula
1.4-Cineole	470-67-7	Monoterpene	154.25	$C_{10}H_{18}O$	
2-methylpentanoic acid	97-61-0	Acid	116.16	$C_6H_{12}O_2$	
3-octanol	589-98-0	Alcohol	130.22	$C_8H_{18}O$	
Dodecane	112-40-3	Alkane	170.33	$C_{12}H_{26}$	$CH_3(CH_2)_{10}CH_3$
Methyl nonanoate	1731-84-6	Methyl ester	172.26	$C_{10}H_{20}O_2$	
Phenyl acetate	122-79-2	Phenol ester	136.15	$C_8H_8O_2$	

[a] CAS: Chemical Abstracts Service; [b] Molecular weight.

Table S2. Absolute area ± standard deviation of the volatile compounds of lager beer evaluated for different extraction times using HS-SPME-GC/MS performed using a commercial DVB/Car/PDMS and a version of this fiber overcoated with an extra PDMS-layer. Experimental conditions are described in section 2.4.

Polar (log P < 2)						
Acetic acid						
Time (min)	**15**	**30**	**45**	**60**	**90**	**120**
PDMS-overcoated	$3.9\ 10^5 \pm 1.6\ 10^4$	$4.5\ 10^5 \pm 3.3\ 10^4$	$6.1\ 10^5 \pm 3.5\ 10^4$	$6.4\ 10^5 \pm 3.4\ 10^4$	$6.4\ 10^5 \pm 3.9\ 10^4$	$6.4\ 10^5 \pm 4.0\ 10^4$
Commercial	$5.4\ 10^5 \pm 3.0\ 10^4$	$5.9\ 10^5 \pm 3.7\ 10^4$	$6.8\ 10^5 \pm 4.1\ 10^4$	$6.8\ 10^5 \pm 5.6\ 10^4$	$6.8\ 10^5 \pm 6.2\ 10^4$	$6.7\ 10^5 \pm 6.7\ 10^4$
p*-value	**0.002**	**0.008**	0.085	0.305	0.378	0.484
2-Furanmethanol						
PDMS-overcoated	$1.5\ 10^4 \pm 9.0\ 10^2$	$2.0\ 10^4 \pm 1.0\ 10^3$	$2.7\ 10^4 \pm 1.2\ 10^3$	$2.7\ 10^4 \pm 1.4\ 10^3$	$2.6\ 10^4 \pm 1.4\ 10^3$	$2.7\ 10^4 \pm 1.6\ 10^3$
Commercial	$2.2\ 10^4 \pm 2.0\ 10^3$	$2.5\ 10^4 \pm 2.0\ 10^3$	$2.9\ 10^4 \pm 2.6\ 10^3$	$2.9\ 10^4 \pm 2.7\ 10^3$	$2.9\ 10^4 \pm 2.8\ 10^3$	$3.0\ 10^4 \pm 2.6\ 10^3$
***p*-value**	**0.005**	**0.011**	0.292	0.331	0.204	0.200
Isoamyl alcohol						
PDMS-overcoated	$1.4\ 10^7 \pm 6.7\ 10^5$	$1.8\ 10^7 \pm 1.0\ 10^6$	$2.3\ 10^7 \pm 1.2\ 10^6$	$2.4\ 10^7 \pm 1.1\ 10^6$	$2.4\ 10^7 \pm 1.1\ 10^6$	$2.4\ 10^7 \pm 1.2\ 10^6$
Commercial	$1.9\ 10^7 \pm 1.5\ 10^6$	$2.3\ 10^7 \pm 1.7\ 10^6$	$2.5\ 10^7 \pm 1.6\ 10^6$	$2.5\ 10^7 \pm 1.8\ 10^6$	$2.6\ 10^7 \pm 3.0\ 10^6$	$2.5\ 10^7 \pm 2.0\ 10^6$
***p*-value**	**0.007**	**0.018**	0.064	0.831	0.435	0.251
Benzaldehyde						
PDMS-overcoated	ND	$2.6\ 10^4 \pm 1.6\ 10^3$	$3.5\ 10^4 \pm 2.3\ 10^3$	$4.3\ 10^4 \pm 2.2\ 10^3$	$4.2\ 10^4 \pm 2.7\ 10^3$	$4.1\ 10^4 \pm 2.6\ 10^3$
Commercial	ND	$3.4\ 10^4 \pm 3.8\ 10^3$	$4.4\ 10^4 \pm 4.7\ 10^3$	$4.5\ 10^4 \pm 5.1\ 10^3$	$4.6\ 10^4 \pm 5.9\ 10^3$	$4.6\ 10^4 \pm 6.2\ 10^3$
***p*-value**	ND	**0.028**	**0.040**	0.490	0.341	0.272
Medium polar (2 < log P < 4)						
Isoamyl acetate						
PDMS-overcoated	$2.2\ 10^6 \pm 1.0\ 10^5$	$2.3\ 10^6 \pm 1.4\ 10^5$	$2.6\ 10^6 \pm 1.3\ 10^5$	$2.6\ 10^6 \pm 1.6\ 10^5$	$2.6\ 10^6 \pm 1.5\ 10^5$	$2.6\ 10^6 \pm 1.0\ 10^5$
Commercial	$2.0\ 10^6 \pm 1.7\ 10^5$	$2.1\ 10^6 \pm 2.4\ 10^5$	$2.3\ 10^6 \pm 2.7\ 10^5$	$2.4\ 10^6 \pm 2.0\ 10^5$	$2.3\ 10^6 \pm 2.2\ 10^5$	$2.3\ 10^6 \pm 2.9\ 10^5$

***p*-value**	0.136	0.198	0.232	0.812	0.166	0.152
Linalool						
PDMS-overcoated	$6.0\ 10^4 \pm 1.9\ 10^3$	$6.5\ 10^4 \pm 4.1\ 10^3$	$6.9\ 10^4 \pm 4.4\ 10^3$	$6.9\ 10^4 \pm 3.3\ 10^3$	$7.2\ 10^4 \pm 3.5\ 10^3$	$7.3\ 10^4 \pm 4.7\ 10^3$
Commercial	$5.1\ 10^4 \pm 5.0\ 10^3$	$5.7\ 10^4 \pm 3.2\ 10^3$	$6.0\ 10^4 \pm 2.9\ 10^3$	$6.1\ 10^4 \pm 5.5\ 10^3$	$6.1\ 10^4 \pm 6.6\ 10^3$	$6.0\ 10^4 \pm 7.6\ 10^3$
***p*-value**	0.060	0.067	0.061	0.085	0.067	0.074
Nonanoic acid						
PDMS-overcoated	$2.3\ 10^5 \pm 6.2\ 10^3$	$3.1\ 10^5 \pm 2.2\ 10^4$	$4.6\ 10^5 \pm 3.1\ 10^4$	$5.8\ 10^5 \pm 3.4\ 10^4$	$5.6\ 10^5 \pm 3.5\ 10^4$	$5.7\ 10^5 \pm 4.1\ 10^4$
Commercial	$8.3\ 10^4 \pm 2.6\ 10^4$	$1.6\ 10^5 \pm 4.1\ 10^4$	$2.8\ 10^5 \pm 3.1\ 10^4$	$4.3\ 10^5 \pm 4.8\ 10^4$	$4.5\ 10^5 \pm 4.7\ 10^4$	$4.6\ 10^5 \pm 7.6\ 10^4$
***p*-value**	**0.001**	**0.050**	**0.005**	**0.014**	**0.029**	0.088
Ethyl octanoate						
PDMS-overcoated	$1.1\ 10^7 \pm 5.3\ 10^5$	$1.4\ 10^7 \pm 8.3\ 10^5$	$1.7\ 10^7 \pm 9.5\ 10^5$	$1.9\ 10^7 \pm 1.2\ 10^6$	$2.0\ 10^7 \pm 1.5\ 0^6$	$2.0\ 10^7 \pm 1.5\ 10^6$
Commercial	$6.7\ 10^6 \pm 1.1\ 10^6$	$7.8\ 10^6 \pm 1.1\ 10^6$	$1.1\ 10^7 \pm 1.7\ 10^6$	$1.3\ 10^7 \pm 2.1\ 10^6$	$1.4\ 10^7 \pm 2.3\ 10^6$	$1.6\ 10^7 \pm 2.5\ 10^6$
***p*-value**	**0.005**	**0.002**	**0.007**	**0.011**	**0.018**	0.062
Nonpolar (log P > 4)						
Decanoic acid						
PDMS-overcoated	$8.7\ 10^6 \pm 2.3\ 10^5$	$1.1\ 10^7 \pm 3.6\ 10^5$	$1.4\ 10^7 \pm 6.7\ 10^5$	$1.6\ 10^7 \pm 9.9\ 10^5$	$1.7\ 10^7 \pm 9.1\ 10^5$	$1.7\ 10^7 \pm 1.1\ 10^6$
Commercial	$4.4\ 10^6 \pm 9.0\ 10^5$	$7.0\ 10^6 \pm 1.0\ 10^6$	$9.9\ 10^6 \pm 8.2\ 10^5$	$1.2\ 10^7 \pm 1.1\ 10^6$	$1.3\ 10^7 \pm 1.1\ 10^6$	$1.3\ 10^7 \pm 1.3\ 10^6$
***p*-value**	**0.001**	**0.002**	**0.018**	**0.007**	**0.009**	**0.017**
Ethyl decanoate						
PDMS-overcoated	$7.3\ 10^6 \pm 4.2\ 10^5$	$8.4\ 10^6 \pm 7.3\ 10^5$	$1.2\ 10^7 \pm 9.0\ 10^5$	$1.4\ 10^7 \pm 1.0\ 10^6$	$1.5\ 10^7 \pm 1.2\ 10^6$	$1.5\ 10^7 \pm 1.2\ 10^6$
Commercial	$3.6\ 10^6 \pm 9.4\ 10^5$	$5.0\ 10^6 \pm 9.6\ 10^5$	$7.6\ 10^6 \pm 1.1\ 10^6$	$9.6\ 10^6 \pm 1.2\ 10^6$	$1.0\ 10^7 \pm 1.2\ 10^6$	$9.9\ 10^6 \pm 2.0\ 10^6$
***p*-value**	**0.003**	**0.008**	**0.005**	**0.007**	**0.008**	**0.025**
Ethyl dodecanoate						
PDMS-overcoated	$3.7\ 10^6 \pm 1.2\ 10^5$	$4.8\ 10^6 \pm 1.9\ 10^5$	$5.3\ 10^6 \pm 3.0\ 10^5$	$5.6\ 10^6 \pm 3.4\ 10^5$	$5.6\ 10^6 \pm 3.8\ 10^5$	$5.6\ 10^6 \pm 3.5\ 10^5$
Commercial	$1.8\ 10^6 \pm 3.0\ 10^5$	$3.0\ 10^6 \pm 3.2\ 10^5$	$3.6\ 10^6 \pm 3.6\ 10^5$	$4.2\ 10^6 \pm 5.3\ 10^5$	$4.4\ 10^6 \pm 6.2\ 10^5$	$4.5\ 10^6 \pm 7.5\ 10^5$
***p*-value**	**0.000**	**0.009**	**0.003**	**0.018**	0.054	**0.041**
Methyl hexadecanoate						

PDMS-overcoated	$2.1\ 10^5 \pm 6.8\ 10^3$	$3.1\ 10^5 \pm 9.6\ 10^3$	$3.4\ 10^5 \pm 2.1\ 10^4$	$4.0\ 10^5 \pm 2.1\ 10^4$	$4.0\ 10^5 \pm 2.1\ 10^4$	$4.0\ 10^5 \pm 2.3\ 10^4$
Commercial	$9.7\ 10^4 \pm 2.3\ 10^4$	$1.7\ 10^5 \pm 3.2\ 10^4$	$2.2\ 10^5 \pm 3.1\ 10^4$	$2.6\ 10^5 \pm 3.0\ 10^4$	$2.7\ 10^5 \pm 3.7\ 10^4$	$2.7\ 10^5 \pm 3.9\ 10^4$
***p*-value**	**0.001**	**0.002**	**0.007**	**0.003**	**0.006**	**0.004**

* $p < 0.05$ in bold green font indicate significant difference between the chromatographic area of the volatile compound extracted by PDMS-overcoated and commercial fibers; ND: not detected.

Table S3. Effect of ethanol concentration on normalized chromatographic area (± standard deviation) of volatile compounds obtained by HS-SPME performed using a commercial DVB/Car/PDMS and this fiber overcoated with an extra PDMS-layer. Experimental conditions are described in section 2.4.

Polar (log P < 2)				
Acetic acid				
Ethanol content (%)	**0**	**4**	**8**	**12**
PDMS-overcoated	0.42 ± 0.04 A	0.42 ± 0.04 A	0.42 ± 0.05 A	0.43 ± 0.08 A
Commercial	0.63 ± 0.06 A	0.51 ± 0.07 B	0.43 ± 0.10 C	0.39 ± 0.10 C
2-Furanmethanol				
PDMS-overcoated	0.20 ± 0.00 A	0.19 ± 0.01 A	0.18 ± 0.02 A	0.17 ± 0.01 A
Commercial	0.40 ± 0.02 A	0.33 ± 0.02 B	0.25 ± 0.03 C	0.21 ± 0.03 C
Isoamyl alcohol				
PDMS-overcoated	4.59 ± 0.07 A	4.56 ± 0.12 A	4.83 ± 0.12 A	4.81 ± 0.13 A
Commercial	6.24 ± 0.14 A	6.00 ± 0.56 B	5.97 ± 0.51 B	4.96 ± 0.59 C
Benzaldehyde				
PDMS-overcoated	2.62 ± 0.19 A	2.52 ± 0.19 A	2.61 ± 0.24 A	2.60 ± 0.24 A
Commercial	4.87 ± 0.35 A	3.52 ± 0.43 B	3.43 ± 0.62 B	3.10 ± 0.72 B
Medium polar (2 > log P > 4)				
Isoamyl acetate				
PDMS-overcoated	16.96 ± 0.47 A	18.04 ± 0.36 A	17.08 ± 1.10 A	15.99 ± 1.38 A
Commercial	24.73 ± 1.90 A	21.47 ± 2.08 A	21.42 ± 2.69 A	20.52 ± 2.98 A
Linalool				
PDMS-overcoated	7.57 ± 0.23 A	7.68 ± 0.42 A	7.64 ± 0.56 A	7.59 ± 0.60 A
Commercial	6.67 ± 0.69 A	5.68 ± 0.74 A	5.62 ±0.95 A	5.59 ± 0.92 A
Nonanoic acid				
PDMS-overcoated	4.34 ± 0.22 A	4.26 ± 0.26 A	4.39 ± 0.23 A	4.31 ± 0.27 A

Commercial	2.71 ± 0.19 A	2.41 ± 0.25 A	2.31 ± 0.44 A	2.32 ± 0.47 A
		Ethyl octanoate		
PDMS-overcoated	79.87 ± 5.24 A	79.92 ± 6.99 A	79.60 ± 7.48 A	78.39 ± 7.99 A
Commercial	55.07 ± 9.85 A	47.85 ± 5.60 A	46.37 ± 8.33 A	48.21 ± 9.41 A
		Nonpolar (log P > 4)		
		Decanoic acid		
PDMS-overcoated	13.21 ± 0.34 A	13.14 ± 0.49 A	13.30 ± 0.65 A	12.72 ± 0.94 A
Commercial	9.76 ± 0.57 A	8.05 ± 0.42 A	7.94 ± 0.96 A	8.10 ± 1.62 A
		Ethyl decanoate		
PDMS-overcoated	66.06 ± 1.70 A	65.71 ± 2.46 A	66.52 ± 3.24 A	63.60 ± 4.70 A
Commercial	48.80 ± 2.84 A	40.27 ± 2.08 A	39.71 ± 4.82 A	40.51 ± 8.09 A
		Ethyl dodecanoate		
PDMS-overcoated	21.35 ± 1.25 A	21.73 ± 1.58 A	21.88 ± 2.11 A	22.03 ± 2.46 A
Commercial	16.77 ± 1.95 A	13.55 ± 2.10 A	12.83 ± 2.67 A	12.61 ± 3.51 A
		Methyl hexadecanoate		
PDMS-overcoated	3.32 ± 0.16 A	3.23 ± 0.19 A	3.36 ± 0.23 A	3.33 ± 0.29 A
Commercial	2.27 ± 0.16 A	1.89 ± 0.26 A	1.80 ± 0.33 A	1.73 ± 0.37 A

In each line, normalized chromatographic areas with the same letters are not significantly different from each other ($p > 0.05$) according to ANOVA followed by Tukey test.

Table S4. Retention indices of the volatile compounds of a lager beer obtained through HS-SPME-GC/MS using PDMS-overcoated and commercial fibers as shown in Table 3. Experimental conditions are described in section 2.4. Compounds were listed according to polarity (polar, medium polar and nonpolar) and in ascending order of log P.

#	Compound[a]	RI_{exp}[b]	RI_{lit}[c]
	Polar (log P < 2)		
1	2,3-Butanediol	1541	1536 [1]
2	3-Methyl-2-cyclopenten-1-one	1508	1507 [2]
3	3-Hydroxy-2-butanone	1288	1287 [3]
4	Acetic acid [d]	1456	1461 [4]
5	3-(Methylthio)-1-propanol	1714	1714 [5]
6	Butanoic acid	1634	1640 [6]
7	Isoamyl alcohol [d]	1219	1211 [7]
8	Diethyl succinate	1679	1675 [8]
9	2-Methyl-2-cyclopenten-1-one	1362	1366 [2]
10	Tetramethyl-pyrazine	1471	1478 [9]
11	2-Hexanone	1084	1088 [9]
12	Benzaldehyde [d]	1517	1519 [3]
13	3-Hexanone	1059	1058 [10]
14	1-Pentanol	1245	1246 [8]
15	3-Methyl-cyclopentanol	1341	1342 [11]
16	3-Hexanol	1208	1207 [12]
17	2-Hexanol	1231	1232 [13]

18	3-Methyl-butanoic acid	1674	1674 [14]
	Medium polar (2 < log P < 4)		
19	1-Hexanol [d]	1357	1359 [7]
20	Dihydro-5-pentyl-2(3H)-furanone	2033	2036 [15]
21	2-Methoxy-4-vinylphenol	2208	2200 [16]
22	Isoamyl acetate [d]	1122	1126 [4]
23	Ethyl phenylacetate	1784	1781 [3]
24	2-Phenylethyl acetate	1820	1822 [7]
25	2-Octanone	1283	1283 [17]
26	Heptanoic acid	1960	1966 [18]
27	Ethyl cinnamate	2143	2139 [5]
28	2-Ethyl-hexanoic acid	1957	1954 [19]
29	Ethyl hydrocinnamate	1888	1892 [20]
30	Ethyl hexanoate [d]	1234	1237 [4]
31	Hexyl acetate	1274	1271 [4]
32	Linalool [d]	1552	1554 [21]
33	1-Octanol	1560	1562 [22]
34	Carvone	1725	1728 [23]
35	Isoamyl octanoate	1661	1657 [3]
36	Nonanal	1392	1394 [24]
37	Ethyl heptanoate	1334	1336 [20]
38	Nonanoic acid [d]	2126	2124 [25]
39	Myrcenol	1617	1620 [26]
40	Nonanoic acid [d]	2172	2173 [9]
41	Ethyl octanoate [d]	1438	1435 [3]
42	Octyl acetate	1470	1467 [27]

43	Undecylenic acid	2342	2351 [28]
	Nonpolar (log P > 4)		
44	Ethyl decanoate	1642	1648 [4]
45	Decanoic acid [d]	2275	2279 [9]
46	2-Undecanol	1724	1716 [25]
47	α-Humulene epoxide	2044	2048 [29]
48	1-Decanol	1766	1767 [30]
49	Dodecanoic acid	2492	2490 [31]
50	Ethyl 9-decenoate	1693	1688 [3]
51	Ethyl nonanoate	1535	1535 [3]
52	α-Cadinol	2247	2251 [32]
53	τ-Cadinol	2180	2188 [32]
54	β-Farnesene	1668	1672 [33]
55	Ethyl dodecanoate [d]	1860	1856 [20]
56	Farnesol	2360	2361 [34]
57	Isoamyl decanoate	1875	1871 [20]
58	Methyl hexadecanoate [d]	2225	2225 [35]
59	Methyl 9-octadecenoate	2456	2455 [36]
60	Ethyl 9-hexadecenoate	2291	2283 [3]
61	Ethyl hexadecanoate	2225	2225 [37]

[a] Compounds positively identified with the use of analytical standards was indicated in Table 3; [b] RI_{exp}: Experimental retention index obtained in a DB-WAX column of GC/qMS; [c] RI_{lit}: Literature retention index obtained in polar column; [d] compounds positively identified with the use of analytical standards.

Table S5. Monitoring of volatile profile (level ± standard deviation, mg L^{-1}) of the five stages of lager craft brewing using the PDMS-overcoated DVB/Car/PDMS commercial fiber and gas chromatography with mass spectrometric detection. Compounds are listed in increasing order of RI for the distinct classes Experimental conditions are described in section

2.4.

#	Compound[a]	Mashing	Boiling	Fermentation	Maturation	Pasteurization	RI_{exp} [r]	RI_{lit} [s]	OT [t]	OAV [u]	FR	%FR [v]	Odor description
	Esters	Level ± SD (mg L^{-1})							(mg L^{-1})				
22	Isoamyl acetate [b]	0.34 ± 0.04	0.37 ± 0.01	5.60 ± 0.32	2.51 ± 0.32	1.96 ± 0.03	1122	1126 [4]	0.72 [38] [1]	2.70	90	23	Banana [38]
30	Ethyl hexanoate [c]	ND	ND	0.20 ± 0.11	2.39 ± 0.62	2.26 ± 0.01	1234	1237 [4]	0.16 [38] [1]	14.10	233	59	Sweet, fruit [38]
37	Ethyl heptanoate [c]	ND	ND	0.11± 0.06	0.92 ± 0.06	0.90 ± 0.09	1334	1336 [20]	132 [39] [2]	<0.00	230	58	Tropical juice, honey [40]
41	Ethyl octanoate [c]	ND	ND	3.18 ± 0.08	16.37 ± 2.93	15.86 ± 0.64	1438	1435 [3]	0.29 [38] [1]	54.70	219	55	Fruity [38]
42	Octyl acetate [c]	ND	ND	0.98 ± 0.01	1.00 ± 0.03	0.98 ± 0.01	1470	1467 [27]	5.00 [41] [4]	0.20	56	14	Floral [42]
51	Ethyl nonanoate [c]	ND	ND	2.16 ± 0.01	3.15 ± 0.10	3.50 ± 0.14	1535	1535 [3]	3.15 [39] [2]	1.12	55	14	Waxy [43]
44	Ethyl decanoate [d]	ND	ND	0.07 ± 0.00	0.14 ± 0.08	<0.05	1642	1648 [4]	1.12 [39] [2]	<0.04	170	43	Wine aroma, pear, brandy [44]
57	Isoamyl octanoate [d]	ND	ND	0.11 ± 0.03	0.07 ± 0.00	0.06 ± 0.00	1661	1657 [3]	0.15 [45] [4]	0.40	170	43	Fruity [46]
62	Ethyl 4-decenoate [d]	ND	ND	0.21 ± 0.02	0.06 ± 0.00	0.05 ± 0.02	1673	1680 [3]	NF	NC	210	53	Waxy, leathery [47]
8	Diethyl succinate [c]	ND	ND	ND	0.20 ± 0.11	0.19 ± 0.02	1679	1675 [8]	353.19 [39] [2]	<0.00	220	56	Fruity [48]
50	Ethyl 9-decenoate [d]	ND	ND	0.37 ± 0.09	<0.05	<0.05	1693	1688 [3]	0.10 [41] [4]	<0.50	370	94	Rose [4]
23	Ethyl phenylacetate [e]	ND	ND	ND	0.16 ± 0.01	0.15 ± 0.01	1784	1781 [49]	0.41 [39] [2]	0.36	55	14	Floral [50]
63	β-Phenylethyl acetate [e]	ND	ND	0.90 ± 0.23	1.58 ± 0.26	0.93 ± 0.04	1810	1810 [51]	2.76 [38] [1]	0.34	219	55	Floral [38]
55	Ethyl dodecanoate [d]	ND	ND	0.18 ± 0.08	0.44 ± 0.17	0.09 ± 0.03	1860	1856 [20]	NF	NC	165	42	Sweet, floral, fruity cream [27]
64	Ethylphenyl propanoate [e]	ND	ND	0.48 ± 0.00	0.27 ± 0.02	0.25 ± 0.06	1884	1886 [52]	NF	NC	90	23	Floral [38]
65	β-Phenylethyl butyrate [e]	ND	ND	ND	0.26 ± 0.00	0.27 ± 0.01	1962	1959 [53]	NF	NC	175	44	Rose [44]
66	Methyl tetradecanoate [d]	ND	ND	ND	0.16 ± 0.00	0.06 ± 0.00	2009	2009 [54]	NF	NC	55	14	Waxy [55]
27	Ethyl cinnamate [d]	ND	ND	0.35 ± 0.02	0.33 ± 0.01	0.36 ± 0.03	2143	2139 [5]	0.0024 [38] [1]	150.00	50	13	Acid red fruits; banana [104]
67	Ethyl octadecenoate [c]	ND	ND	ND	0.09 ± 0.12	0.02 ± 0.00	2412	2409 [56]	NF	NC	189	48	NF

58	Methyl hexadecanoate [f]	ND	0.14 ± 0.00	0.15 ± 0.00	0.16 ± 0.01	0.15 ± 0.02	2225	2225 [35]	NF	NC	25	6	Fruit [50]
61	Ethyl hexadecanoate [f]	ND	ND	0.17 ± 0.02	0.56 ± 0.23	0.16 ± 0.02	2225	2225 [37]	1.50 [45] [4]	0.10	26	7	Wax, cream [44]
60	Ethyl 9-hexadecenoate [f]	ND	0.14 ± 0.00	0.16 ± 0.01	0.31 ± 0.10	0.14 ± 0.00	2291	2283 [3]	NF	NC	89	23	NF
59	Methyl 9-octadecenoate [f]	ND	ND	ND	0.51 ± 0.08	0.25 ± 0.00	2456	2455 [36]	NF	NC	180	46	NF
	Alcohols												
68	1-Butanol [g]	ND	ND	ND	0.25 ± 0.01	<0.25	1155	1152 [3]	2.73 [39] [2]	0.09	44	11	Fossil oil [57]
7	Isoamyl alcohol [g]	0.42 ± 0.16	0.32 ± 0.04	7.10 ± 0.41	20.07 ±0.98	6.29 ± 0.32	1219	1211 [7]	16.80 [38] [1]	0.37	55	14	Fusel alcohol [38]
69	2-Heptanol [h]	0.20 ± 0.00	<0.14	<0.14	<0.14	<0.14	1321	1321 [58]	0.20 [45] [4]	0.70	398	101	Lemon, orange, copper [45]
19	1-Hexanol [h]	0.35 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	1357	1359 [7]	5.37 [39] [2]	0.04	10	3	Green, leafy [59]
70	2-Octanol [h]	0.16 ± 0.00	<0.14	<0.14	<0.14	<0.14	1421	1421 [58]	NF	NC	195	49	Spicy [60]
71	1-Octen-3-ol [h]	0.17 ± 0.00	<0.14	<0.14	<0.14	<0.14	1449	1448 [61]	0.61 [39] [2]	0.23	396	100	Green, woody, mushroom [40]
72	1-Heptanol [h]	<0.14	<0.14	0.39 ± 0.03	<0.14	<0.14	1454	1454 [62]	0.2-0.3 [45] [4]	<0.47	56	14	Oily [63]
73	2-Ethyl-1-hexanol [h]	0.21 ± 0.02	<0.14	<0.14	<0.14	<0.14	1486	1486 [51]	0.80 [45] [4]	<0.17	390	98	Sweet, fruity [45]
33	1-Octanol [h]	<0.14	<0.14	<0.14	<0.14	<0.14	1560	1562 [22]	0.90 [45] [4]	0.15	5	1	Sweet [64]
1	2,3-Butanediol [g]	2.30 ± 1.90	<0.25	0.27 ± 0.01	0.34 ± 0.02	0.31 ± 0.12	1541	1536 [1]	50.00 [65] [3]	0.01	388	98	Fruit, floral [50]
74	2-Octenol [h]	0.18 ± 0.00	ND	ND	ND	ND	1610	1615 [23]	NF	NC	399	100	Sweet, Floral [66]
75	1-Nonanol [h]	0.16 ± 0.00	ND	ND	ND	ND	1658	1661 [3]	1.00 [65] [3]	NC	395	100	Floral [4]
46	2- Undecanol [h]	0.20	0.19	0.17	0.17	0.15	1724	1716 [8]	0.0041 [67] [4]	36.58	25	6	Fruity [67]
48	1-Decanol [h]	0.18 ± 0.01	0.18 ± 0.02	ND	ND	ND	1766	1767 [30]	0.40 [68] [4]	NC	250	63	Fruit, floral, sweet [50]
76	9-Decen-1-ol [h]	ND	ND	0.66 ± 0.11	0.70 ± 0.17	0.67 ± 0.10	1820	1828 [69]	NF	NC	44	11	NF
77	Phenoxyethanol [e]	ND	ND	ND	0.25 ± 0.00	0.25 ± 0.00	2142	2142 [70]	NF	NC	366	93	Floral [71]
78	Benzyl alcohol [e]	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.41 ± 0.00	0.26 ± 0.00	1876	1876 [72]	40.90 [39] [2]	0.01	25	6	Sweet, fruity [63]
79	Phenylethyl alcohol [e]	0.32 ± 0.01	0.31 ± 0.03	<0.25	ND	ND	1902	1902 [73]	7.74 [38] [1]	NC	244	62	Floral [38]
80	1-Dodecanol [i]	ND	ND	0.45 ± 0.02	0.39 ± 0.05	1.02 ± 0.02	1969	1969 [74]	1.00 [27] [4]	1.02	46	12	Unpleasant in higher levels, flowery in low levels [27]
81	1-Hexadecanol [i]	ND	ND	ND	ND	<0.25	2374	2377 [74]	NF	NC	50	13	Fruity, flowery [75]
	Acids												
4	Acetic acid [j]	ND	ND	ND	358.96 ± 15.54	302.20 ± 5.83	1456	1461 [4]	130 [103] [1]	2.33	227	57	Vinegar [50]

82	3-Methyl-pentanoic acid [k]	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	1671	1780 [76]	NF	NC	10	3	NF
83	Hexanoic acid [k]	0.14 ± 0.00	0.15 ± 0.01	<0.05	<0.05	<0.05	1862	1863 [20]	0.50 [77] [1]	<0.10	243	62	Rancid, sweaty [38]
26	Heptanoic acid [k]	ND	ND	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	1960	1966 [18]	NF	NC	45	11	Sweaty, cheese [79]
28	2-Ethyl-hexanoic acid [k]	0.07 ± 0.00	0.07 ± 0.00	0.1± 0.04	0.17 ± 0.04	<0.05	1957	1954 [19]	NF	NC	50	13	Sweaty [80]
84	Octanoic acid [k]	0.18 ± 0.01	0.17 ± 0.00	ND	ND	ND	2070	2070 [81]	2.71 [39] [2]	NC	242	61	Sweaty, rancid [38]
38	Nonanoic acid [L]	ND	ND	<0.75	<0.75	<0.75	2172	2173 [9]	3.56 [39] [2]	<0.21	46	12	Fruit, floral, ripe, sweet [50]
45	Decanoic acid [m]	0.64 ± 0.05	0.65 ± 0.01	<0.25	ND	ND	2275	2279 [9]	13.74 [39] [2]	NC	245	62	Rancid, sweaty [38]
85	9-Decenoic acid [m]	0.38 ± 0.01	0.39 ± 0.04	0.08 ± 0.02	ND	ND	2327	2335 [16]	0.004 [68] [4]	NC	240	61	Waxy, fatty, soapy [68]
	Terpenes												
86	β-Myrcene [n]	ND	ND	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	1150	1150 [82]	NF	NC	64	16	Peppery, terpene, spicy, balsam [83]
32	Linalool [o]	ND	0.25 ± 0.00	0.26 ± 0.00	0.28 ± 0.00	0.25 ± 0.00	1552	1554 [21]	0.001 [38] [1]	250.00	45	11	Floral [38]
87	α-Humulene [n]	ND	0.25 ± 0.01	0.34 ± 0.00	0.25 ± 0.00	ND	1652	1655 [84]	NF	NC	66	17	Woody [85]
54	β-farnesene [n]	ND	ND	ND	ND	<0.25	1668	1672 [33]	NF	NC	50	13	Fruity [33]
88	α-Terpineol [n]	ND	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.26 ± 0.01	1690	1690 [86]	1.00 [48] [4]	0.26	65	16	Fresh, clean, woody, pine, floral, lime [83]
89	Nerol [n]	ND	ND	0.25 ± 0.00	0.26 ± 0.01	0.27 ± 0.02	1796	1796 [11]	0.40 [87] [4]	0.67	77	19	Citrus, rose, fresh [83]
47	α-Humulene epoxide [n]	ND	ND	ND	ND	0.25 ± 0.00	2044	2048 [29]	NF	NC	363	92	Herbal [83]
90	Nerolidol [n]	ND	ND	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	2042	2039 [88]	1.00 [48] [4]	0.25	80	20	Rose, apple, green, citrus [48]
34	Carvone [n]	ND	ND	0.28 ± 0.00	0.29 ± 0.01	0.28 ± 0.02	1725	1728 [23]	NF	NC	85	22	(4*R*)-(−)carvone: minty, (4*S*)-(+)-carvone: caraway [89]
53	τ-Cadinol [n]	ND	ND	0.29 ± 0.00	0.26 ± 0.01	0.28 ± 0.01	2172	2167 [32]	NF	NC	86	22	Astringent [90]
52	α-Cadinol [n]	ND	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.26 ± 0.01	2247	2251 [28]	NF	NC	70	18	Herb, woody [91]
91	Farnesol [n]	ND	ND	0.26 ± 0.00	0.27 ± 0.01	0.26 ± 0.01	2360	2361 [34]	2.40 [48] [4]	0.10	78	20	Floral [48]

	Phenols												
92	Phenol [e]	ND	ND	0.25 ± 0.00	0.27 ± 0.01	<0.25	1999	2000 [74]	18.90 [39] [2]	<0.01	56	14	NF
93	4-Ethylguaiacol [e]	ND	ND	0.25 ± 0.00	1.34 ± 0.32	1.30 ± 0.20	2028	2028 [92]	0.12 [39] [2]	10.83	218	55	Clove [93]
21	2-Methoxy-4-vinylphenol [e]	0.36 ± 0.02	0.35 ± 0.00	0.66 ± 0.00	0.77 ± 0.13	0.74 ± 0.05	2208	2200 [16]	0.10 [94] [3]	7.4	55	15	Roasted, caramel [59]
94	2-Tert-butyl-4-methylphenol [e]	ND	ND	0.25 ± 0.00	0.26 ± 0.01	0.26 ± 0.00	2226	2235 [95]	0.10 [96] [3]	2.6	50	13	NF
	Lactones												
95	γ-Nonalactone [p]	ND	0.29 ± 0.00	0.25 ± 0.02	0.26 ± 0.00	<0.25	2026	2025 [97]	0.034[38] [1]	<7.35	195	49	Sweet [38]
96	γ-Lactone [p]	ND	0.25 ± 0.00	ND	ND	ND	2150	2152 [98]	NF	NC	40	10	NF
	Furans												
97	2-Furanmethanol [q]	ND	0.28± 0.01	<0.25	<0.25	<0.25	1659	1659 [99]	2.00 [39] [2]	<0.12	379	96	Popcorn, socks/musty, earthy, feet [100]
98	2,3-Dihydro-benzofuran [e]	ND	0.36 ± 0.01	<0.25	<0.25	<0.25			50.00 [45] [4]	0.00	380	96	Milk, cream [45]
	Pyrazines												
10	Tetramethyl pyrazine [e]	ND	0.32 ± 0.00	0.24 ± 0.01	0.25 ± 0.02	<0.25	1471	1478 [9]	80.00 [39] [2]	0.00	374	95	Baked potato, nutty [57]
	Ketones												
99	Acetoin [g]	<0.25	<0.25	<0.25	<0.25	<0.25	1280	1279 [70]	>50 [77] [1]	0.00	0	0	Fatty [101]
25	2-Octanone [g]	ND	ND	0.36 ± 0.05	ND	ND	1283	1283 [17]	0.005 [65] [3]	NC	368	93	Earthy [60]
	Aldehydes												
12	Benzaldehyde [e]	ND	ND	0.26 ± 0.00	<0.25	<0.25	1512	1512 [102]	4.20 [39] [2]	<0.06	56	14	Almond, burnt sugar [38]
	Sulfur compounds												
5	3-Methylthio-1-Propanol [g]	ND	<0.25	<0.25	<0.25	<0.25	1714	1714 [5]	1.0 [45] [4]	<0.25	0	0	Soy sauce, fusel [38]

[a] The analytical curves shown in Table 2 were used for the quantification of volatile compounds as follows: [b] isoamyl acetate; [c] ethyl octanoate; [d] ethyl dodecanoate; [e] benzaldehyde; [f] methyl hexadecanoate; [g] isoamyl alcohol; [h] 1-hexanol; [i] 1-dodecanol; [j] acetic acid; [k] octanoic acid; [L] nonanoic acid; [m] decanoic acid; [n] nerol; [o] linalool; [p] furfural; [q] 2-furanmethanol; [r] RI_{exp}: Experimental retention index obtained in a DB-WAX column of GC/qMS; [s] RI_{lit}: Literature retention index obtained in polar column; [t] Odor threshold (OT); [1] OT determined in beer; When OT data were not available in the literature, other matrices were mentioned as follows: [2] aqueous solution containing 46% ethanol; [3] water; [4] aqueous solution containing 10-12% ethanol; [u] Odor active value (OAV): the ratio between the OT and the measured concentration of each compound; [v] Percentage of the Fisher ratio (FR) obtained in relation to the most discriminant compound (with higher Fisher ratio value). The highest FR value is defined as 100% and the others correspond to x%; ND: not detected; which means that concentration was less than LOD. Porque não colocamos o valor de LOD nestes casos ao invés de ND?

NF: not found; NC: not calculated.

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Artigo II - Carbonyl compounds and furan derivatives with toxic potential evaluated in the brewing stages of craft beer – an exposure risk assessment study

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Abstract

The objective of this study was, for the first time, to validate a method based on headspace-solid phase microextraction using and gas chromatography with mass spectrometric detection in selected ion monitoring mode (HS-SPME-GC/MS) to investigate the free form of target toxic carbonyl compounds [acetaldehyde, acrolein, ethyl carbamate (EC) and formaldehyde] and furan derivatives [including furfural and furfuryl alcohol (FA)] during the brewing stages of craft beer of the types ale and lager. In addition, the exposure risk to these compounds through the consumption of the commercial beers under study was assessed. The validation parameters showed that the method is suitable to simultaneously quantify these compounds, which presented recovery from 90 to 105%, relative standard deviation obtained in repeatability, and precision assays that were lower than 12 and 13.6%, respectively. Limits of detection and quantification for all compounds (values lower than 0.5 and 2.5 μg L^{-1}, respectively) demonstrated the adequate sensitivity of the method. Acetaldehyde, acrolein, formaldehyde and furfuryl alcohol were found in all brewing stages of both beer types, while EC and furfural were not detected [levels lower than the limit of detection (LOD) for these compounds: 0.1 and 0.01 μg L^{-1}, respectively]. Boiling and fermentation of ale brewing seem to be important steps for the formation of acrolein and acetaldehyde, respectively, while boiling resulted in the increase of furfuryl alcohol in both types of beer. Contrary, maturation and/or pasteurization reduced the levels of these compounds in both types of beer. Acrolein was the only compound found in 27% of the commercial samples (1 sample of ale beer and 3 samples of lager) at

concentration (3.8 to 5.4 μg L^{-1}) capable of causing health risk, according to margin of exposure (MOE) approach (MOE values lower than 10,000). This aldehyde may be an environmental contaminant and in the case of ale beer may be formed in boiling step. The increase in concentration of the free form of acrolein has not been verified in lager brewing probably due to the difference between in boiling time between these two types of beer (60 and 90 min for ale and lager, respectively). Besides furfural and FA, other four furan-containing compounds (5-methyl-2-furanmethanethiol, acetylfuran, 5-methylfurfural and γ-nonalactone) were also found in beers, however at levels with no potential for health risk.

Key words: Carbonyl compounds; risk assessment; beer elaboration; toxic compounds.

1. Introduction

Beer is the most consumed alcoholic beverage, with emphasis on the consumption of artisanal beer that has increased worldwide (FAOstat, 2018). Craft beer has been chosen by consumers according to different flavor preferences and is perceived to be of higher quality than beers produced in large scale, due to the raw materials used for brewing (Aquilani, Laureti, Poponi, & Secondi, 2015).

Beers can be classified according to the type of fermentation in ale and lager. Ale beers are considered high fermentation due to fluctuation of yeasts in the fermentation tank during the process, which occurs at 15-25 °C for 5 to 10 days and results in more complex and noticeable aromas. Lager beers are named of low fermentation, which process occurs at 7-14 °C during 7 to 14 days. In this condition, the yeasts remain in the bottom of the fermentation tank, resulting in beers with subtle aroma (Buglass, 2011; Buiatti, 2009). In addition to the fermentation conditions (time and temperature), the yeast strain and the wort composition (types of cereals used in malting, mashing/boiling conditions) also may influence the chemical profile of beer, resulting in a wide range of components, including compounds with toxic potential such as carbonyl compounds [acetaldehyde, acrolein, ethyl carbamate (EC) and formaldehyde] and furan derivatives [especially furfural and furfuryl alcohol (FA)] (Parker, 2012).

These compounds may be present as free species or bound to beer constituents. When these compounds are free form, they are readily available to form adduct with

DNA or cause oxidative stress to human cells (Zamora, Aguilar, Granvogl, & Hidalgo, 2016; Zhu et al., 2009). Acetaldehyde, acrolein and formaldehyde, are highly reactive due to their electrophilic nature, and are able to easily react with the biological nucleophilic targets such as proteins, RNA and DNA. EC requires enzymatic biological activation, via cytochrome P450, into vinyl carbamate epoxide, to covalently bind to DNA, RNA and proteins (Forkert, 2010). In the case of the furan derivatives, the carcinogenic effects were hypothesized to originate from sulfotransferase (SULT)-mediated bioactivation yielding a sulphate ester, which can induce genotoxic and mutagenic effects through a highly electrophilic allyl carbocation (Sachse et al., 2016).

In addition, the International Agency for Research on Cancer (IARC) classifies the acetaldehyde ingested specifically through alcoholic beverages as carcinogenic to humans (group 1). Formaldehyde is also in this group, while EC is classified as probable carcinogenic (group 2A) and furfuryl alcohol was recently considered possible carcinogenic for humans (group 2B). Acrolein and furfural are in group 3, in which the IARC needs further study to classify this compound regarding carcinogenic effects (WHO, 2018).

However, the risk of exposure to these compounds through beer consumption has been poorly studied. Lachenmeier et al. (2012) evaluated the risk of exposure to acetaldehyde, formaldehyde and EC using margin of exposure (MOE) approach from quantitative data disclosed in the IARC monographs for the respective compounds found in beers. MOE is obtained by the ratio of the dose that produces a specific toxic effect in studies using animals and the estimated daily intake. According this study, acetaldehyde present in beer may pose risk to health, EC may pose risk only when present at levels around 33 μg kg^{-1}, while formaldehyde appears not to be a health concern (Lachenmeier, Przybylski, & Rehm, 2012). Formaldehyde also showed no health risk when literature data on the occurrence of this compound were used in the calculation of the MOE by Monakhova et al. (2012). No studies on risk assessment regarding to the exposure to acrolein, furfural and furfuryl alcohol through beer consumption were found in literature.

In beer, acetaldehyde may be formed by yeasts, acetic acid bacteria, and by the oxidation of ethanol and phenolic compounds. Acrolein may arise as a thermal degradation product (temperature higher than 180 °C) from various precursors such as

glycerol, amino acids, carbohydrates and triglycerides or by metabolic activity of microorganisms (Kächele, Monakhova, Kuballa, & Lachenmeier, 2014). In addition, acrolein is formed during combustion processes, particularly of fuels, wood or plastics, therefore, this aldehyde may be present in raw materials due to the environmental contamination (Burcham, 2017). Ethyl carbamate is mainly formed by the reaction of urea with ethanol produced during alcoholic fermentation (Zhao et al., 2013). Formaldehyde is generated from the oxidation of methanol that is derived from Strecker degradation of the amino acid glycine or from the lipid oxidation of polyunsaturated fatty acids present in malt by chemical and enzymatic reactions (Jeong et al., 2015). Furfural and furfuryl alcohol are Maillard products, mainly formed during the kilning of malt and the boiling of wort, in which high levels of sugars and amino acids are subjected to high temperatures. Moreover, furfuryl alcohol may be formed through furfural reduction by the yeast action (Vanderhaegen et al., 2004).

The evaluation of carbonyl and furan derivative compounds is challenging due to their low concentrations, high volatility, high reactivity and the presence of other major compounds. In a previous study of this research group, these compounds were simultaneous quantified in Syrah wines through comprehensive two-dimensional GC with a time-of-flight mass spectrometric detection (GC×GC/TOFMS) (Lago, Nicolli, Marques, Zini, & Welke, 2017). The second chromatographic dimension allowed resolving two co-elutions involving (i) acetaldehyde, 4-methyl pentanoate and limonene and (ii) acrolein and methyl hexanoate. Although GC×GC is an effective analytical tool to analyze complex matrices (Nicolli et al., 2018), it still imposes high costs, which may limit its routinely use. In order to employ a simpler and less expensive method, a GC/MS in selected ion monitoring mode (SIM) approach was developed to simultaneously quantify toxic carbonyl compounds throughout the stages of Merlot winemaking in another previous study (Ferreira, Hernandes, et al., 2018). The matrix effect, related to the presence of ethanol in the samples collected in the subsequent stages to fermentation, was surpassed through the use of analytical curves prepared in model solutions with similar ethanol content of the different stages of vinification (Ferreira, Nicolli, et al., 2018). However, this strategy is laborious and costly. An extra layer of polydimethylsiloxane (PDMS) was added to commercial SPME fiber and proved to overcome the displacement effect of minor compounds, which is caused by

the major matrix constituent (ethanol), in addition to increase the fiber extraction capacity of medium and nonpolar compounds related to the volatile profile along lager brewing (Hernandes, Souza-Silva, Assumpção, Zini, & Welke, 2018).

Therefore, the objective of this study was, for the first time, to evaluate simultaneously the levels of the free forms of carbonyl compounds (acetaldehyde, acrolein, ethyl carbamate and formaldehyde) and furan derivatives, which present toxic potential, in 5 stages of the production of ale and lager beer through a validated HS-SPME-GC/MS-SIM using a PDMS-overcoated fiber. In addition, to investigate whether the consumption of the samples under study may represents risk to the consumer's health.

2. Materials and Methods

2.1. Reagents and chemical standards

Individual stock solutions (1,000 mg L^{-1}) of formaldehyde (Vetec, Rio de Janeiro, Brazil), acetaldehyde (Fluka, Ronkonkoma, USA), acrolein, furfural, FA, EC, 2-furfurylthiol and 2-octanone (Sigma-Aldrich, St. Louis, USA), with purity higher than 98% were prepare in double distilled ethanol (Nuclear, São Paulo, Brazil).

2-Furfurylthiol and 2-octanone were used as internal standards (IS) having in mind that their chemical class and/or structure should be similar to the toxic compounds evaluated in beer. Table S1 of Supplementary Material contains information about the toxic compounds and the IS used to normalize the area of each analyte, including their chemical structures, molecular weight and retention time, in addition to the qualifier and quantifiers ions used for the identification and quantification of the toxic compounds, respectively. In addition, preliminary tests were performed to verify the absence of these compounds in beer samples (results not shown). Ten microliters of a solution containing the two IS (10 mg L^{-1}) was added in 1 mL sample, as well as in the analytical curves before HS-SPME.

2,2,2-Trifluoroethyl hydrazine (TFEH, Aldrich, Steinheim, Germany) was employed as derivatizing agent for determination acetaldehyde, acrolein and formaldehyde. An aqueous solution of 10,000 mg L^{-1} of TFEH was prepared in ultrapure water (Millipore purification system, Bedford, MA, USA) and 100 µL were added in each extraction.

A beer model solution was prepared with (+)-tartaric acid (6 g L^{-1}, Synth, São Paulo, Brazil) in ultrapure water and the pH was adjusted to 4.6 with sodium hydroxide (Nuclear, São Paulo, SP, Brazil) according to CAMPILLO et al. (2009). This value corresponds to the mean pH value of the samples evaluated at each stage of brewing of lager and ale, respectively: mashing (4.7 and 4.5), boiling (4.4 for both), fermentation (4.4 and 4.6), maturation (4.8 and 4.9) and carbonation (4.5 and 4.6). This model solution was used to perform the analytical curves. Solutions containing all the toxic analytical standards were prepared in model solution according to the concentrations showed in Table 1.

2.2. Samples and beer elaboration

In order to verify the influence of brewing stages on the levels of toxic compounds, samples of five stages of the production of ale and lager beers, including mashing, boiling, fermentation, maturation and pasteurization, were obtained from a microbrewery from Porto Alegre, Rio Grande do Sul, Brazil. In addition, others 30 samples from different brands of beers obtained at local supermarkets or donated by the manufacturers were analyzed. Samples were obtained in triplicate and degassed by sonication for 5 min using ice in the water of ultrassom (Ultronique, model Q3.0/40A) to prevent the loss of volatile compounds.

Beers were produced following the scheme of Figure S1 of Supplementary Material as explained in detail in a previous study (Hernandes et al., 2018). Briefly, milled malt (Monster Mill 3 roller, Fayetteville, USA) was solubilized in water (73 °C) and mashed to the complete conversion of the starch into smaller sugars, such as maltose and maltodextrose, which was verified using a refractometer (Akso, model RHB32, São Leopoldo, Brazil). The wort was filtered and then boiled during 60 or 90 minutes for ale beer and lager beer, respectively. Hop was gradually added during boiling. Suspended particles were grouped through mechanical agitation (15 minutes) followed by separation of decanted aggregates. Wort was cooled through plate exchangers (Indupropil, Chiller, São Paulo, Brazil) and *Belgian Strong Ale* yeast (*Saccharomyces cerevisiae var. diastaticus*, Bio4, Fazenda Rio Grande, Brazil) or dry lager yeast *Saccharomyces cerevisiae* (W-34/70, Fermentis, Marcq-en-Baroeul, France) was added to produce lager or ale beer, respectively. Fermentation occurred for 7 days at 20

ºC or 12 °C for the production of ale or lager beer, respectively. The fermentation was considered finished when the readings of °Brix measurement in refractometer remained constant for 3 days, which is equivalent to a density 1.008 and ethanol 4.5%. Maturation was performed at 5 °C for 3 days and a clarifier (Spindasol, AEB Group, Bento Gonçalves, Brazil) was added for decantation of the remaining particles. Beer was kept at 0 °C for 5 to 8 days and CO_2 (White Martins, Triunfo, Brazil) was added followed by bottling and pasteurization (62°C for 2 min).

2.3. Determination of the toxic compounds using HS-SPME-GC/qMS-SIM

Extractions were performed with divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) Stable Flex 2cm fiber (Supelco, Bellefonte, USA). An extra PDMS coating was added to the commercial fiber to minimize ethanol effect in extraction performance as discussed in a previous study (Hernandes et al., 2018). Headspace extraction was performed with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) according to Ferreira et al. (2018) using 1 mL of sample, 30 % of NaCl (m/v), without agitation and extraction temperature of 55 °C.

Toxic compounds were evaluated by a Shimadzu gas chromatograph coupled to a mass spectrometer detector QP 2010S (GC/qMS) and separated by a DB-Wax (30 m × 0.25 mm × 0.25 µm) polar column. Oven temperature was kept at 35 °C for 5 min and it was heated up to 140 °C at a rate of 3 °C min^{-1}, reaching a final temperature of 240 °C at 20 °C min^{-1}. Injector and detector temperature were kept at 240 °C, helium (analytical purity 99.999%, Linde Gases, Canoas, RS, Brazil) flow rate was 1 mL min^{-1} and desorption was made in the splitless mode. The MS parameters included electron ionization at 70 eV and ion source temperature at 250 °C in SIM mode.

Analytes were positively identified comparing the retention times (t_R) and mass spectra of unknown compounds with those of standard compounds through co-injections. The qualifier and quantifier ions of toxic compounds and IS used in SIM mode of GC/MS for identification and quantification, respectively, were presented in Table S1 of the Supplementary Material. Analytical curves were prepared according item 2.1 of Material and Methods section. Quantitative data were obtained by the internal standard method using the ratio between the area of quantifier ion of each toxic compound and the area of the quantifier ion of the appropriate IS. In order to verify the

incidence of other furan-containing compounds (besides furfural and furfuryl alcohol), samples were also evaluated in the scan mode using Total Ion Current (TIC) and furan-containing compounds were tentatively identified by the retention index and mass spectra.

Validation parameters of the method used to quantify toxic compounds were: linearity, recovery, precision, repeatability, limit of quantification (LOQ) and detection (LOD), which were verified according (ICH, 2005). The lowest, intermediate and highest concentrations of the analytical curve of each toxic compound were used to determine recovery, repeatability and precision. Repeatability and precision were obtained by the coefficient of variation (CV) of four independent assays performed under the same analytical conditions on the same and in four different days, respectively.

2.4. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey test (Statistica 7.1 software, StatSoft, Inc.Tulsa, USA) was used to verify if there is a significant difference between the levels of toxic compounds found in the brewing stages. In addition, the t-test was used to compare lager and ale beer in relation to the levels of toxic compounds found at each stage of brewing. Differences were considered significant when $p < 0.05$ for both Tukey and t-test.

2.5. Determination of the estimated daily intake (EDI) of toxic compounds through beer consumption and risk characterization of the samples under study

The exposure of toxic compounds found in beer samples under study was estimated as follows:

$$EDI\ (\mu g\ kg^{-1}\ b.w.\ day^{-1}) = \frac{concentration\ of\ toxic\ compound\ (\mu g\ mL^{-1}) \times beer\ consumption\ (mL\ day^{-1})}{body\ weight\ (kg)}$$

The concentration of toxic compounds was obtained as mentioned in item 2.3. The beer consumption of 300 and 600 mL for women and men, respectively, was used in the calculation of EDI. This amount has been mentioned in studies that show the

positive effect of the moderate beer consumption (containing 4.5% of ethanol) (de Gaetano et al., 2016; Matsumoto, Miedema, Ofman, Gaziano, & Sesso, 2014; Nogueira, do Rio, Lollo, & Ferreira, 2017). This concept of moderate consumption is also adopted by Health Agency of Canada (CAMH, 2015), United States of America (USDA, 2015) and the International Alliance for Responsible Drinking (IARD, 2018). The protective effect occurs due to the alcoholic content of beer, which is reported to increase the high-density lipoprotein (HDL), in addition to the polyphenols presence, which has antioxidant potential (Matsumoto et al., 2014). Body weight of 60 and 72 kg was considered the average weight of Brazilian women and men, respectively, according the Analysis of Personal Food Consumption done by Brazilian government (IBGE, 2011).

Since genotoxic compounds, as formaldehyde, acetaldehyde, acrolein and EC, have no safe intake parameters established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), MOE must be used to the risk characterization (WHO, 2010). In this study, the lowest limit of the 95% confidence interval of the dose required to give a 10% increase in the occurrence of a toxic effect compared to the control (benchmark dose lower confidence limit, BMDL10) was used in MOE calculation and are presented in Table S2 of the Supplementary Material. EDI of toxic compounds was obtained as mentioned in Section 2.5. MOE was calculated as follows:

$$MOE = \frac{BMDL10\ (\mu g\ kg^{-1}\ BW\ day^{-1})}{EDI\ (\mu g\ kg^{-1}\ BW\ day^{-1})}$$

MOE lower than 10,000 has been proposed as an indication of concern from the perspective of public health (WHO, 2010).

Non-genotoxic compounds, as furfural and FA (considered food additives with flavoring function), present an acceptable daily intake (ADI) of 0.5 mg per kg of body weight (BW) established by JECFA (JECFA, 2000). In this study, the comparison of EDI of furfural and FA (calculated as mentioned above) with their ADI was used to assess the potential risks to human health. Risk may exist if the estimated intake exceeds the ADI.

3. Results and Discussion

3.1. Validation of the HS-SPME-GC/MS-SIM method

The performance of the method used for simultaneous quantification of toxic compounds during brewing using the PDMS-overcoated fiber is shown in Table 1. The calibration curves showed adequate linearity with determination coefficients (r^2) ranging from 0.9731 to 0.9960 for acetaldehyde and EC, respectively. LOD and LOQ demonstrated the adequate sensitivity of the method. The lowest LOD was found for acetaldehyde (0.03 µg L^{-1}), while acetaldehyde, EC, formaldehyde and furfural present the lowest LOQ value (1.0 µg L^{-1}). Furthermore, the relative standard deviation obtained in repeatability and precision assays were lower than 12.0 and 11.5%, respectively, and recoveries ranged from 90 to 100%, indicating the efficiency of the proposed method.

Table S3 shows a comparison between the HS-SPME-GC/MS-SIM with other methods found in the literature for the determination of the target compounds in beer. It is important to note that some studies have not reported some important method validation parameters, such as LOQ and/or recovery. Furthermore, the values of LOD for acetaldehyde (0.03 µg L^{-1}), acrolein (0.3 µg L^{-1}), formaldehyde (0.1 µg L^{-1}) and furfural (0.1 µg L^{-1}) obtained in the HS-SPME-GC/MS-SIM herein presented were lower than those reported in the literature for acetaldehyde evaluated by headspace-GC with flame ionization detector (FID) (5 µg L^{-1}) (Liu, Li, Niu, Zheng, & Zhao, 2018) or MS detection (30 µg L^{-1}) (Zapata, Mateo-Vivaracho, Lopez, & Ferreira, 2012), formaldehyde quantified using solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) with diode array detection (3000 µg L^{-1}) (Hu & Wang, 2015), acrolein (0.51 and 14 µg L^{-1} as shown by Moreira et al., 2013 and Kächele et al., 2014, respectively) and furfural (1.6 and 2.8 µg L^{-1} as reported by (Carrillo, Bravo, & Zufall, 2011) analyzed using HS-SPME-GC/MS. LOD obtained for the EC in the method under study was the same than reported by Li et al. (2014) that used SPE-GC / MS (0.1 µg L^{-1}), whereas the methods available in the literature for furfural quantification have not mentioned validation parameters (Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014).

In addition, the proposed method requires less time and labor for the analysis of each sample when compared to the literature methods (Table S3). This vantageous

throughput is related to the simultaneous quantification of six compounds in about 90 minutes (mean of 15 minutes for each compound), whereas the literature presents only methods focused on the individual determination of these compounds in beer with analysis times of the acetaldehyde, acrolein, EC, formaldehyde, furfural and FA at least 27 min (Gonçalves et al., 2010), 57 min (Kächele et al., 2014), 21 min (G. Li, Zhong, Wang, & Gao, 2017), 20 min (Deng et al., 2016), 24 min (M. Li, Yang, Yang, Shan, & Dong, 2009) and 61.5 min (Vanderhaegen et al., 2003).

3.2. Carbonyl compounds and furan derivatives throughout the brewing stages

Figure 1 shows the levels of the toxic compounds found after mashing, boiling, fermentation, maturation and pasteurization of lager and ale brewing. EC and furfural were not detected along brewing (values lower than LOD of the method for these compounds: 0.1 and 0.01 μg L^{-1}, respectively), therefore these compounds were not included in Figure 1. Acetaldehyde, acrolein, formaldehyde and FA were found in all brewing stages of ale (Figure 1A) and lager (Figure 1B).

The levels of the toxic compounds found throughout the brewing stages were discussed based on the results of ANOVA followed by Tukey test performed for each type of brewing (ale and lager) as presented in Figure 1 and Table S4. In addition, comparisons between the levels of the toxic compounds quantified in ale and lager beers were made considering the *p*-values shown in Table S5.

Acetaldehyde was found at levels statistically higher (15.2 μg L^{-1}) after fermentation than the other brewing stages (2.2, <1.0 μg L^{-1}, 1.5 and 1.8 μg L^{-1} for mashing, boiling, maturation and pasteurization stages, respectively) of ale brewing. However, in lager beer, the levels of this aldehyde after fermentation (1.3 μg L^{-1}) were statistically similar to those found in the previous stages (mashing and boiling, <1.0 μg L^{-1} for both stages, which is the LOQ of the method), as well as in the subsequent stages of brewing (maturation and pasteurization, 2.0 and 1.3 μg L^{-1}, respectively). It should be noted that the levels of this aldehyde after fermentation were statistically higher in the ale (15.2 μg L^{-1}) than lager (1.3 μg L^{-1}, $p = 0.02$ according to the t test shown in Table S5), indicating that the conditions of fermentation (yeast strain and temperature) play an important role in the formation of this compound. Fermentation of ale and lager

beer occurred, respectively, with *Belgian Strong Ale* yeast (*S. cerevisiae var. diastaticus*) at 20 ºC and *S. cerevisiae* yeast at 12 ºC. Webersinke et al., (2018) also verified that the fermentation temperature had a significant impact on the acetaldehyde level found in ale beer, since the concentration of this aldehyde was significantly higher when the wort was fermented at 25.7 ºC (14400 µg L^{-1}) than when fermentation occurred at 14.3 ºC (3700 µg L^{-1}).

It is important to note that after maturation of ale beer, significant reduction (around 90%) in acetaldehyde levels (1.5 µg L^{-1}) was verified in relation to the previous stage (fermentation, 15.2 µg L^{-1}). The ligation between acetaldehyde and phenolic compounds due to the electrophilic and nucleophilic character, respectively, of these compounds (Sheridan & Elias, 2016) may explain the reduction of free acetaldehyde levels (analyzable by the HS-SPME-GC/MS method). This reduction had not been found after lager maturation, since the concentration of this aldehyde was similar after fermentation and maturation (1.3 and 2.0 µg L^{-1}, respectively). The concentration of phenolic compounds depends mainly on the malt (type of cereal such as barley, corn and wheat) and hop varieties and quantities used in brewing (Šimić et al., 2017). According to information provided by the brewery partner of this study, ale beer was produced using barley and wheat malts (66 and 34%, respectively), while in lager brewing as used barley and melanoidin (95 and 5%, respectively) malts. In addition, ale beer is produced with 25% more hop than is used in lager. These differences result in distinct phenolic profile between ale and lager brewing (Fogarasi, Kun, Tankó, Stefanovits-Bányai, & Hegyesné-Vecseri, 2015), and may explain the differences of acetaldehyde levels after maturation of these beer types. Despite differences in acetaldehyde levels after fermentation and maturation of ale and lager beer, in the last stage of brewing (after pasteurization), the levels of this aldehyde found in beer ale and lager were statistically similar (1.8 and 1.3 µg L^{-1}, respectively; $p = 0.17$ according t-test, Table S5).

Acrolein levels found after mashing and boiling of ale beer production were statistically similar (6.1 and 10.0 µg L^{-1}, respectively), while in the subsequent brewing stages occurred a significant reduction in the levels of this aldehyde reaching 4.1 µg L^{-1} after pasteurization. Similarly, in lager brewing, the acrolein levels were also higher after mashing and boiling (24.8 and 17.7 µg L^{-1}, respectively) than other stages

decreasing to 2.9 μg L^{-1} after pasteurization. The binding of acrolein to other beer compounds such as amino acids and phenolic compounds can occur throughout beer elaboration, which may justify the decrease of the levels of this compound along brewing stages. Baert et al., (2012) reported that imine adduct formation (interaction between carbonyl group of an aldehyde, such as acrolein, with the amino group of amino acid of raw materials) may occur throughout the brewing process. Similarly, the binding between acrolein and phenolic compounds has been reported due to the interaction electrophic/nucleophilic between these compounds (Zamora et al., 2016).

Mashing and boiling steps, in which occur malt heating, may result in acrolein derived from the thermal degradation of amino acids, carbohydrates and triglycerides (Burcham, 2017). The discrepancy of acrolein levels found after mashing in ale (6.1 μg L^{-1}) and lager (24.8 μg L^{-1}) may be related to the differences in the malt composition (concentration of Maillard precursors, amino acids and proteins) used in brewing (levels significantly different according of t-test as presented in Table S5, $p = 0.03$). As previously mentioned, lager brewing is predominantly performed using barley and a small portion of melanoidin malt, while in ale brewing, barley malt was used in higher proportion than wheat malt.

Furthermore, an important aspect to highlight is that the presence of acrolein may also indicate the environmental contamination of the cereal used to produce malt and/or the formation of this compound during thermal stages of malting. In addition, the influence of malting of the cereal, which includes steeping, germination, kilning and roasting, had not been evaluated in this study, since breweries had not performed these stages (only the large breweries produce their own malt). Among these malting stages, kilning and roasting involve heating that is suggested degrade constituents of the cereal used as raw material for malt production (Bagchi & Swaroop, 2016).

Formaldehyde was found in statistically similar levels throughout the stages of ale brewing (from 1.5 to 2.2 μg L^{-1}). In lager beer, significantly higher levels were found after boiling (4.0 μg L^{-1}) than the other stages (2.6 μg L^{-1} after both mashing and fermentation stages, 2.8 and <1.0 μg L^{-1} after maturation and pasteurization). The longest boiling time may justify the higher levels of this aldehyde in lager (90 min) than ale brewing (60 min). Furthermore, as mentioned previously, the type and proportion of malt used in lager and ale are different. Consequently, distinct levels of

formaldehyde precursors can be verified, including amino acids and polyunsaturated fatty acids, which through the degradation and oxidation reactions, respectively, give rise to formaldehyde (Jeong et al., 2015). In the last stage of lager production (pasteurization), formaldehyde levels were significantly reduced (level lower than LOQ of the method, 1.0 μg L^{-1}), probably due to the high reactivity of this compound, which may readily to react with phenolic compounds, as has been to reported to occur in wine (Aleixandre-Tudo et al., 2016).

Furfural was detected at levels lower than the LOQ (1.0 μg L^{-1}) in all stages of brewing of ale and lager, while FA was found in all stages of both types of brewing at levels ranging from 6.9 to 21.9 and 4.7 to 9.0 μg L^{-1} in ale and lager production, respectively. According Vanderhaegen et al. (2004), furfural is formed through Maillard reaction during malt production (kilning or roasting steps) and the reduction of this aldehyde during brewing increases the FA content. Boiling seems to play an important role in FA formation, since the levels of this compound were significantly higher after boiling (21.9 and 9.0 μg L^{-1} for ale and lager, respectively) than the previous stage (mashing; 8.9 and 4.7 μg L^{-1} for ale and lager, respectively) and the subsequent steps (fermentation, maturation and pasteurization; levels in these steps ranging from 9.5 to 6.9; 6.9 to 5.0 and 8.9 to 5.8 μg L^{-1} for ale and lager, respectively).

According to t-test presented in Table S5, FA levels were significantly higher in all stages of ale than lager brewing, indicating that the differences between raw material (ale beer: 66% barley and 34% wheat malts and 25% more hop than lager beer; lager beer: 95% barley and 5% melanoidin malts), boiling time (60 and 90 min), yeast strain (ale beer: *S. cerevisiae var. diastaticus;* lager beer: *S. cerevisiae*) and temperature of fermentation (20 and 12 °C), respectively, may also influence on the levels of this compound. Melanoidin malt is rich in products from Maillard reaction, including furan-containing compounds (Carvalho, Correia, Lopes, & Guido, 2014).

3.3. EDI and MOE of toxic compounds through the consumption of commercial beers

Table 2 shows the levels, EDI and MOE calculated for the toxic compounds under study evaluated in commercial beers (both ale and lager).

Acetaldehyde, acrolein, formaldehyde and FA were detected in all samples. Acetaldehyde was found at quantifiable levels in one ale beer (1.8 µg L^{-1}) and 2 lager samples (1.3 and 2.5 µg L^{-1}). Other samples presented this aldehyde between 0.03 and 1.0 µg L^{-1}, which is the LOD and LOQ of the method, respectively. Higher levels of acetaldehyde were found in Chinese beers, which type was not mentioned by the authors, ranging from 1.42 to 8.16 mg L^{-1} (Liu et al., 2018) and in Korean beers varying from 2.72 to 11.63 mg L^{-1} (Kim et al., 2017).

Acrolein was found in one ale beer (4.1 µg L^{-1}) and eight lager beers at levels ranging from 2.6 µg L^{-1} to 5.4 µg L^{-1}. This aldehyde was found from 0.3 to 2.5 µg L^{-1}, which are the LOD and LOQ of the method, respectively. In lager beers from Portugal, acrolein was found in concentration varying from 0.976 to 4.61 in 60% of samples (Moreira, Meireles, Brandão, & de Pinho, 2013).

EC was detected in three samples (one ale and two lager beers) at levels ranging between the LOD and LOQ of the method (0.1 to 1 µg L^{-1}). In Chinese beer (the authors have not specified the type of beer), EC was found at levels ranging between the LOD (0.1 µg L^{-1}) and 19.6 µg L^{-1} (Choi et al., 2017). Mo et al. (2014) investigated the concentration of EC in beers (type was also not specified) were lower (<2-6 µg kg^{-1}) than those in the other fermented products such as grape wine (8-46 µg kg^{-1}), rice wine (13-575 µg kg^{-1}) and soy sauce (<2-93 µg kg^{-1}).

Formaldehyde was quantified in only one commercial lager beer sample (2.6 µg L^{-1}). In ale beer and other lager samples this aldehyde was detected at levels ranging from 0.3 to 1.0 µg L^{-1}, which is the LOD and LOQ of the method, respectively. In Chinese beers, lower levels of formaldehyde were found in lager beers (0.062-0.453 µg L^{-1}) (Hu and Wang, 2015), Wang et al. (2012) found higher levels of this compound ranging from 172 to 385 µg L^{-1} (the authors have not specified the type of evaluated beer).

Furfural was the toxic compound found in higher concentrations in both type of beers. This compound was detected in 3 ale beers at levels between 523.1 and 4264.3 µg L^{-1} and in 18 lager samples at levels ranging from 1.15 to 4116.0 µg L^{-1}. In Belgian ale beers, levels of furfural ranged from 48 to 2535 µg L^{-1} in all samples (Vanderhaegen et al., 2003).

FA was quantified in all ale and lager samples at levels ranging from 4.2 to 20.7 µg L^{-1} and 5.8 to 30.9 µg L^{-1}, respectively. This alcohol was found in lager beers from

Turkey at levels around 1.5 μg L^{-1} (concentration range of this compound found in the samples was not mentioned by the authors) (Akillioglu, Mogol, & Gökmen, 2011). In Spanish beers (the beer type was not mentioned in this study), FA levels ranged from 0.8 to 1.2 μg L^{-1}, while furfural levels were found from 0.9 to 3.5 μg L^{-1} (Riu-Aumatell et al., 2014).

In order to assess if the beer samples under study may pose risk due to the occurrence of carbonyl compounds and furan derivatives, the EDI was determined according the approach mentioned in section 2.5. Furfural showed the highest EDI (12.6 and 5.2 μg kg^{-1} of BW for men and women, respectively, Table 2), followed by acrolein (2.9 and 2.6 μg kg^{-1} of BW for men and women, respectively), FA (0.1 and 0.06 μg kg^{-1} of BW for men and women, respectively), acetaldehyde (0.03 and 0.02 μg kg^{-1} of BW for men and women, respectively) and formaldehyde (0.03 and 0.008 μg kg^{-1} of BW for men and women, respectively). EDI to EC was not calculated since this compound was not detected at levels higher than the LOQ of the method (1.0 μg L^{-1}).

In addition to furfural and FA, other four furan-containing compounds (5-methyl-2-furanmethanethiol, acetylfuran, 5-methylfurfural and γ-nonalactone) were found in ale and lager commercial beers (Table 3). γ-Nonalactone was the most frequently detected furan-containing, which was found in 6 ale (resulting in EDI from 2.4 to 17.2 μg kg^{-1} of BW for men and from 1.5 to 10.3 μg kg^{-1} of BW for women) and 21 lager beers (resulting in EDI from 2.1 to 59.0 μg kg^{-1} of BW for men and from 1.2 to 35.4 μg kg^{-1} of BW for women). None of these furan-containing compounds found is the samples under study have been included in the safe ingestion parameter of furan-derived compounds (500 μg kg^{-1} of BW) established by JECFA (2000). Even if the other furan-compounds detected in this study were included in ADI, the EDI would not exceed the safe parameter, indicating that the exposure to these compounds through the consumption of these samples does not represent a risk to human health.

Since acetaldehyde, acrolein, EC and formaldehyde are genotoxic compounds, the risk characterization was performed through the determination of MOE. Exposure to acrolein may pose risk on the consumer health only for men, since 1 ale sample and 3 lager samples showed calculated MOE value lower than 10,000 as shown in Table 2. On the other hand, exposure to acetaldehyde, EC and formaldehyde do not represent risk to consumers health through the consumption of beers under study.

It is worth mentioning that in addition to beer, other fermented products are exposure source of carbonyl compounds, such as wine, yogurt, cheese and kefir. Furthermore, coffee, breakfast cereals, fried food items and bakery products are well known to contain furan derivatives. Specifically regarding acetaldehyde, the endogenous formation of this aldehyde in the oral cavity, gastrointestinal tract and liver from the oxidation of ethanol by alcohol dehydrogenase is recognized as the major exposure source to this aldehyde (Pflaum et al., 2016).

Furthermore, the calculation of EDI and risk assessment approach were performed using the concept of moderate consumption of beer, which also considers some contraindications (i.e, the consumption of alcoholic beverage, at any dosage, is not recommended for children, adolescents, pregnant women, individuals at risk of alcoholism, those with cardiomyopathy, cardiac arrhythmias, depression, or liver and pancreatic diseases, or during performing actions that require concentration, skill or coordination) and in the context of healthy eating/lifestyle may reduce the risk of cardiovascular diseases (de Gaetano et al., 2016; Nogueira et al., 2017). It should be noted that the alcoholic drinking has been also associated to the increase of risk of oral and pharyngeal, esophageal and breast cancer and, the excessive alcohol (beer) consumption exerts deleterious effects on the human body, with increased risks for many organs, but most primarily for the liver. In addition, there are social problems such as addiction, accidents, violence and crime that may be associated to alcoholism (Nogueira et al., 2017). Despite the deleterious effects of alcohol exposure, moderate consumption of beer need not necessarily be discouraged for healthy individuals and, therefore the concept of moderate consumption was adopted as a hypothetical approach to evaluate the risk of exposure to carbonyl compounds and furan derivatives present in beers under study.

4. Conclusion

The HS-SPME-GC/qMS method showed adequate linearity, repeatability, recovery and precision as well as limits of detection and quantification satisfactory to quantify simultaneously six target compounds during brewing. Boiling and fermentation seems to be important steps in toxic compound formation, while maturation and pasteurization are crucial in the reducing of these compounds in both

ale and lager production. Therefore, differences in the composition of the raw material, boiling time and temperature during fermentation may be related to the differences in the levels of toxic compounds found in lager and ale brewing.

Acrolein was the only compound found in concentration capable of causing health risk in 27% of the samples under study. This aldehyde was found in all stages of the production of both types of beer. Acrolein may be an environmental contaminant and the differences in quality/quantity/type of raw material used to produce lager and ale beer may contribute to the different concentrations of acrolein found in the first stage of production of these two types of beer. In addition, the boiling step employed in ale brewing was the only step that resulted in increased acrolein concentration. Therefore, boiling conditions (time/temperature) should be studied to reduce the formation of acrolein.

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Zhu, Q., Zheng, Z. P., Cheng, K. W., Wu, J. J., Zhang, S., Yun, S. T., … Wang, M. (2009). Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chemical Research in Toxicology*, *22*(10), 1721–1727. https://doi.org/10.1021/tx900221s

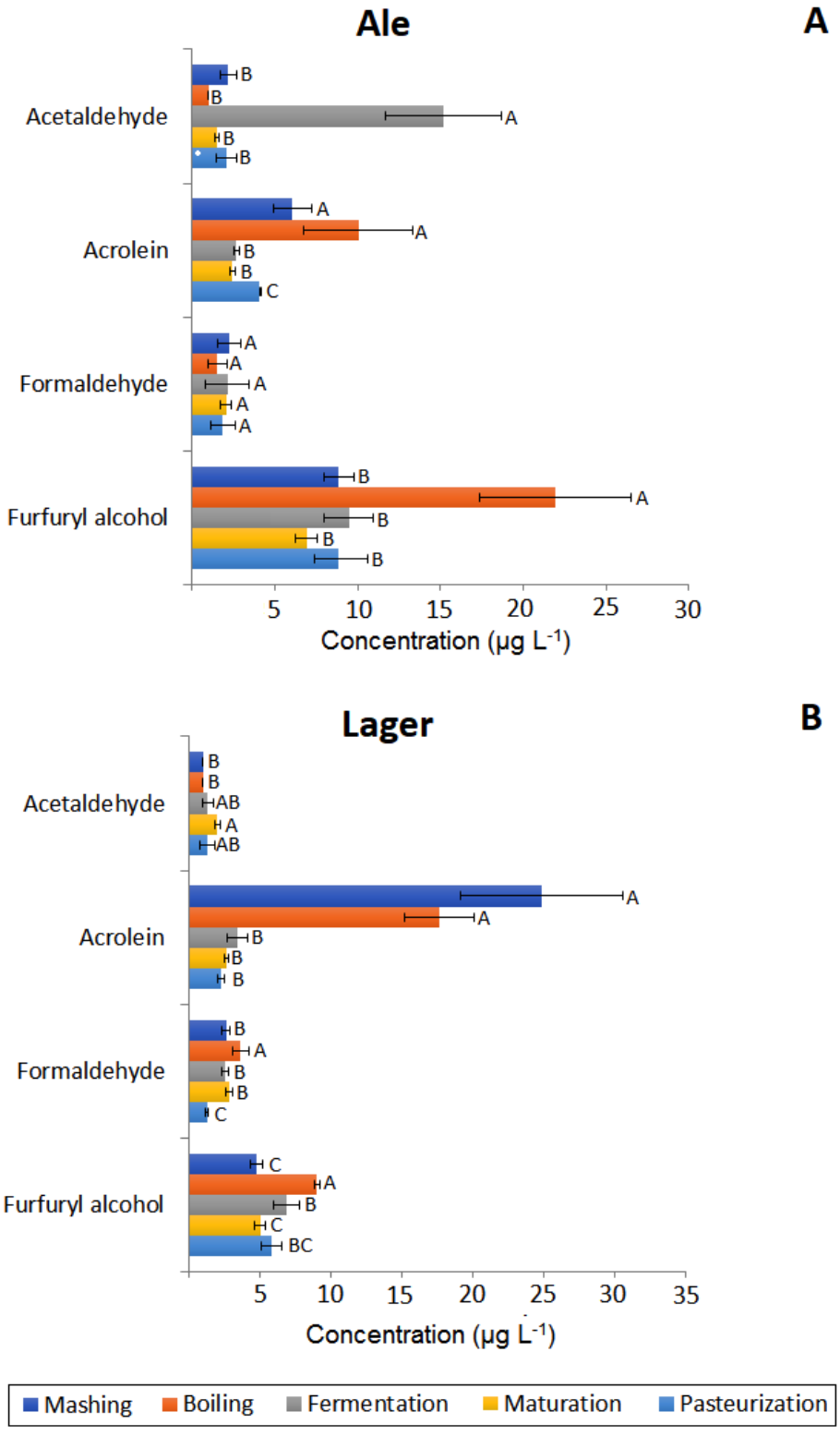


Figure 1. Levels of acetaldehyde, acrolein, formaldehyde and furfuryl alcohol found in five stages of brewing (mashing, boiling, fermentation, maturation and carbonation) of ale (A) and lager (B) beers. Bars related to a compound in the brewing stages followed by the same letter indicate that the levels are not statistically different ($P < 0.05$) by Tukey test.

Table 1. Figures of merit of the analytical method employed for the determination of toxic compounds found during beer elaboration using gas chromatography with mass spectrometric detection (HS-SPME-GC/MS) in selected ion monitoring mode (SIM) mode.

Compound	Range (µg L^{-1})	Regression equation	r^2 [a]	LOD (µg L^{-1})[b]	LOQ (µg L^{-1})[c]	Conc. (µg L^{-1})[d]	Rec. (%)[e]	RSD Rep.(%)[f]	RSD Prec.(%)[g]
Acetaldehyde	1.0-25	y = 32852x + 910106	0.9731	0.03	1.0	1.5	98	6.3	11.5
						73	91	5.2	9.8
						146	95	9.0	7.0
Acrolein	2.5-100	y = 180355x + 7739	0.9948	0.3	2.5	2.5	91	8.2	10.9
						10	90	1.0	10.5
						100	96	0.9	8.3
Ethyl carbamate	1.0-100	y = 1002740x + 14248	0.9960	0.1	1.0	1.0	98	6.9	13.6
						10	97	0.9	11.0
						100	100	2.9	9.1
Formaldehyde	1.0-100	y = 1347.3x + 134500	0.9945	0.3	1.0	1.0	105	5.3	7.8
						10	100	12.0	10.8
						100	90	5.6	7.4
Furfural	1.0-100	y = 852.94x + 35579	0.9917	0.1	1.0	1.0	98	3.7	3.6
						70	100	7.4	9.8
						100	93	3.1	7.0
Furfuryl alcohol	2.5-100	y = 175.790x + 1678.7	0.9937	0.5	2.5	2.5	100	2.8	4.2
						10	94	11.1	10.7
						100	94	5.5	9.2

[a]r^2: Determination coefficient; [b]LOD: Limit of detection; [c]LOQ: Limit of quantification; [d]Conc.: Concentration corresponding to the lowest, intermediate and higher concentration of analytical curve of each toxic compound used for determining the percentage of repeatability, reproducibility and recovery; [e]Rec.: Recovery; [f]Rep.: Repeatability (n=7): coefficient of variation of seven independent assays performed under the same analytical conditions on the same day; [g]Prec.: Intermediate precision (n=16): coefficient of variation of four independent assays performed under the same analytical conditions in four different days.

Table 2. Levels ± standard deviation (μg L^{-1}), estimated daily intake (EDI, μg kg^{-1} of body weight)* and margin of exposure (MOE) for men and for women calculated for toxic compounds found in beers analyzed by HS-SPME-GC/qMS.

	Sample	Acetaldehyde			Acrolein			Ethyl carbamate (EC)			Formaldehyde			Furfural		Furfuryl alcohol	
		Level	EDI	MOE	Level	EDI	MOE	Level	EDI	MOE	Level	EDI	MOE	Level	EDI	Level	EDI
Ales	A1	1.8 ± 0.9	0.02	2687238; 5374475	4.1 ± 1.8	0.04; 0.01	**8804**; 28883	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	11.9 ± 5.5	0.12; 0.06
	A2	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	4.2 ± 0.4	0.04; 0.02
	A3	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	523.1 [a] ± 414.3	5.23; 2.6	18.0± 2.5	0.18; 0.09
	A4	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	11.2 ± 1.1	0.11; 0.06
	A5	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	20.7 ± 1.2	0.21; 0.10
	A6	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	12.5 ± 1.0	0.12; 0.07
	A7	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<1.0	<0.025	>14400	<1.0	<0.025	>1120000	4264.3 [a] ± 3438.1	42.64; 21.3	14.5 ± 1.4	0.15; 0.13
	A8	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	417.7 [a] ± 218.9	4.18; 2.1	5.7 ± 0.9	0.06; 0.03
Lagers	L1	<1.0	<0.01	>5600000	3.1 ± 0.9	0.03	11616; 23231	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	8.3 ± 0.5	0.08; 0.04
	L2	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	427.7[a] ± 124.3	4.28; 2.14	9.4 ± 3.7	0.09; 0.05
	L3	1.3 ± 0.5	0.01	4194764; 7095753	2.9 ± 1.2	0.03	12361; 31958	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	5.8 ± 0.7	0.06; 0.03
	L4	<1.0	<0.01	>5600000	2.6 ± 0.6	0.03	13793; 31746	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	370.7[a] ± 87.9	3.71; 2.50	9.7 ± 0.7	0.10; 0.05
	L5	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	1581.6 [a] ± 964.4	15.82; 6.76	9.4 ± 1.5	0.09; 0.05;
	L6	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	7.1 ± 1.2	0.07; 0.04
	L7	<1.0	<0.01	>5600000	3.8 ± 0.6	0.04	**9556**; 19112	<1.0	<0.025	>14400	<1.0	<0.025	>1120000	1351.5 [a] ± 526.8	13.51; 2.50	8.7 ± 2.0	0.09; 0.04
	L8	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	1232.4 [a] ± 395.6	12.32; 2.50	10.7 ± 1.3	0.11; 0.05

L9	2.5 ± 0.6	0.03	2013009; 4026017	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	4264.3 [a] ± 267	4.35; 2.62	6.6 ± 0.7	0.06; 0.03
L10	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	1.15 ± 0.91	1.14; 0.5	26.5 ± 3.0	0.26; 0.13
L11	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	2082.3 [a] ± 507.0	20.82; 10.4	30.9 ± 6.5	0.31; 0.15
L12	<1.0	<0.01	>5600000	2.6 ± 0.3	0.03	14207; 43265	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	4116.0 [a] ± 3043.6	41.16; 20.6	9.1 ± 1.1	0.09; 0.05
L13	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	767.4 [a] ± 88.3	7.67; 3.8	16.5 ± 1.5	0.16; 0.08
L14	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	2403.4 [a] ± 1205.3	24.03; 12.0	11.6 ± 0.9	0.12; 0.06
L15	<1.0	<0.01	>5600000	2.6 ± 0.5	0.03	14061; 33087	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	364.3 [a] ± 94.2	3.64; 1.8	6.2 ± 0.9	0.06; 0.03
L16	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	1524.1 [a] ± 516.2	15.24; 7.6	28.3 ± 4.0	0.28; 0.14
L17	<1.0	<0.01	>5600000	4.5 ± 3.8	0.04	**8001**; 28415	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	545.5 [a] ± 76.2	5.46; 2.7	9.8 ± 1.2	0.10; 0.05
L18	<1.0	<0.01	>5600000	5.4 ± 5.5	0.05	**6679**; 25400	<1.0	<0.025	>14400	<1.0	<0.025	>1120000	<0.1	<1.00	13.3 ± 1.2	0.13; 0.07
L19	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	1341.1 [a] ± 1650.2	13.41; 6.7	9.7 ± 0.8	0.10; 0.05
L20	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	2.6 ± 1.8	0.03; 0.008	1056925; 3443504	421.8 [a] ± 43.5	4.22; 2.1	11.1 ± 8.3	0.11; 0.06
L21	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	575.4 [a] ± 68.0	5.75; 2.9	10.6 ± 2.4	0.11; 0.06
L22	<1.0	<0.01	>5600000	<2.5	<0.025	>10000	<LOD	<0.025	>14400	<1.0	<0.025	>1120000	628.3 [a] ± 293.2	6.28; 3.1	6.9 ± 0.5	0.07; 0.03
Averege	1.9	0.1; 0.02	2965004; 4190943	3.4; 1.9	0.04	10661; 29142	-	<0.025	>14400	2.6	0.03; 0.008	1056925; 3443504	1187.6	12.6; 5.2	12.2	0.1; 0.06
Median	1.8	0.03; 0.02	2687238; 5374475	2.9; 2.6	0.03	10586; 28649	-	<0.025	>14400	2.6	0.03; 0.008	1056925; 3443504	628.3	7.7; 2.56	10.2	0.1; 0.05

* EDI calculated based on the recommended maximum daily intake of beer of 600 mL for men and 300 mL for woman, which corresponds to moderate consumption associated with the beneficial properties of beer; LOQ of acetaldehyde, formaldehyde, EC and furfural: 1.0 µg L^{-1}; LOQ of Acrolein and furfuryl alcohol: 2.5 µg L^{-1}; Furfural LOD: 0.1 µg/L; In red, MOE values that represent risk to consumer health (MOE < 10,000). LOD of EC: 0.1 µg L^{-1}. In samples with toxic compounds levels lower than LOQ, EDI and MOE were calculated using the LOD values of these compounds, since these compounds have not been quantified in this sample (levels within LOD and LOQ). [a] Samples were diluided for analysis since furfural content was higher then the last point of calibration curve.

Table 3. Concentration of furan-containing compounds in ale and lager commercial beers analyzed using HS-SPME-GC/qMS in scan mode.

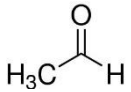
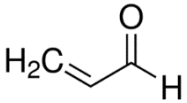
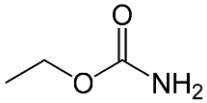
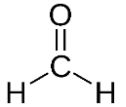
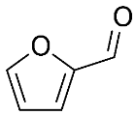
	Sample	5-Methyl-2-furanmethanethiol (μg L^{-1})	Acetylfuran (μg L^{-1})	5-Methylfurfural (μg L^{-1})	γ-Nonalactone (μg L^{-1})	Total concentration per sample (μg L^{-1})	EDI (μg kg^{-1} of body weight)* men	EDI (μg kg^{-1} of body weight)* women
Ales	A1	<0.1	<0.1	<0.1	2059.0 ± 87.3	2059.0	17.2	10.3
	A2	<0.1	<0.1	<0.1	<0.1	<0.1	NC	NC
	A3	259.5 ± 16.3	996.8 ± 36.3	<0.1	273.7 ± 18.6	1530.0	12.7	7.6
	A4	389.3 ± 5.1	<0.1	<0.1	305.6 ± 7.7	694.9	5.8	3.5
	A5	<0.1	<0.1	<0.1	<0.1	NC	NC	NC
	A6	<0.1	<0.1	<0.1	289.5 ± 6.8	289.5	2.4	1.5
	A7	<0.1	<0.1	<0.1	371.6 ± 3.0	371.6	3.1	1.9
	A8	<0.1	<0.1	<0.1	309.3 ± 4.9	309.3	2.6	1.5
Lagers	L1	<0.1	<0.1	<0.1	255.5 ± 2.3	255.5	2.1	1.3
	L2	<0.1	<0.1	<0.1	2352.1 ± 834.6	2352.1	19.6	11.8
	L3	<0.1	<0.1	<0.1	647.5 ± 15.6	647.5	5.4	3.2
	L4	<0.1	<0.1	<0.1	6110.2 ± 82.5	6110.2	50.9	30.5
	L5	<0.1	<0.1	<0.1	287.0 ± 6.4	287.0	2.4	1.4
	L6	<0.1	<0.1	<0.1	284.0 ± 3.2	284.0	2.4	1.4
	L7	<0.1	<0.1	<0.1	299.3 ± 4.1	299.3	2.5	1.5
	L8	<0.1	<0.1	<0.1	262.5 ± 2.3	262.5	2.2	1.3
	L9	<0.1	<0.1	<0.1	1310.5 ± 78.0	1310.5	11.0	6.5
	L10	<0.1	<0.1	<0.1	312.7 ± 7.0	312.7	2.6	1.6
	L11	<0.1	633.7 ± 13.7	<0.1	6449.6 ± 205.6	7083.3	59.0	35.4
	L12	<0.1	<0.1	<0.1	249.4 ± 0.1	249.4	2.1	1.2
	L13	<0.1	<0.1	<0.1	260.0 ± 0.8	260.0	2.2	1.3
	L14	<0.1	1263.3 ± 59.2	<0.1	276.4 ± 5.9	1539.7	12.8	7.7
	L15	<0.1	<0.1	<0.1	272.4 ± 0.8	272.4	2.3	1.4

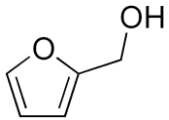
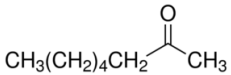
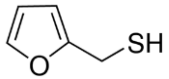
L16	<0.1	556.8 ± 16.6	242.3 ± 66.7	346.9 ± 16.4	1146.0	9.5	5.7
L17	<0.1	<0.1	<0.1	279.8 ± 3.2	279.8	2.3	1.4
L18	<0.1	<0.1	<0.1	<0.1	NC	NC	NC
L19	<0.1	<0.1	<0.1	288.0 ± 9.1	288.0	2.4	1.4
L20	<0.1	<0.1	<0.1	281.9 ± 1.8	281.9	2.3	1.4
L21	<0.1	<0.1	<0.1	295.4 ± 1.8	295.4	2.5	1.5
L22	<0.1	<0.1	<0.1	249.4 ± 0.0	249.4	2.1	1.2
				Total	29321.0	244.4	146.6

* EDI calculated based on the recommended maximum daily intake of beer of 600 mL for men and 300 mL for woman, which corresponds to moderate consumption associated with the beneficial properties of beer; NC: not calculated

Supplementary Material

Table S1. Toxic compounds and their respective retention times (t_R), monitored ions and internal standards (IS) used in the quantification by HS-SPME-GC/qMS-SIM.

	Toxic compound	Chemical structure	MW (g mol^{-1})[a]	t_R (min)[b]	Qualifiers ions	Quantifier ion	Internal standard
Toxic compounds	Acetaldehyde		44.05	13.9	71; 140	71	2-Octanone
	Acrolein		56.06	13.6	83; 55; 152	83	2-Octanone
	Ethyl carbamates		89.08	36.4	62; 74; 89	62	2-Octanone
	Formaldehyde		30.03	12.9	57; 126	57	2-Octanone
	Furfural		96.07	25.9	96; 95	96	2-Furfurylthiol

	Furfuryl alcohol		98.10	29.3	98; 81; 53	98	2-Furfurylthiol
IS[c]	2-Octanone		128.21	18.8	58; 71	58	-
	2-Furfurylthiol		114.17	28.8	81; 53	81	-

[a] Molecular weight; [b] Retention time (minutes).

Table S2. The lower limit of the 95% confidence interval of the dose required to give a 10% increase in the occurrence of a toxic effect compared to the control (BMDL10) and their toxic effects observed after oral exposure to toxic compounds.

Compound	BMDL10 (mg kg^{-1} of body weight per day)	Toxic effect	Reference
Acetaldehyde	56	Tumors in rats (breast, stomach, bowel, testicular)	Lachenmeier et al. (2009)[a]
Acrolein	0.36	Forestomach squamous epithelial hyperplasia in mice	ATSDR (2007)[b]
Ethyl carbamate	0.25	Alveolar and bronchiolar adenoma or carcinoma in male and female mice	Schlatter et al. (2010)[c]
Formaldehyde	28	Chronic atrophic gastritis in rats	Monakhova et al. (2012)[d]

[a] Lachenmeier, D.W., Kanteres, F., Rehm, J. (2009). Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. *Addiction*, 104(4), 533-550; [b] ATSDR, Agency for Toxic Substances and Disease Registry, Toxicological profile for acrolein. *U.S. Department of Health and Human Services*, 2007; [c] Schlatter, J., DiNovi, M., Setzer, R. W. (2010). Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic Example: Ethyl carbamate. *Food and Chemical Toxicology*, 48, S63–S68; [d] Monakhova, Y.B., Jendral, J.A., Lachenmeier, D.W. (2012). The margin of exposure to formaldehyde in alcoholic beverages. *Archives of Industrial Hygiene and Toxicology*. 63(2), 227-237.

Table S3. Comparison of analytical performance of the current HS-SPME-GC/MS-SIM method (gray line) used to simultaneous evaluate carbonyl and furan-derivative compounds in beer with literature methods, which have been focused only on the individual evaluation of these compounds.

Toxic	Method	Extraction	LOD[a]	LOQ[b]	Recovery (%)	Extraction time (min)	Time of instrumental analysis (min)	Reference
				($\mu g\ L^{-1}$)				
Acetaldehyde	GC-FID[c]	HS[i]	5.0	NR[e]	99.8–108.9	30	22.5	(Liu, Li, Niu, Zheng, & Zhao, 2018)
	HPLC–UV[d]	GDME[j]	12.3	41.0	NR[o]	15	12	(Gonçalves et al., 2010)
	GC-FID[c]	HS[i]	NR[o]	NR[o]	NR[o]	40	47	(Kucharczyk & Tuszyński, 2018)
	GC-FID[c]	HS[i]	0.036	NR[o]	98.1	30	~39.5	(Huimin, Hongjun, Xiuhua, & Bing, 2012)
	GC/MS[e]	HS[i]	30.0	NR[o]	80.4	25	~37.25	(Zapata, Mateo-Vivaracho, Lopez, & Ferreira, 2012)
	GC-FID[c]	SPE[k]	39.0	240.0	NR[o]	10-15	48	(Hrivňák, Šmogrovičová, Nádaský, & Lakatošová, 2010)
	GC-FID[c]	HS[i]	NR[o]	NR[o]	NR[o]	NR[o]	NR[o]	(Tian, 2010)
	GC/MS[e]	**HS-SPME[m]**	**0.03**	**1.0**	**91-98**			
Acrolein	GC/MS[e]	HS-SPME[m]	0.51	1.55	98-102	20	56	(Moreira, Meireles, Brandão, & de Pinho, 2013)
	GC/MS[e]	HS-SPME[m]	0.24	0.81	NR[o]	30	~48.5	(Saison, De Schutter, Delvaux, & Delvaux, 2009)
	GC/MS[e]	HS-SPME[m]	14.0	40.0	90.2-94.7	30	27	(Kächele, Monakhova, Kuballa, & Lachenmeier, 2014)
	GC/MS[e]	**HS-SPME[m]**	**0.3**	**2.5**	**90-96**			
Ethyl carbamate	GC/MS[e]	SPE[k]	NR[o]	NR[o]	90-95	NR[o]	21	(Wu, Pan, Wang, Shen, & Yang, 2012)
	GC/MS[e]	SPE[k]	0.1	0.3	95.3-105.2	10	21	(G. Li, Zhong, Wang, & Gao, 2017)
	GC/MS[e]	**HS-SPME[m]**	**0.1**	**1.0**	**97-100**			
Formaldehyde	HPLC-DAD[g]	SD[n]	3000	NR[o]	97.9-99.9	NP[p]	14	(Hu & Wang, 2015)
	HPLC-DAD[g]	SD[n]	0.016	0.042	91.3-104.3	20	12	(Zhao, Wang, Cao, & Guo, 2015)
	HPLC–UV[d]	SPE[k]	3.0	10.0	75-84	NR[o]	20	(Deng et al., 2016)
	GC/MS[e]	**HS-SPME[m]**	**0.1**	**1.0**	**90-105**			
Furfural	HPLC–UV[d]	GDME[j]	1.5	4.9	NR[o]	NR[o]	12	(Gonçalves et al., 2010)
	GC/MS[e]	HS-SPME[m]	1.6	5.2	97-102	30	31.7	(Carrillo, Bravo, & Zufall, 2011)
	GC/MS[e]	HS-SPME[m]	2.8	9.304	NR[o]	30	53	(Saison, De Schutter, Delvaux, & Delvaux, 2008)
	HPLC-PDA[h]	SPE[k]	5.0	NR[o]	99.7-100.1	NR[o]	24	(M. Li, Yang, Yang, Shan, & Dong, 2009)
	LC-UV	NP[p]	NR[o]	NR[o]	91-98	NP[p]	20	(Lo Coco, Valentini, Novelli, & Ceccon, 1995)

	GC/MS[e]	**HS-SPME[m]**	**0.1**	**1.0**	**93-100**			
Furfuryl alcohol	GC/MS[e]	HS-SPME[m]	NR[o]	NR[o]	NR[o]	20	~66.7	(Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas, & López-Tamames, 2014)
	GC/MS[e]	P&T[l]	NR[o]	NR[o]	NR[o]	20	41.5	(Vanderhaegen et al., 2003)
	GC/MS[e]	**HS-SPME[m]**	**0.5**	**2.5**	**94-100**			

[a] LOD: Limit of detection; [b] LOQ: Limit of quantification; [c] GC-FID: Gas chromatography with flame ionization detector; [d] HPLC-UV: high-performance liquid chromatography with ultraviolet detection; [e] GC/MS: Gas chromatography with mass spectrometry; [g] HPLC-DAD: High-performance liquid chromatography with diode array detection; [h] HPLC-PDA: High-performance liquid chromatography with protodiode array detection; [i] HS: Static headspace extraction; [j] GDME: Gaseous diffusion microextraction; [k] SPE: Solid phase extraction; [l] P&T: Purge and trap extraction; [m] HS-SPME: Solid phase microextraction in headspace mode; [n] SD: Steam distillation; [o] NR: Not reported; [p] NP: Extraction not performed.

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Table S4. Levels ± standard deviation (µg L^{-1}) of acetaldehyde, acrolein, formaldehyde, furfural and furfuryl alcohol found in five steps of brewing (mashing, boiling, fermentation, maturation and carbonation) of lager and ale beer. Letters correspond to ANOVA followed by the Tukey test (p = 0.05). Different letters, for each type of beer, indicate significant difference in toxic compounds levels between stages of brewing.

Beer	Stage	Acetaldehyde	Acrolein	Ethyl carbamate Level ± SD (µg L^{-1})	Formaldehyde	Furfural	Furfuryl alcohol
Ale	Mashing	2.2 ± 0.5 ^{B}	6.1 ± 1.2 ^{A}	<0.1	2.2 ± 0.7 ^{A}	<0.1	8.9 ± 0.9 ^{B}
	Boiling	<1.0 ^{B}	10.0 ± 3.3 ^{A}	<0.1	1.5 ± 0.6 ^{A}	<0.1	21.9 ± 4.6 ^{A}
	Fermentation	15.2 ± 3.5 ^{A}	2.7 ± 0.2 ^{B}	<1.0	2.1 ± 1.3 ^{A}	<0.1	9.5 ± 1.2 ^{B}
	Maturation	1.5 ± 0.1 ^{B}	2.5 ± 0.2 ^{B}	<0.1	2.0 ± 0.3 ^{A}	<0.1	6.9 ± 0.7 ^{B}
	Pasteurization	1.8 ± 0.7 ^{B}	4.1 ± 0.0 ^{C}	<0.1	1.9 ± 0.7 ^{A}	<0.1	8.9 ± 1.5 ^{B}
Lager	Mashing	<1.0 ^{B}	24.8 ± 5.7 ^{A}	<0.1	2.6 ± 0.3 ^{B}	<0.1	4.7 ± 0.4 ^{C}
	Boiling	<1.0 ^{B}	17.7 ± 2.5 ^{A}	<0.1	4.0 ± 0.1 ^{A}	<0.1	9.0 ± 0.2 ^{A}
	Fermentation	1.3 ± 0.4 ^{AB}	3.4 ± 0.7 ^{B}	<0.1	2.6 ± 0.4 ^{B}	<0.1	6.9 ± 0.9 ^{B}
	Maturation	2.0 ± 0.2 ^{A}	2.7 ± 0.2 ^{B}	<0.1	2.8 ± 0.2 ^{B}	<0.1	5.0 ± 0.4 ^{C}
	Pasteurization	1.3 ± 0.5 ^{AB}	2.9 ± 1.2 ^{B}	<0.1	<1.0 ^{C}	<0.1	5.8 ± 0.7 ^{BC}

Table S5. Levels ± standard deviation (µg L^{-1}) of acetaldehyde, acrolein, ethyl carbamate, formaldehyde, furfural and furfuryl alcohol with the results of t-test used to compare the levels of toxic compounds found in each stage of lager and ale brewing. In bold green, *p*-value < 0.05 indicate significant difference between the levels of toxic compounds found in the stages of elaboration comparing both ale and lager beer.

Stage	Beer	Acetaldehyde		Acrolein		Ethyl carbamate	Formaldehyde		Furfural	Furfuryl alcohol	
		Level ± SD (µg L^{-1})	*p*-value	Level ± SD (µg L^{-1})	*p*-value	Level ± SD (µg L^{-1})	Level ± SD (µg L^{-1})	*p*-value	Level ± SD (µg L^{-1})	Level ± SD (µg L^{-1})	*p*-value
Mashing	Ale	2.2 ± 0.5	**0.02**	6.1 ± 1.2	**0.03**	<0.1	2.2 ± 0.7	**0.01**	<0.1	8.9 ± 0.9	**0.00**
	Lager	<1.0		24.8 ± 5.7		<0.1	2.6 ± 0.3		<0.1	4.7 ± 0.4	
Boiling	Ale	<1.0	0.28	10.0 ± 3.3	**0.02**	<0.1	1.5 ± 0.6	**0.01**	<0.1	21.9 ± 4.6	**0.00**
	Lager	<1.0		17.7 ± 2.5		<0.1	4.0 ± 0.1		<0.1	9.0 ± 0.2	
Fermentation	Ale	15.2 ± 3.5	**0.01**	2.7 ± 0.2	0.15	<1.0	2.1 ± 1.3	**0.01**	<0.1	9.5 ± 1.2	**0.00**
	Lager	1.3 ± 0.4		3.4 ± 0.7		<0.1	2.6 ± 0.4		<0.1	6.9 ± 0.9	
Maturation	Ale	1.5 ± 0.1	**0.04**	2.5 ± 0.2	0.22	<0.1	2.0 ± 0.3	**0.01**	<0.1	6.9 ± 0.7	**0.00**
	Lager	2.0 ± 0.2		2.7 ± 0.2		<0.1	2.8 ± 0.2		<0.1	5.0 ± 0.4	
Pasteurization	Ale	1.8 ± 0.7	0.17	4.1 ± 0.0	**0.01**	<0.1	1.9 ± 0.7	**0.01**	<0.1	8.9 ± 1.5	**0.00**
	Lager	1.3 ± 0.5		2.9 ± 1.2		<0.1	<1.0		<0.1	5.8 ± 0.7	

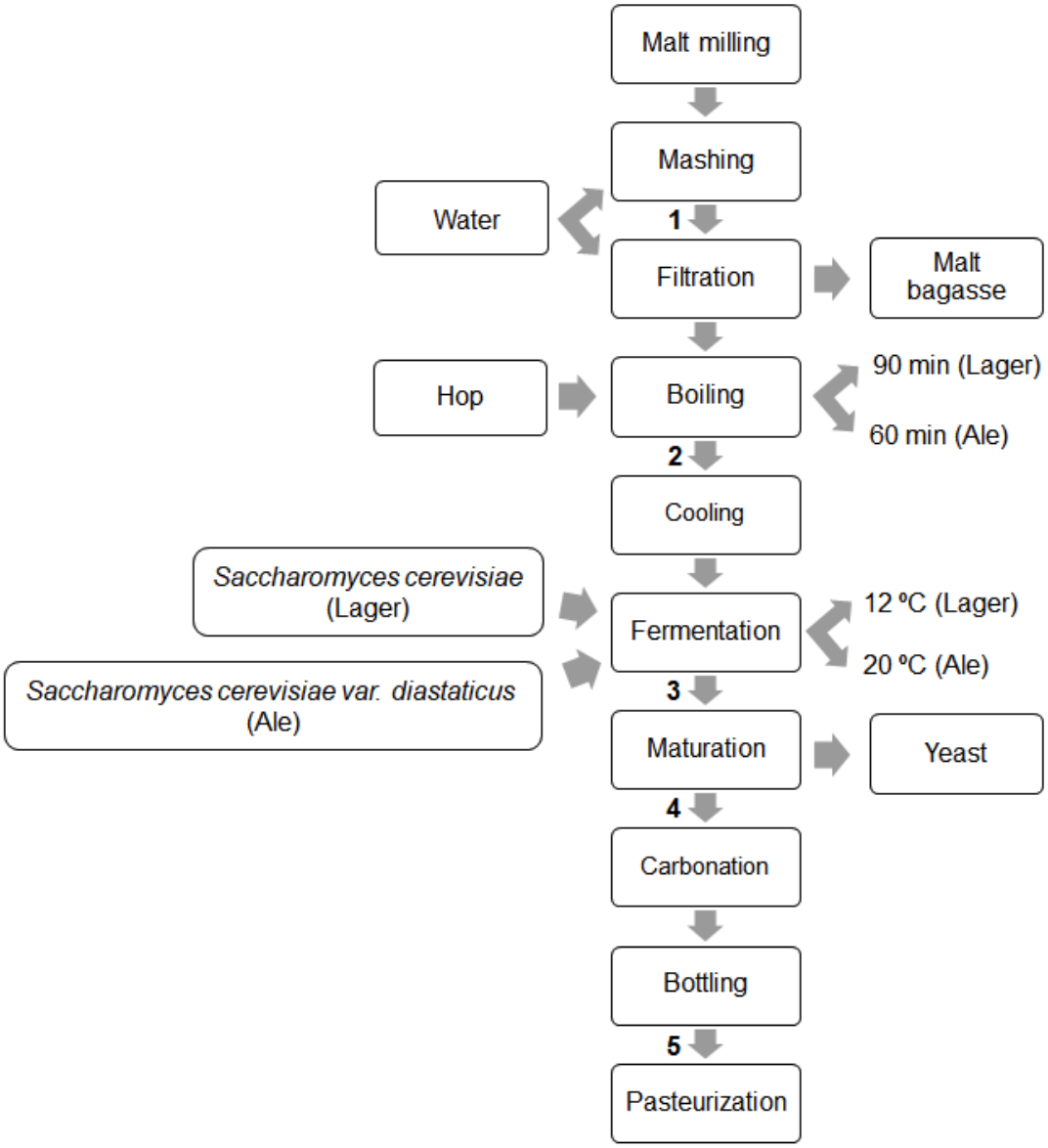

Figure S8. Stages of ale and lager craft brewing indicating the points in which samples were collected for analysis: after (1) mashing, (2) boiling, (3) fermentation, (4) maturation and (5) pasteurization.

5. DISCUSSÃO GERAL

A adição de uma camada extra de PDMS a uma fibra comercial de DVB/Car/PDMS foi avaliada com o objetivo de minimizar o efeito do etanol, que é o composto majoritário de cervejas, em relação à extração de compostos voláteis minoritários. Além disso, o efeito de 5 etapas da produção de cerveja (mosturação, fervura, fermentação, maturação e pasteurização) sobre os níveis de compostos voláteis, que conferem aroma a cervejas artesanais, e de compostos carbonílicos e álcool furfurílico potencialmente tóxicos em cervejas foi verificado. A avaliação do risco da exposição aos compostos tóxicos através do consumo moderado de cervejas comercialmente disponíveis também foi investigada de acordo com protocolos de estimativa de risco da Organização Mundial da Saúde (OMS).

Na primeira etapa deste estudo, a performance de uma fibra DVB/Car/PDMS comercial e de sua versão revestida com PDMS foram comparadas. Os efeitos do tempo de extração (15, 30, 45, 60, 90 e 120 min) e do teor de etanol (0, 4, 8 e 12%) sobre a extração de 12 compostos voláteis de cerveja de diferentes classes químicas (entre ácidos, ésteres, álcoois, aldeídos e terpenos), pesos moleculares (de 60,0 a 270,4 g mol^{-1}) e polaridades (log P entre -0,2 e 7,4) foram avaliados.

Apesar dos compostos polares (log P < 2) apresentarem menor afinidade pela camada extra de PDMS (apolar), que compostos de média polaridade e apolares (2 < log P < 4 e log P > 4, respectivamente), diferenças significativas ($p < 0,05$) nas áreas extraídas entre a fibra modificada e não modificada foram verificadas apenas para os tempos de extração de 15 e 30 min. Para tempos de extração superiores a 45 min, não houve diferença entre as fibras ($p > 0,05$). Para compostos de média polaridade e apolares, a fibra PDMS-modificada apresentou um aumento na capacidade extratora quando comparada à fibra não modificada. Além disso, foi verificado que o equilíbrio é atingido com 60 min de extração tanto para compostos polares quanto apolares.

Em relação ao efeito da concentração do etanol na extração de compostos voláteis, verificou-se que a adição da camada extra de PDMS impediu o efeito do deslocamento do etanol sobre os compostos polares (log P < 2), visto que não houveram diferenças entre as áreas extraídas com o aumento da concentração do etanol das soluções modelo contendo os padrões analíticos. Além disso, um ganho na capacidade extratora de compostos de média polaridade e

apolares foi verificado quando a fibra modificada foi utilizada em todas as concentrações de etanol (0, 4, 8 e 12%) avaliadas.

O perfil volátil de uma cerveja *lager* avaliado por meio da HS-SPME-GC/MS usando ambas fibras foi determinado. A capacidade extratora da fibra revestida com PDMS foi superior à versão comercial, o que foi verificado pelo número de compostos extraídos (61 e 45, respectivamente) e pelo aumento em 20% na área cromatográfica total obtida pela fibra revestida com PDMS comparada à sua versão não modificada. O desempenho superior da fibra PDMS-modificada em comparação com a fibra comercial foi verificado principalmente em relação aos compostos apolares (18 e 8 compostos extraídos, respectivamente) e de média polaridade (25 e 19 compostos extraídos, respectivamente), mostrando que estes compostos possuem maior afinidade pela camada extra de PDMS.

Entre os 16 compostos extraídos exclusivamente pela fibra revestida com PDMS (10 apolares e 6 de média polaridade), pode-se destacar compostos que impactam tanto positiva quanto outros que tiveram contribuição negativa para o aroma de cervejas. Entre os ésteres, nonanoato de etila (odor descrito como frutado), hexadecanoato de metila (frutado), 9-octanoato de metila (verde/doce), 9-hexadecenoato de etila (frutado), hexadecanoato de etilo (frutado), cinamato de etila (morango/doce) e acetato de octila (flor de laranjeira/jasmim) foram extraídos exclusivamente pela fibra modificada com PDMS. Os ésteres são o maior grupo de compostos identificado na cerveja e são destacados especialmente pelas notas frutadas, florais e/ou doces de aroma e, portanto, constituem um importante grupo de compostos com influência positiva para a qualidade da cerveja (OCVIRK; MLINARIČ; KOŠIR, 2018). Alguns terpenos, incluindo τ-cadinol, β-farneseno, farnesol, α-humuleno epóxido II e carvona, também foram extraídos exclusivamente pela fibra modificada com PDMS. Estes compostos são derivados do lúpulo e podem conferir propriedades organolépticas desejáveis à cerveja (PRIEST; STEWART, 2006). Entre os demais compostos extraídos exclusivamente pela fibra revestida, a 2-octanona apresenta contribuição positiva para a percepção sensorial com um aroma descrito como floral, verde ou frutado (FENG et al., 2015), assim como o 2-undecanol, cujo aroma é frutado. No entanto, a presença de ácido heptanoico e ácido 2-etil-hexanoico pode ser indesejável, uma vez que ambos os ácidos apresentam um odor descrito como de suor (MALFONDET et al., 2016).

O método HS-SPME-GC/MS utilizado para quantificação de voláteis presentes em diferentes estágios da elaboração de cervejas, utilizando a fibra revestida com PDMS mostrou parâmetros de validação adequados. As curvas analíticas apresentaram linearidade satisfatória com coeficientes de determinação (r^2) variando de 0,98 para o hexadecanoato de metila a 0,99 para os demais compostos. Os limites de detecção (LOD) e quantificação (LOQ) demonstraram a sensibilidade do método, em que os menores valores de LOD (0,001 mg L^{-1}) foram obtidos para o benzaldeído e o dodecanoato de etila e os menores valores de LOQ (0,05 mg L^{-1}) para ácido octanoico, dodecanoato de etila, hexanoato de etila e octanoato de etila. Além disso, os coeficientes de variação relacionados aos ensaios de repetibilidade e precisão intermediária foram inferiores a 12,4 e 14,9%, respectivamente, e as recuperações variaram de 92 a 100%, demonstrando a eficiência do método proposto. Deve-se destacar que apenas uma curva analítica foi construída para cada composto, uma vez que a adição da camada extra de PDMS permitiu a quantificação de amostras com diferentes teores de etanol, dispensando a necessidade de preparação de curvas com teores de etanol semelhantes aos encontrados nas amostras analisadas.

Setenta e seis compostos foram positiva (18) ou tentativamente (58) identificados ao longo dos estágios de elaboração de cerveja *lager*, sendo 23 ésteres, 20 álcoois, 11 terpenos, 9 ácidos, 4 fenóis, 2 compostos derivados de furano, 2 lactonas, 2 cetonas, 1 pirazina, 1 aldeído e 1 composto sulfurado. As razões de Fisher foram calculadas considerando os compostos voláteis quantificados nas cinco etapas da produção de cerveja. Quarenta e oito compostos que apresentaram razão de Fisher correspondente a pelo menos 15% do valor do coeficiente Fisher do composto mais discriminante (2-octenol, razão Fisher: 399) foram selecionados para traçar o mapa de calor com agrupamento hierárquico.

Através destas técnicas quimiométricas, foi possível verificar que a mosturação destacou-se em relação às demais etapas pelos maiores teores de álcoois, como 2-octenol, 2-heptanol, 1-octen-3-ol, 2-etil-1-hexanol, 1-nonanol, 2,3 -butanodiol e 2-octanol. Esses álcoois podem ser formados por anabolismo ou catabolismo (via Ehrlich) de aminoácidos (HUGHES; BAXTER, 2001). Além disso, uma diminuição dos níveis destes compostos foi observada após a etapa de fervura, seja devido sua volatilidade ou às reações que sofrem para formar outros compostos (STEWART; RUSSELL; ANSTRUTHER, 2018).

A fervura foi caracterizada pelos maiores níveis de produtos da reação de Maillard (2-furanometanol, 2,3-dihidrobenzofurano e tetrametilpirazina) e γ-nonalactona. Esses compostos podem ter origem na malteação de cereais e são liberados para o mosto durante aquecimento (YU et al., 2014).

A fermentação, maturação e pasteurização foram discriminadas das demais etapas pela presença majoritária de ésteres. A formação de ésteres durante estas etapas de processamento através da condensação de ácidos e etanol pode resultar em uma mudança positiva no aroma, uma vez que o caráter gorduroso/rançoso dos ácidos é então substituído pelas características frutadas de seus ésteres correspondentes (STEWART; RUSSELL; ANSTRUTHER, 2018). Além disso, terpenos são incorporados ao mosto durante a fervura (β-mirceno, linalol, α-humuleno e α-cadinol) ou fermentação (τ-cadinol, carvona, nerolidol, farnesol e nerol) e a concentração destes compostos permanece semelhante nas etapas subsequentes da elaboração da cerveja.

Uma vez que a adição de uma camada extra de PDMS em uma fibra DVB/Car/PDMS comercial mostrou-se ser uma alternativa promissora na mitigação do efeito de deslocamento de etanol na extração de voláteis durante os estágios de elaboração de cerveja por HS-SPME, esta abordagem foi empregada na determinação quantitativa de compostos tóxicos na elaboração de cervejas do tipo *ale* (alta fermentação) e *lager* (baixa fermentação), bem como em cervejas comerciais.

Neste estudo, a cerveja *ale* foi fermentada a 20 ºC pela levedura *Belgian Strong Ale* (*S. cerevisiae var. diastaticus*), enquanto que a cerveja *lager* foi fermentada a 12 ºC pela levedura *S. cerevisiae*. Além disso, a elaboração das cervejas *ale* e *lager* diferiu quanto ao tempo de fervura (60 e 90 min, respectivamente) e ao malte empregado: a cerveja *ale* foi produzida usando maltes de cevada e trigo (66 e 34%, respectivamente), enquanto que na cerveja *lager* foi utilizado malte de cevada e melanoidina (95 e 5%, respectivamente). Ainda, a cerveja *ale* foi produzida com 25% de lúpulo a mais do que o usado na *lager*.

A quantificação de compostos carbonílicos e derivados do furano é desafiadora devido às suas baixas concentrações, altas volatilidades e reatividades, além da presença de outros compostos em maiores níveis. Devido à essas dificuldades, um método baseado no uso da HS-SPME-GC/MS-SIM para a determinação simultânea de compostos tóxicos (acetaldeído, acroleína, carbamato de etila, formaldeído, furfural e álcool furfurílico) previamente

desenvolvido para análise de vinhos (FERREIRA et al., 2018) foi avaliado quanto sua aplicabilidade na análise de cervejas.

O desempenho do método HS-SPME-GC/MS-SIM utilizado para a quantificação simultânea de tóxicos durante a elaboração de cervejas *ale* e *lager* utilizando a fibra recoberta por uma camada extra de PDMS também apresentou parâmetros de validação adequados, assim como comentado anteriormente para os compostos voláteis. As curvas analíticas apresentaram linearidade com r^2 variando de 0,9731 a 0,9960 para acetaldeído e CE, respectivamente. O LOD mais baixo foi encontrado para o acetaldeído (0,03 µg L^{-1}), enquanto o menor valor de LOQ (1,0 µg L^{-1}) foi verificado tanto para o acetaldeído, quanto CE, formaldeído e furfural. Além disso, os ensaios de repetibilidade e precisão obtiveram desvios padrão relativos inferiores a 12,0 e 11,5%, respectivamente, e as recuperações variaram de 90 a 100%.

CE e furfural não foram detectados ao longo da elaboração das cervejas *ale* e *lager* (valores inferiores ao LOD do método para estes compostos: 0,1 e 0,01 µg L^{-1}, respectivamente). Enquanto que acetaldeído, acroleína, formaldeído e álcool furfurílico foram encontrados em todos os estágios de fabricação de ambas as cervejas.

Na cerveja *ale*, o acetaldeído foi encontrado em níveis estatisticamente maiores ($p < 0,05$) após a fermentação (15,2 µg L^{-1}) do que os demais estágios (2,2; < 1,0; 1,5 e 1,8 µg L^{-1} após fervura, maturação e pasteurização, respectivamente). Entretanto na cerveja *lager*, os níveis deste aldeído após a fermentação (1,3 µg L^{-1}) foram estatisticamente semelhantes aos encontrados nos estágios pré-fermentativos (mosturação e fervura: < 1,0 µg L^{-1}), bem como nos estágios subsequentes à fermentação (maturação e pasteurização: 2,0 e 1,3 µg L^{-1}, respectivamente). É importante ressaltar que os níveis deste aldeído após fermentação foram estatisticamente maiores na *ale* (15,2 µg L^{-1}) do que na cerveja *lager* (1,3 µg L^{-1}, p = 0,02 de acordo com o teste t), indicando que as condições de fermentação (cepa de levedura e temperatura) desempenham um papel importante na formação deste composto. Além disso, após a maturação da cerveja *ale*, verificou-se redução significativa (em torno de 90%) nos níveis de acetaldeído (1,5 µg L^{-1}) em relação a etapa anterior (fermentação: 15,2 µg L^{-1}). Este comportamento não foi verificado após a maturação da cerveja *lager*, o que indica que além das condições de fermentação (temperatura e levedura), a matéria-prima também desempenha um papel importante tanto na formação quanto na redução deste composto (ŠIMIĆ et al., 2017).

Os níveis de acroleína encontrados após a mosturação e a fervura tanto na cerveja *ale* (6,1 e 10,0 µg L^{-1}, respectivamente) quanto na *lager* (24,8 e 17,7 µg L^{-1}, respectivamente) foram superiores aos demais estágios e apresentaram uma redução significativa após a pasteurização (4,1 µg L^{-1} e 2,9 µg L^{-1}, respectivamente). As etapas de mosturação e fervura, nas quais ocorre o aquecimento do malte, podem resultar na formação de acroleína proveniente da degradação térmica de aminoácidos, carboidratos e triglicerídeos (BURCHAM, 2017), aumentando os níveis deste composto. Enquanto que a ligação da acroleína a outros compostos da cerveja, como aminoácidos e compostos fenólicos, pode ocorrer, o que pode justificar a diminuição dos níveis desse composto ao longo dos estágios de elaboração da cerveja (PRAET et al., 2014; ZAMORA et al., 2016).

O formaldeído foi encontrado em níveis estatisticamente semelhantes ao longo dos estágios da produção da cerveja *ale* (de 1,5 a 2,2 µg L^{-1}). Na cerveja *lager*, níveis significativamente mais elevados foram encontrados após a fervura (4,0 µg L^{-1}) do que nas demais etapas (2,6 µg L^{-1} após os estágios de mosturação e fermentação; 2,8 e <1,0 µg L^{-1} após a maturação e pasteurização). O aumento significativo nos níveis de formaldeído após a fervura em relação ao estágio anterior (mosturação) na cerveja *lager* não foi verificado na produção de cerveja *ale* e pode ocorrer devido a diferenças na duração da fervura. O maior tempo de fervura da cerveja *lager* (90 min) pode resultar em níveis mais elevados deste aldeído do que *ale* (60 min). Consequentemente, níveis distintos de precursores de formaldeído podem ser verificados, incluindo aminoácidos e ácidos graxos poliinsaturados, que através das reações de degradação e oxidação, respectivamente, dão origem ao formaldeído (JEONG et al., 2015). No último estágio da produção de cerveja (pasteurização), os níveis de formaldeído foram significativamente reduzidos (níveis inferiores ao LOQ do método, 1,0 µg L^{-1}), provavelmente devido à alta reatividade deste composto, que podem prontamente reage com compostos fenólicos, como foi relatado ocorrer em vinho (ALEIXANDRE-TUDO et al., 2016).

Furfural foi detectado em níveis menores que o LOQ (1,0 µg L^{-1}) em todos os estágios de elaboração tanto da cerveja *ale* quanto da *lager*, enquanto o álcool furfurílico foi encontrado em todas as etapas em níveis variando de 6,9 a 21,9 µg L^{-1} e de 4,7 a 9,0 µg L^{-1} na produção *ale* e *lager*, respectivamente. Segundo Vanderhaegen et al. (2004), o furfural é formado através da reação de Maillard durante a produção de malte e a redução desse aldeído durante a fermentação aumenta o teor de álcool furfurílico. A fervura parece desempenhar um papel importante na formação de álcool furfurílico, uma vez que os níveis deste composto foram

significativamente maiores após esta etapa (9,0 e 21,9 µg L^{-1} para cerveja *lager* e *ale*, respectivamente) do que na etapa anterior (mosturação; 4,7 e 8,9 µg L^{-1} para *lager* e *ale*, respectivamente) e etapas subsequentes (fermentação, maturação e pasteurização; níveis nestas etapas variando de 5,0 a 6,9 µg L^{-1} e 6,9 a 8,9 µg L^{-1} para *lager* e *ale*, respectivamente). Além disso, os níveis de álcool furfurílico foram significativamente maiores em todos os estágios da cerveja *ale* do que na *lager*, indicando que as diferenças entre as matérias-prima utilizadas nestes dois tipos de cerveja podem influenciar os níveis deste composto. O malte melanoidina, usado na cerveja *ale*, é rico em produtos da reação de Maillard, incluindo compostos contendo furano (CARVALHO et al., 2014).

Amostras de cervejas comerciais, do tipo *ale* (n=8) e *lager* (n=22), também foram avaliadas, em relação à ocorrência de compostos carbonílicos e derivados do furano. O risco da exposição a esses compostos através do consumo moderado (600 mL para homens e 300 mL para mulheres) das cervejas em estudo, foi avaliado através da determinação da IDE e da MOE.

Nas amostras de cerveja, nas quais o CE foi encontrado, os níveis foram menores que o LOQ do método (1,0 µg L^{-1}). A ocorrência de CE foi verificada em apenas uma cerveja *ale* (4,5% das amostras deste tipo em estudo) e em duas cervejas *lager* (9,0%). Acetaldeído, acroleína, formaldeído e álcool furfurílico foram detectados em todas as amostras avaliadas. Os acetaldeído foi encontrado em níveis quantificáveis (superiores ao LOQ, 1,0 µg L^{-1}) em 12,5% das amostras *ale* (1,8 µg L^{-1}) e em 9,0% das amostras *lager* (1,3 e 2,5 µg L^{-1}). Acroleína foi encontrada em uma amostra de cerveja ale (4,1 µg L^{-1}). Em 36,4% das amostras *lager*, a acroleína foi detectada em níveis variando de 2,5 a 5,4 µg L^{-1}. O formaldeído foi detectado, mas não quantificado, em todas as amostras de cerveja ale, uma vez que os níveis encontrados foram inferiores ao LOQ (1,0 µg L^{-1}). Em cervejas *lager*, este aldeído foi quantificado em apenas 4,5% das amostras *lager* (2,6 µg L^{-1}).

O álcool furfurílico foi quantificado em todas as amostras de *ale* e *lager* em níveis variando de 4,2 a 20,7 µg L^{-1} e 5,8 a 30,9 µg L^{-1}, respectivamente. Furfural foi o composto tóxico encontrado em maiores concentrações nos dois tipos de cervejas, sendo detectado em 37,5% das cervejas *ale* em níveis entre 523,1 e 4264,3 µg L^{-1} e em 81,8% das amostras *lager* em níveis que variaram de 1,15 a 4116,0 µg L^{-1}. Além de furfural e álcool furfurílico, outros quatro compostos contendo furano (5-metil-2-furanmetanotiol, acetilfurano, 5-metilfurfural e γ-nonalactona) foram encontrados em cervejas *ale* e *lager* comerciais. A γ-nonalactona foi o

composto contendo furano mais frequentemente detectado, encontrado em 75 e 95,4% das cervejas *ale* e *lager*, respectivamente.

O furfural apresentou o maior valor de IDE (12,6 e 5,2 µg kg^{-1} de PC para homens e mulheres, respectivamente), seguido pela acroleína (2,9 e 2,6 µg kg^{-1} de PC para homens e mulheres, respectivamente), álcool furfurílico (0,1 e 0,06 µg kg^{-1} de PC para homens e mulheres, respectivamente), acetaldeído (0,03 e 0,02 µg kg^{-1} de PC para homens e mulheres, respectivamente) e formaldeído (0,03 e 0,008 µg kg^{-1} de PC para homens e mulheres, respectivamente). IDE para CE não foi calculado, uma vez que este composto não foi detectado em níveis superiores ao LOQ do método (1,0 µg L^{-1}).

Os valores IDE indicaram que a exposição ao furfural e ao álcool furfurílico através do consumo moderado de cerveja, juntamente aos demais compostos contendo furano, não representam risco para ambos os sexos, visto que a IDE não excedeu a ingestão diária aceitável (500 µg kg^{-1} de PC) estabelecido pelo JECFA (2000).

Como o acetaldeído, acroleína, CE e formaldeído são compostos genotóxicos, a caracterização do risco foi realizada através da determinação do MOE. A exposição à acroleína quantificada em quatro amostras (níveis variando entre 3,8 E 5,4 µg L^{-1}) pode representar risco para a saúde do consumidor, considerando o consumo dos homens, visto que a MOE foi de 6679 a 9556 (um valor de MOE igual ou inferior a 10.000 têm sido proposto como uma indicação de situação de rico sob a ótica da saúde pública). Por outro lado, os níveis de acetaldeído, CE e formaldeído quantificados nas amostras em estudo não representam risco à saúde do consumidor. Contudo, é importante notar que outros alimentos e bebidas, além da contaminação ambiental, podem ser fonte de exposição a estes compostos.

6. CONCLUSÃO

A adição de uma camada extra de PDMS a uma fibra comercial de DVB/Car/PDMS foi uma importante alternativa para minimizar o efeito do deslocamento causado pelo etanol na extração de compostos voláteis minoritários e tóxicos por HS-SPME nas etapas de elaboração de cervejas. A modificação na fibra de SPME permitiu a análise quantitativa e simultânea destes compostos de forma mais rápida e mais simples, diminuindo o número de análises, bem como o custo.

Os parâmetros de validação dos dois métodos (validados para a determinação dos compostos voláteis e dos tóxicos), incluindo LOD, LOQ, recuperação, repetibilidade e reprodutibilidade, mostraram que o uso da fibra PDMS-modificada e o método HS-SPME-GC/MS são adequados para a determinação e monitoramento do perfil volátil de cervejas, bem como dos níveis de compostos tóxicos.

A mosturação destacou-se em relação às demais etapas pelos maiores teores de álcoois superiores. A fervura foi caracterizada pelos maiores níveis de produtos da reação de Maillard, enquanto que a fermentação, maturação e pasteurização foram discriminadas pela presença majoritária de ésteres e incorporação de alguns terpenos ao mosto. Além disso, a fervura e a fermentação parecem ser etapas importantes na formação dos compostos tóxicos avaliados, enquanto a maturação e a pasteurização reduzem seus níveis nas cervejas *ale* e *lager*.

A avaliação do perfil volátil e dos níveis de compostos potencialmente tóxicos durante as etapas de elaboração de cervejas *ale* e *lager* permitiu a identificação dos pontos críticos da produção desta bebida, bem como para a geração de dados que poderão servir como base para a criação de uma legislação nacional que estabeleça limites máximos para os níveis dos compostos tóxicos avaliados. Cabe ressaltar que apenas a acroleína foi detectada em níveis suficientes para causar risco para a saúde do consumidor.

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