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Determination of ethanol in low-alcohol fermented beverages

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Abstract – Due to the exaggerated worldwide consumption of alcoholic beverages the development of special fermented beverages with low alcohol content or without alcohol is an interesting market niche. Thus, this study aims to quantify the ethanol content of commercial fermented beverages labelled "low alcohol content" or "without alcohol" and evaluate it in order to guarantee the safety of the beverage for its consumers. For this, the ethanol quantification was performed in eleven samples by gas chromatography with headspace and flame ionization detection (HS-GC-FID) and the method has to be successful. Four samples were in agreement with the legislation, six presented ethanol above recommendations and one of them presented ethanol content below the value labelled. These results show that it is necessary an improvement on analytical controls by industries and also a more active inspection of regulatory organizations to guarantee the consumers safety.
Keywords: Alcoholic beverages. Ethanol content. Gas chromatography. Headspace.

Determinação de etanol em bebidas fermentadas com baixo teor de álcool

Resumo – Devido ao exagerado consumo mundial de bebidas alcoólicas, o desenvolvimento de bebidas fermentadas especiais com baixo teor alcoólico ou sem álcool é um nicho de mercado interessante. Assim, este estudo tem como objetivo quantificar o teor de etanol de bebidas fermentadas comerciais rotuladas "baixo teor alcoólico" ou "sem álcool" e avaliá-lo de forma a garantir a segurança da bebida para seus consumidores. Para isso, a quantificação do etanol foi realizada em onze amostras por cromatografia gasosa com headspace e detecção de ionização de chama (HS-GC-FID) e o método mostrou ser bem sucedido. Quatro amostras estavam de acordo com a legislação, seis apresentaram etanol acima das recomendações e uma delas apresentou teor de etanol abaixo do valor indicado no rótulo. Esses resultados mostram que é necessário um aprimoramento dos controles analíticos por parte das indústrias e também uma fiscalização mais ativa dos órgãos reguladores para garantir a segurança dos consumidores.
Palavras-chave: Bebidas alcoólicas. Teor de etanol. Cromatografia a gás. *Headspace*.

Introduction

The World Health Organization (WHO) together with its Member States, presented strategies to reduce the harmful use of alcohol, due to its frequency and excessive consumption by the population. In the "Global status report on alcohol and health 2018" report, an estimated average per capita consumption of 6.4 liters of pure alcohol worldwide and 7.8 liters in Brazil was presented. It is known that high alcohol consumption can cause several diseases such as cirrhosis of the liver, depression, in addition to other ailments such as chemical dependency, etc. Taking into account the WHO recommendations, the beverage market started to offer traditionally alcoholic beverages, with low alcohol content or without alcohol. According to the

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"No- and Low-Alcohol Strategic Study 2021" issued by the market research organization International Wine & Spirit Research (IWSR), the market for low-alcohol and non-alcoholic beverages will grow by 31% by 2024. In addition, the market also highlights that due to the lower concentration of ethanol presented, these beverages have a lower caloric content, in addition to having in their composition beneficial compounds to human health, such as antioxidants (WHO, 2010; LIGUORI *et al.*, 2018; WHO, 2019; IWSR, 2021).

Given this growing market for low-alcohol or non-alcoholic beverages, there is also a growing need to carry out the analytical control of these beverages, in order to maintain a standard of identity and quality (PIQ) according to the country's legislation, in the case of Brazil, as presented in the annex of Operational Standard No. 1/2019. In addition to maintaining the PIQ of the drink, analytical control allows guaranteeing the safety of drink for consumption by the population, since through this control, it is possible to present reliable labeling information as required by the country, thus avoiding risks to consumer health (BRAZIL, 2019c; IWSR, 2021).

A very important analytical parameter to be verified in beverages with low alcohol content or without alcohol is the concentration of ethanol present in them, as it is the compound responsible for their classification. To monitor ethanol, there are several chemical analysis techniques available, such as electrochemical, spectrophotometric, spectroscopic and chromatographic, and in this scenario, chromatography is noteworthy because it is widely used to perform efficient and specific separations, identification and determination of chemical components in complex mixtures (SKOOG *et al.*, 2017; BRAZIL, 2019c; WACHEŁKO, SZPOT & ZAWADZKI, 2021).

Thus, this work aimed to analyze different fermented beverages, classified as alcohol free or low in alcohol, from the determination of ethanol concentration using gas chromatography with headspace sampling. The alcohol content found in comparison with the values stated on the labels and its agreement with current legislation was also evaluated.

Materials and methods

The n-hexanol was used as the internal standard and the ethanol was used to construct the calibration curve. Both are from Sigma-Aldrich. The calibration curve was prepared with deionized water (conductivity of 18.2 MΩ cm at 25 ºC).

The fermented beverages with low alcohol content analyzed in this work were beers and kombuchas. All of them were acquired in the local Market at Piracicaba city, from São Paulo State, Brazil.

The samples of low-alcoholic or "non-alcoholic" beverages were analyzed for ethanol concentration, after being previously degassed for 5 minutes under mechanical agitation (GOMES, YOSHINAGA & BORTOLETO, 2020). A PerkinElmer model Clarus 600 gas chromatograph was used, with headspace sampling using an automatic sampler model CTC Analytics, Pal System. The chromatographic column was NOVA-WAX (30 m × 0.25 mm × 0.25 µm) from Nova Analytics, and the conditions were optimized, according to Bortoleto & Gomes (2020).

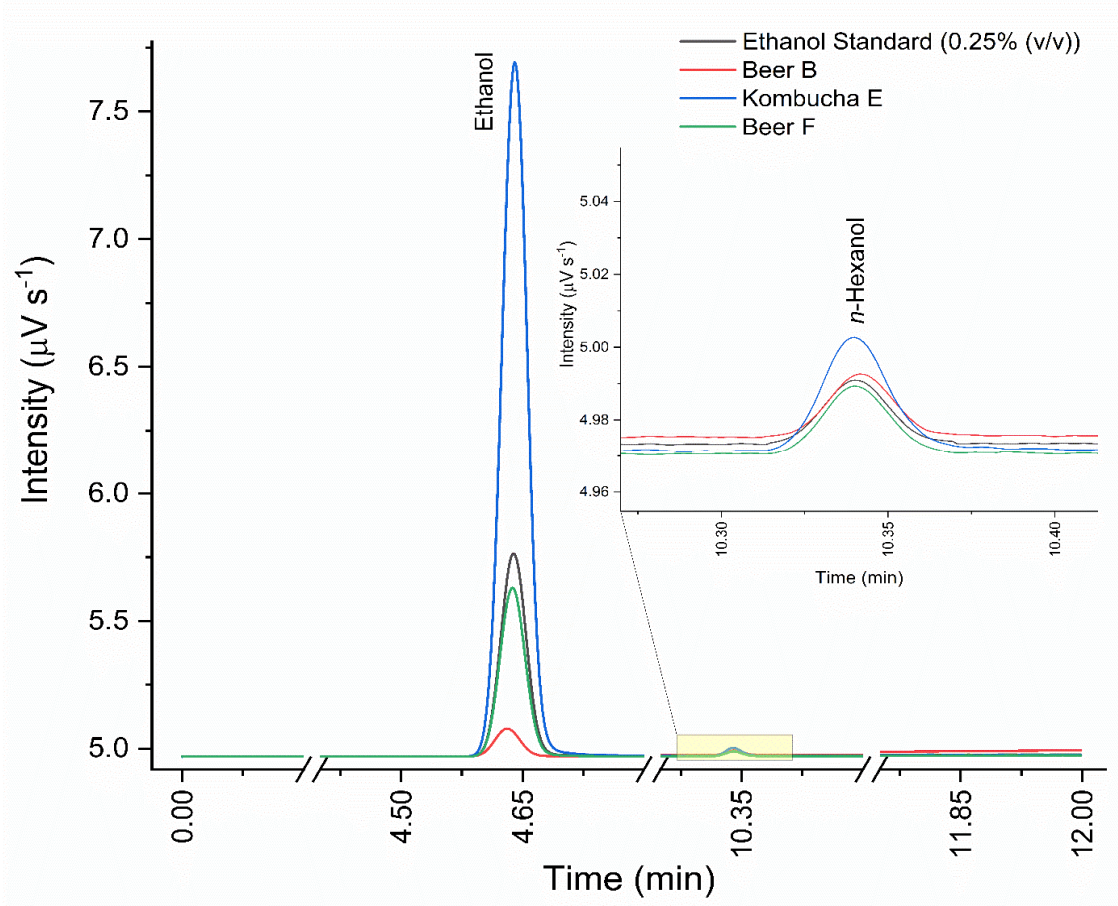
Ethanol quantification was performed based on the analytical curve with n-hexanol as internal standard, of five concentration points, all prepared in deionized water. The analytical curve constructed until 1.00% (v/v) of ethanol with the linear coefficient $R^2 = 0.999$ and the equation: Area = 6.62 + 253.41 C (%, v/ v).

Results and discussions

The headspace gas chromatography (HS-GC-FID) method for the determination of ethanol in fermented beverages has been applied with successfully. Wachełko, Szpot &

Zawadzki, also presented recently (2021) a HS-GC-FID/FID method for quantifying ethanol in beers and it was fully validated. They described the technique as sensitive and precise with a sufficiently wide range to be applied for quality control of beverages in accordance with both, EU standards and BJCP recommendation. In the same way, the method presented at this work attends the Brazilian recommendation, considering in this case the n-hexanol has been used as an internal standard, differently from the Wachełko and partners proposed. The chromatographic profile obtained from the analytical standard and the internal standard allowed the identification and quantification of ethanol in the samples. As shown in Figure 1, the compound of interest identified to be ethanol was eluted in 4.64 min and n-hexanol (internal standard) was eluted in 10.34 min.

Figure 1 - Chromatographic profile obtained for sample and identification of the ethanol and *n*-hexanol. Beer B: alcohol free, Kombucha E: low alcohol and Beer F: low alcohol.



Source: Personal data (2020).

For this work, the samples of commercial fermented beverages analyzed were beers and Kombuchas. Considering beer samples with low ethanol concentrations, it is possible to classify them in alcohol free (A, B, C, D) and low alcohol (E, F). In Brazil while "No Alcohol" beers may present until 0.05 % (v/v) of ethanol, in the "Low Alcohol" beers, allows a maximum of 0.5% (v/v) of alcohol in its composition (BRAZIL, 2019b).

For Kombuchas, in Brazil they can be classified in "Low Alcohol" (with less than 0.5% (v/v) of ethanol) or "Alcoholics", but in this last case, the ethanol concentration must be expressed in the label and be less than 8 % (v/v) (BRAZIL, 2019a).

Table 1 shows the values of alcohol found in labels and the values of ethanol found by HS-GC-FID in samples of beers and kombucha.

Table 1 - Ethanol values informed on labels and determined by HS-GC-FID in sample of beverages with low alcohol content or no alcohol content.

Samples	Labelled alcohol (%, v/v)	Found alcohol (%, v/v)
Beer A	0.0	0.02 ± 0.01
Beer B	0.0	0.01 ± 0.01
Beer C	0.0	0.04 ± 0.01
Beer D	0.0	0.03 ± 0.01
Beer E	0.5	0.87 ± 0.03
Beer F	0.4	0.18 ± 0.01
Kombucha A	0.5*	3.29 ± 0.30**
Kombucha B	0.5*	0.68 ± 0.02
Kombucha C	0.5*	0.75 ± 0.03
Kombucha D	0.5*	0.83 ± 0.25
Kombucha E	0.5*	0.61 ± 0.10

*The label indicates that the drink may contain until 0.5% (v/v) of alcohol.
** This value is out of the range studied but even so shows that the drink presents a high ethanol content, diverging from the value mentioned on its label and outside the legal limit.
Source: Personal data (2020).

Considering the samples A, B, C and D, although these beers are labelled “No alcohol”, the values of ethanol concentrations obtained agree to Agriculture Ministry from Brazil, because all of them presented less than 0.05% (v/v) of ethanol in its composition. But, analyzing the result of the sample E, it is possible to observe a value above that presented in the label and above that allowed by the current law. Besides, the F sample presented an ethanol content below the value informed on their label, even considering the RDC 360/2003 that allows a content variation until 20% of value labelled (BRASIL, 2003).

In 1980, Morad, Hikal & Buchanin (1980) applied the chromatography techniques to determine ethanol concentrations in beverages that they used to call “Alcohol Free”, to validate the labels from commercial samples. The authors evaluated five samples of beers and found ethanol concentrations between 0.009 until 0.385 % (v/v). According to the authors, these type of beers normally contains 0.5 to 2.5% (v/v) of alcohol in the final process and must be called “Alcohol-low” or “Alcohol-poor”, because, in fact, they are not completely free of ethanol.

In 1993, Buckee & Mundy also used the chromatography for ethanol quantification in beer, and, among the samples analyzed, one was a type considered “Alcohol Free” and presented 0.93% (v/v) of ethanol. Today, it is important to mention that some of these beers must be called “Low Alcohol”, especially in Brazil, because, in fact, they present more than 0.05% (v/v) of ethanol and it is not in agreement with the legislation.

In 2017, Vargas *et al.* (2017) developed a new automated bioanalyzer for ethanol content determination in low alcohol beer, able to operate for on line analysis, with advantages of simplicity, sensitivity, and assay time. They show the potential for commercialization as an affordable and useful analytical tool for routine quality control of low-alcohol beer either during

the manufacturing process or for the final product. The researches also mention the possibility to use the equipment for other beverage that presents low ethanol content.

Considering the Kombucha samples analyzed, it is important to highlight that all five samples presented ethanol content above that the current law allows. In 2017, Talebi *et al.*, from the Chemistry and Biochemistry department of the Texas University, also applied a headspace gas chromatography technique for determining the ethanol content in complex commercial kombucha products. The ethanol concentration in all commercial kombucha products examined was higher than the federal limit of 0.5% ABV. Moreover, the authors mention that the ethanol concentration of commercial kombucha products changes with time. Longer storage times resulted in formation of higher ethanol concentrations in both sample products at 4 °C and at room temperature. They suggest using the method HS-GC for food industries and regulatory agencies to monitor the alcohol content in kombucha products.

Liu *et al.*, in 2019, attend a multilaboratory study and demonstrate that the evaluated headspace GC-FID method is suitable for the detection of ethanol in Kombucha. They also found commercial samples with the ethanol content above 0.5% ABV legal limit.

All these results, especially the ones above of legal limit or even in the labels of the products show the neglect of some food industries with the consumers. The consumption of ethanol without to knowing about it is very serious and can be dangerous. It includes people that can not make use because of some drug interaction, people that will drive and may have problems in the breathalyzer test, or worse, children that drink kombucha because of the beneficial properties.

All these researchers are showing the regulatory agencies also must do an efficient oversight, because if some industries are not worried with the consumers' safety, the government must be.

Conclusions

This work presented a simple and efficient method using HS-GC-FID to be applied in fermented beverages analysis with low ethanol content. From eleven samples, six were with ethanol concentration above the limit of 0.5% (v/v) and one of them presented ethanol below the value informed on the label.

The production of popular and alcoholic beverages like beers, with low or no alcohol is a good alternative for beer consumers and it is according to WHO recommendations. The production of other fermented beverages with no-ethanol or poor-ethanol, with healthy proprieties are also encouraged. However, the beverage industries, that are making a lot of money with these products, should also guarantee the safety of the consumers. In this scenery, the authorities must also fulfill their role and improve enforcement.

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