Food Chemistry 346 (2021) 128924

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The application of headspace gas chromatographic method for the determination of ethyl alcohol in craft beers, wines and soft drinks

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ARTICLE INFO

Keywords:
Craft beer
Wine
Soft drink
Ethanol content
Headspace
Gas chromatography

ABSTRACT

The increasing interest of consumers in the still-developing craft beer market and the strict tax-related legal regulations concerning alcoholic beverages require precise methods for quality control. Determination of ethyl alcohol concentration was performed in 167 samples of alcoholic beverages (craft beers, soft drinks, wines, and cider). We applied headspace gas chromatography using a dual column/dual flame ionization detector (HS-GC-FID/FID), a technique routinely used in forensic toxicology. The method was linear in range, from 0.01 to 20.0%, with a coefficient of determination of 0.999 (R2). The limit of quantification was 0.01%; the detection limit was 0.003%. Furthermore, very good validation parameters were achieved (precision and accuracy below 5%). The samples were analyzed for compliance with EU standards and recommendations of The Beer Judge Certification Program. Moreover, the content of trace quantities of volatile compounds and fusel alcohols (1-propanol, 2-propanol, acetone, and acetaldehyde) was found in the majority of alcoholic beverages.

1. Introduction

Ethyl alcohol in the main product of the alcoholic fermentation that occurs during the technological processes involved in the production of alcoholic beverages. The concentration of ethyl alcohol determines the classification of an alcoholic beverage, according to strictly defined criteria, as a high-percentage (absinthe, cognac, whiskey, vodka) or medium- or low-percentage (wine, beer, "soft drinks") beverage. This classification is of key importance in many countries for tax-related legal regulations such as the Council Directive 92/84/EEC of October 19, 1992 (Council Directive, 1992) on the approximation of the rates of excise duty on alcohol and alcoholic beverages and the Craft Beverage Modernization (CBMA) portion of the US Tax Cuts and Jobs Act of 2017 (The Craft Beverage, 2017). Furthermore, a number of clinical trials have confirmed a correlation between the amount of ethanol consumed and the state of human health, as well as the impact of alcohol on mortality; for example, the Copenhagen City Heart Study (an examination of a large prospective cardiovascular population) has shown that small amounts of ethyl alcohol in the daily diet protect against ischemic heart disease (Gronbaek et al., 1995). On the other hand, the latest research suggests that there is no level of consumption that minimizes health losses (GBD 2016 Alcohol Collaborators, 2018).

The beer industry is one of the fastest growing industries at the moment, mainly due to the growing interest of consumers in craft beers. The "craft beer revolution" started in the late 1970s with the establishment of the first modern microbrewery in California. Since 2000 the pace of the creation of small local breweries has accelerated on an unprecedented scale. Over the course of several years, the number of breweries in the European Union has doubled, while in the United States it has increased fivefold. The opportunity to import goods and significant progress in globalization have had a major impact on the development of the beer market. In China alone, 489.9 million hectoliters of beer are drunk every year, making it the largest beer-consuming country in the world, followed by the USA (241.7 million), Brazil (131.5 million), and Russia (100.1 million). The populations of European Union member countries drank 359 million hectoliters in 2016 alone (Pokrivcak et al., 2019; Cabras, 2020). On one hand, regional specialties have the chance to reach a larger customer base, as is the case with fruit beers, traditionally from Flemish regions. Belgian beverages such as Trappist and lambic beers have also become a unique symbol of this country thanks to the efforts of the breweries, both small and large, which have promoted them in many countries around the world. As a result, a distinctive perception of the brand associated with Belgian beers is now dominant among consumers around the world, which has

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https://doi.org/10.1016/j.foodchem.2020.128924
Received 20 July 2020; Received in revised form 14 December 2020; Accepted 19 December 2020
Available online 27 December 2020
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led to Belgian beers and beer culture being officially recognized by UNESCO as part of the Intangible Cultural Heritage of Humanity in 2016 (Cabras, 2020). On the other hand, craft beer is currently an important sector for so-called beer tourism. Consumers now spend much more money on locally brewed products, mainly due to their authenticity and long tradition. Microbreweries mainly use regional water, hops, and yeast, which influences the unique taste composition of the drink (Kraftchick, Byrd, Canziani, & Gladwell, 2014). The clear increase in consumer interest in craft beers is best illustrated by Google trends, which show that the number of searches for the term *craft beer* in the United States alone has increased by 70% in just a few years. Wine, by comparison, has not followed this trend (Depenbusch, Ehrich, & Pfizenmaier, 2018). In 2018 the beer market was worth US $114.2 billion, of which 24% represented craft beers (Brewers Association, 2020). Considering these trends, this tendency will increase and local breweries will thus stand a chance of experiencing their biggest expansion in history.

A number of methods are currently used in the analysis of ethanol content in alcoholic beverages. Among instrumental methods, the following should be mentioned: electrochemical (Paixão, Corbo, & Bertotti, 2002), spectrophotometric (Pilone, 1985), and near-infrared spectrometry (NIR) (Gallignani, Garrigues, & de la Guardia, 1993). There are also a number of chromatographic methods: full evaporation headspace gas chromatography (HS-GC-FID) (Li, Chai, Deng, Zhan, & Fu, 2009; Zhang, Lin, Chai, Zhong-Li, & Barnes, 2015), gas chromatography with flame ionization detection (GC-FID) (Stackler & Christensen, 1974; Jamieson, 1979; Clarkson, Ormrod, & Sharpe, 1995; Wang, Choong, Su, Lee, & Hsiang, 2003), direct analysis in real time mass spectrometry (DART-MS) (Sisco & Robinson, 2020), high-performance liquid chromatography with refractive index detection (HPLC-RI) (Calull, Marcé, & Borrull, 1992; Lopez & Gomez, 1996), and high-performance liquid chromatography with ultraviolet/visible and refractive index detection (HPLC-UV-RI) (Castellari, Sartini, Spinabelli, Riponi, & Galassi, 2001).

1.1. Beers

The main ingredients used in the production of beer are water, barley malt, yeast, and hops, which, as a result of many chemical processes, form more than 800 chemical compounds responsible for the characteristic aroma, taste, and color of this drink. Moreover, the use of different varieties of hops (American, Old World, New World, Saazer-type hops) and malt (Munich, Vienna, Pilsner) modifies the aroma of beer by giving it a characteristic taste composition (BJCP, 2015). The predominant reactions leading to carbonyl compounds are alcoholic fermentation, lipid oxidation, Strecker degradation, aldol condensation and Maillard's reaction, which is responsible for the brown color and rich and malty compounds in beer (BJCP, 2015). A perceptible note of sweetness or bitterness or an acidity or buttery flavor indicates the type of beer and helps to assess its quality, while the concentration of ethyl alcohol determines the classification of beer: from 0.0–0.5% (v/v) for non-alcoholic to 12.5% (v/v) for strong. Furthermore, according to the guidelines developed by the Beer Judge Certification Program, all beer styles must be cleanly fermented and free from technical faults (including acetaldehyde and fusel alcohols) (BJCP, 2015), which proves the importance of analyzing the content of ethyl alcohol and other volatile compounds in the quality control of products of the brewing industry. The range of formation of organic compounds also results from natural biochemical degradation processes during the aging of beer. The substances formed at this stage, such as furfural, diacetyl, *n*-hexanal, and acetaldehyde, give rise to successive stages of the chain reaction leading to the formation of a number of other carbonyl compounds (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006; Vanderhaegen, Delvaux, Daenen, Verachtert, & Delvaux, 2007). Moreover, the varied composition of chemical compounds also influences the taste perception of consumers and their preference for specific beer types (Gonzalez Viejo, Fuentes, Torrico, Godbole, & Dunshea, 2019). Frequently, in order to improve the taste of beer, fruit flavors are added, which, in addition to aromas, provide beverages with antioxidant properties, mainly due to the content of phenolic acids, flavonoids, catechins, carotenoids, tocopherols, and ascorbic acid (Nardini & Garaguso, 2020).

According to the AOAC, reference methods for measuring the ethanol content of beer include the gravimetric, refractometric, and gas chromatographic methods, which use 1-propanol as an internal standard solution (also recommended by the American Society of Brewing Chemists) (Gales, 1990).

1.2. Soft drinks

Soft drinks in most EU countries are divided into alcohol-free beers (AFBs) containing less than 0.5% (v/v) and low-alcohol beers (LABs) with no more than 1.2% (v/v), whereas in the United States, the term alcohol-free beer is used to describe beers containing no ethyl alcohol at all, while those containing less than 0.5% are considered "near-beer" (Montanari, Marconi, Mayer, & Fantozzi, 2009). These varieties were most widespread in the years between 1919 and 1933 (the Prohibition era in the USA), during which it became necessary to modify the production of beer in favor of ethanol-free types due to the legal regulations at that time. The processes of producing low-alcohol beers can be divided into two groups: physical or biological. The former group consists of removing fully produced ethanol (via rectification, evaporation, dialysis, or reverse osmosis); the latter is based on limited ethanol formation during beer fermentation (via modification of the mashing process or the use of special yeast) (Brányik, Silva, Baszczyński, Lehnert, & Almeida e Silva, 2012). Along with the use of physical methods, other beer ingredients, such as acetaldehyde or fusel alcohols, can also be easily removed. In beer de-alcoholized via falling-film evaporation the concentration of 1-propanol is reduced from 7.7 to 0.6 (mg/l); in dialysis, the concentrations of both acetaldehyde (from 5.4 to 3.7 mg/l) and 1-propanol (from 9.4 to 0.5 mg/l) are reduced (Montanari et al., 2009).

1.3. Wines

Wine production is based on alcoholic fermentation of grape juice, an anaerobic process of sugar degradation mainly carried out by *Saccharomyces cerevisiae*. In addition to ethanol, the resulting products are glycerol, succinic acid, diacetyl, acetoin, 2,3-butanediol, and, in particular, higher levels of alcohols and esters, which contribute most to the unique composition of taste and aroma of wine (Zamora, 2009). The grape strain type is closely linked to the corresponding amounts of fusel alcohols, fusel alcohol acetates, isoacids, and their ethyl esters. At low concentrations of these substances, the aroma of the wine is soft and delicate, so that the content of the various compounds correlates closely with the taste preferences of consumers (Ferreira, López, & Cacho, 2000). Furthermore, there are many interactions between sweet, acidic, and salty tastes and ethyl alcohol. Research carried out by the Institute of Chemical Technology in Prague has shown that the concentration of ethyl alcohol in wines has a significant influence on sensory perceptions of these beverages. The degree of sweetness increases in direct proportion to increases in the amount of ethanol up to 16% (v/v), while the level of bitterness is reduced (Panovská, Šedivá, Jedelská, & Pokorný, 2008). Other investigations have shown that global climate changes lead to increased temperatures during the growing season, resulting in higher levels of sugar formation in the grapes. In turn, higher sugar levels result in the formation of more alcohol during fermentation. This is likely one of the reasons for the growing interest over the decades in analyzing the composition of wine and, in particular, in improving methods for determining alcoholic composition (Schultz, 2016). One of the most important stages of wine production is aging after bottling. Over time, more and more oxidation reactions occur, usually initiated by an electron transfer reaction which is itself triggered by a catalyst, consisting, e.g., of iron and copper ions, which transfers the charge to an oxygen

atom. In the presence of catechol, hydrogen peroxide is formed (Danilewicz, 2011). The same pathway may produce a number of other oxidation products, such as glyoxylic acid from tartaric acid or acetaldehyde from ethanol, which is both a product and a substrate for additional reactions, especially with wine phenolics. This has led to an increase in the polymeric pigments and tannins produced by the acetaldehyde-mediated condensation reactions, which are responsible for the deep red color of wine following a long period of aging (Han, Webb, & Waterhouse, 2019).

The reference methods for measuring the ethanol content of wine according to the AOAC are the gravimetric, refractometric, spectrophotometric (using dichromate oxidation), and gas chromatographic methods, using 2-propanol as an internal standard solution (Caputi, 1990).

This paper aims to apply a novel method for determination of ethyl alcohol with the use of *tert*-butanol as an internal standard. The samples were analyzed by headspace gas chromatography using a dual column/dual flame ionization detector. This method was applied to control the quality of alcoholic beverages, as well as to monitor fusel alcohols and other volatile compounds contained in craft beers, soft drinks and wines.

2. Experimental

2.1. Chemicals and reagents

Water (Chromasolv LC-MS) was purchased from Sigma-Aldrich (Steinheim, Germany), *tert*-Butanol (internal standard, ISTD), as well as the standard aqueous ethanol solution used to create the calibration curve, from Sigma-Aldrich (St. Louis, USA). The standard solutions and ISTD were stored at 5 °C.

2.2. Instrumentation

A Shimadzu GC-2010 Plus AF IVD (Kyoto, Japan), equipped with an advanced flow controller (AFC), a split/splitless injector (SPL), and two Flame Ionization Detectors (FID), was used in this research. A static headspace sampler (Shimadzu HS-20, Kyoto, Japan) was used for sample preparation and introduction into the GC through a single SPL. Effluent from the HS-20 was divided between two columns: a Zebron ZB-BAC1, 0.32 mm × 30 m × 1.8 μm and a Zebron ZB-BAC2, 0.32 mm × 30 m × 1.2 μm (both from Phenomenex, Torrance, California, USA), using a SilFlow microfluidic platform (Trajan, Ringwood, Victoria, Australia), presumably at a 1:1 ratio. Each column was connected to a separate FID and analyzed simultaneously. Operating parameters applied in the method are presented in Table 1.

Table 1
HS-GC-FID/FID operating parameters.

HS-20	GC-2010 Plus
Oven Temperature: 65ºC	Carrier Gas: He
Sample Line Temperature: 150ºC	Column Temperature: 40 °C
Transfer Line Temperature: 150ºC	Column Flow: 2.57 mL/min
Shaking Level: 1	Linear Velocity: 40 cm/sec
Multi Injection Count: 1	Total Flow: 55.1 mL/min
Pressurize Gas Pressure: 60.0 kPa	FID1 and FID2 Temperature: 240 °C
Equlib. Time: 10 min	FID1 and FID2 Makeup Flow: 30 mL/min
Pressurizing Time: 0.5 min	FID1 and FID2 H2 Flow: 40 mL/min
Pressure Equlib. Time: 0.1 min	FID1 and FID2 Air Flow: 400 mL/min
Load Time: 0.5 min	APC1 Pressure: 60 kPa
Load Equlib. Time: 0.1 min	Total Program Time: 6.40 min.
Injection Time: 1 min	
Needle Flush Time: 1 min	
Injection mode: Split	
Sampling Time: 1 min	

2.3. Standard solutions and calibration standards

For the calibration curve (in triplicate) of the standard sample, 0.2 mL of standard aqueous ethanol solutions (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0%) were added to headspace vials (10 mL, Shaoxing ALWSCI Technologies, China). Next, 1 mL of the internal standard aqueous *tert*-butanol solution (0.5 g/L) was added and the vials immediately sealed with headspace caps (aluminum cap: butyl rubber/PTFE, Polygen, Gliwice, Poland).

2.4. Sample preparation

In total, 167 samples of alcoholic beverages were collected, including 140 beer, 13 soft-drink, 13 wine (7 white, 6 red), and one cider sample. As in the calibration, 0.2 mL of the alcoholic beverage to be tested was added to a headspace vial. Next, 1 mL of internal standard aqueous *tert*-butanol solution (0.5 g/L) was added and the vial immediately sealed with a cap.

2.5. Validation

Linearity: A calibration curve was created within a range of ethanol concentrations from 0.01 to 20.0%. The coefficient of determination (R^2) was determined.

Precision and accuracy: Inter-day and intra-day validation was performed for three concentration levels: low, medium, and high (0.01%, 5%, and 15%). For this purpose, validation of each concentration value was performed with five repetitions; subsequently, the results from both detectors (FID1 and FID2) were averaged. To determine precision and accuracy values for the method, relative standard deviation (RSD) and relative error (RE) were calculated.

LOQ and LOD: The limit of quantification (LOQ) was defined as the lowest measurable concentration which could be determined with a RSD below 20% and a signal-to-noise ratio (S/N) of at least 10 (Peters, Drummer, & Musshoff, 2007). The limit of detection (LOD) was determined from the equation below:

$$\mathrm{LOD} = \frac{\mathrm{LOQ}}{3}$$

Selectivity: Five different lots of blank beers and wines were tested for possible endogenous interference peaks at the retention times of the *tert*-butanol.

3. Results and discussion

3.1. Validation process

Using the described method, very good validation parameters were achieved. Intra-day precision and accuracy at three concentration levels (0.01%, 5%, and 15%) were: 4.8, 1.6, 1.2 (RSD%) and 5.0, 2.1, 1.3 (RE %), respectively. Inter-day precision and accuracy at three concentration levels (0.01%, 5%, and 15%) were: 4.5, 1.8, 1.0 (RSD%) and 4.9, 2.3, 1.1 (RE%), respectively. The value of the coefficient was 0.999 (R^2). The limit of quantification (LOQ) was 0.01%; the limit of detection (LOD) was 0.003%. With the use of the described method, no substances from the matrix interfered with ethanol or with the internal standard (*tert*-butanol). The chromatograms of two blank samples (beer and wine), ISTD, and ethanol standard at the concentration of LOQ, are presented below (Fig. 1). The retention time of *tert*-butanol (ISTD) on the first column (Zebron ZB-BAC1) was 2.848 min, on the second (Zebron ZB-BAC2), 3.131 min. The retention time of ethanol was 2.038 min. and 2.248 min, respectively.

Chromatographic methods are definitely among the most reliable methods for the determination of ethanol content in samples of alcoholic beverages. A comparison of these methods is presented in Table 2. Chromatographic-as opposed to gravimetric and refractometric-

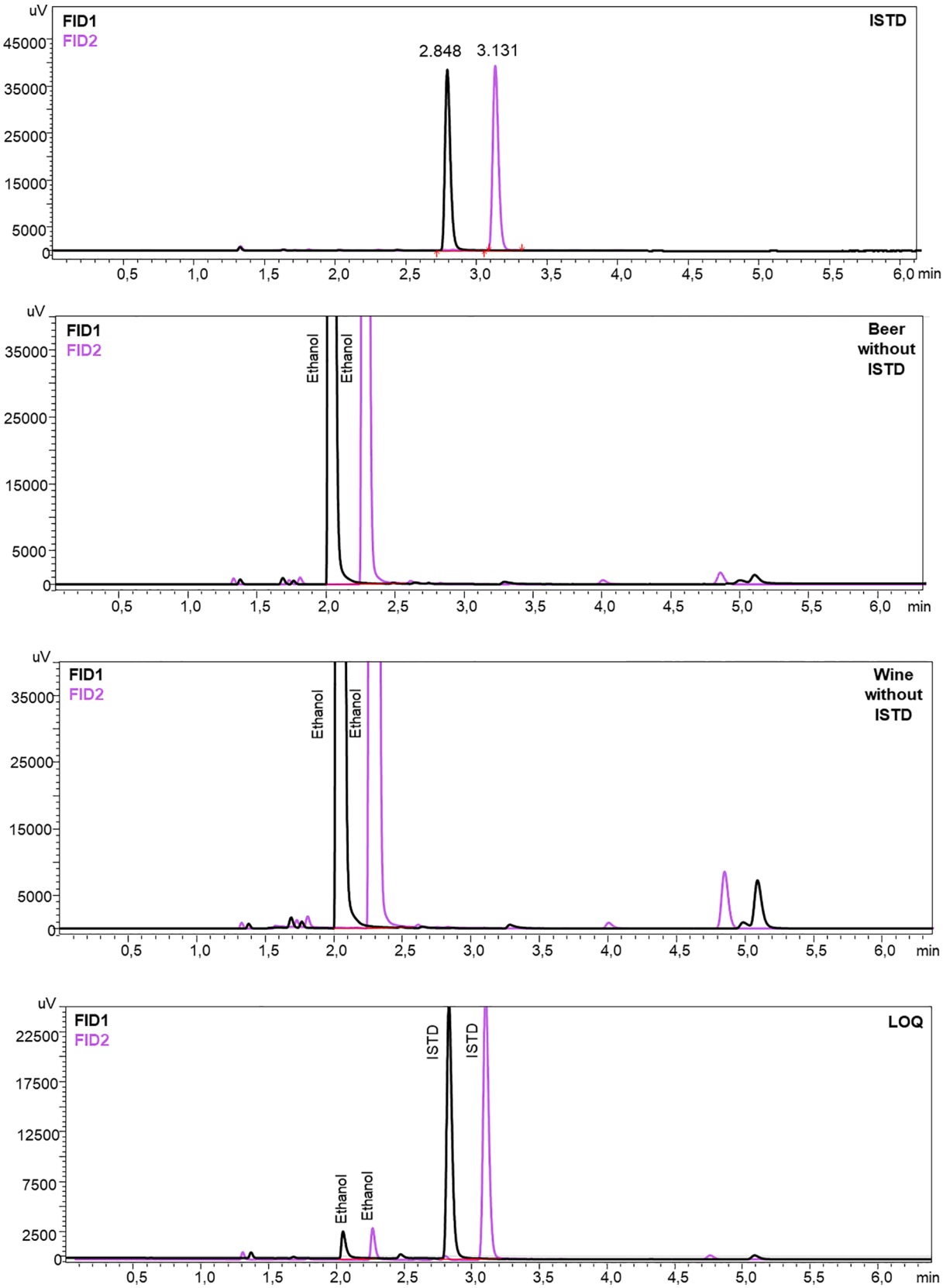

Fig. 1. Chromatograms of ISTD, beer without internal standard, wine without internal standard and ethanol standard at the concentration of LOQ. Black line is illustrating the results from FID1 and violet line from FID2.

Table 2
Comparison of chromatographic methods for determination of ethanol in beers, wines and soft drinks.

No	Sample (volume)	Method	Internal standard	LOQ/LOD [%]	Year	References
1.	Beer (750 µl)	GC-FID	1-PrOH	–	1979	Jamieson
2.	Wine (100 µl)	GC-FID	1-BuOH	–	1974	Stackler & Christensen
3.	Beer (1000 µl)	GC-FID	2-PrOH	–	1995	Clarkson et al.
4.	Alcoholic beverage (500 µl)	GC-FID	ACN	LOQ: 0.00005 LOD: –	2003	Wang et al.
5.	Beer (5000 µl)	HPLC-UV-RI	–	LOQ: 0.002 LOD: 0.008	2001	Castellari et al.
6.	Wine (10 µl)	HPLC-RI	–	LOQ: – LOD: 0.3	1996	López & Gómez
7.	Wine (1000 µl)	HPLC-RI	–	LOQ: 0.5 LOD: –	1992	Calull et al.
8.	Alcoholic beverage (200 µl)	HS-GC-FID/FID	*tert*-BuOH	LOQ:0.01 LOD: 0.003	2020	This method

Abbreviations: GC – Gas Chromatography; HPLC – High Performance Liquid Chromatography; FID – flame-ionization detector; RI – refractive index detector; UV – Ultra-Visable detector; ACN – acetonitrile; 2-PrOH – 2-propanol (isopropanol); 1-PrOH – 1-propanol (n-propanol); 1-BuOH – 1-butanol (n-butanol); *tert*-BuOH – *tert*-butanol; LOD – limit of detection; LOQ – limit of quantification.

methods are characterized by high levels of sensitivity and accuracy, which is essential for estimating the quality of products of the wine and brewing industries. The legal regulations relating to the trading of alcoholic beverages impose strict standards for ethanol content, whereby the permissible deviation between their actual concentration and that declared on labels is slight. Therefore, it is necessary to implement increasingly precise methods of determining ethanol content. Wang et al. (2003) and Castellari et al. (2001) have reached very low limits of quantification using the methods they describe, however, our LOQ is sufficient to determine ethanol in the context of quality control of alcoholic beverages. Organic compounds such as alcohols can be easily analyzed via gas chromatography with a flame ionization detector due to their volatility and the content of carbon atoms in a molecule. However, our research shows that the use of substances such as 1-propanol (Jamieson, 1979; AOAC method 984.14) or 2-propanol (Clarkson et al., 1995; AOAC method 983.13, Sisco & Robinson, 2020) as internal standards may lead to the underestimation of ethanol concentration via gas chromatography methods. These substances are found in a majority of beer samples and soft drinks as well. The standard we used, *tert*-butanol, does not form spontaneously in the processes of oxidation and degradation of components of alcoholic beverages and therefore possesses great potential for use in further analyses. Additionally, chemical compounds formed during fermentation have the significant influence on liquid viscosity. In the case of gas chromatography methods, viscosity has a key influence on the proper transition of sample components into the gas phase. However, with the use of the headspace autosampler, neither the viscosity nor the content of macromolecular substances (such as polysaccharides and proteins) in alcoholic beverages affects the determination of volatile compounds. Furthermore, when a single chromatographic column is used, it may be difficult to distinguish individual chemicals. In one of our columns (Zebron ZB-BAC1), the retention time of 2-propanol and acetonitrile is the same; thus an analysis of the retention times in the second column (Zebron ZB-BAC2) is needed to confirm the presence of these substances in the samples. This proves that methods using two columns with different polarities enable the avoidance of analytical mistakes related to the overlapping of peaks of several substances in a given method.

3.2. Application of the method

3.2.1. Beers

The 140 beer samples (mainly craft beers from small local breweries, as well as imported ones) were sorted according to style family; then each was analyzed in order to determine its actual ethanol content and to compare it with the content indicated on the label, its compatibility with the characteristic strength of each beer style, and its compliance with the maximum permissible deviation in alcohol concentration for the beverage according to regulations in EU countries. The results are presented in Supplementary material (Table S1).

The Beer Judge Certification Program (BJCP) recommends that each style of beer should contain a specific ethanol concentration, known as alcohol by volume (ABV). However, in the case of speciality (fruit and spice) beers, there are no specific ranges related to the content of ethyl alcohol, as ABV depends on the underlying base beer. Detailed information on recommended alcohol concentrations [%] for specific beer styles are given in Table S2 in the Supplementary material.

Of all collected samples, 131 beers were analyzed for compliance with the guidelines. In most cases, the ethyl alcohol concentration was within the characteristic range for a style family. However, as many as 41% of the tested samples were characterized by an overly high or low ethanol level (Fig. 2).

Another criterion for beer quality assessment was a comparison of its actual concentration to that declared in accordance with Regulation (EU) No 1169/2011 of October 25, 2011 on the provision of food information to consumers, which allows for a maximum deviation (positive or negative) of 0.5% (v/v) (for beers with alcohol content of 1.2–5.5 vol%) and 1.0% (v/v) (for beers with content above 5.5 vol%). According to these regulations, all beers (whether produced in the EU or imported) should fall within the limits of established standards. The analysis showed that, of 140 beer samples, in 83 cases the actual concentration was within the limits of error; however, as many as 57 beer samples (i.e. 41% of all tested samples) failed to comply with EU standards (Fig. 2).

Furthermore, for each beer sample the relative error value ERROR [%] was calculated on the basis of the ratio of the average real concentration to the declared concentration. Beer samples were classified into five groups: A: relative error value below 5%; B: relative error value within the range 5–10%; C: relative error value within the range 10–20%; D: relative error value within the range 20–30%; group E: relative error value above 30%. Of all examined samples, only in the case of 34 samples (less than 25% of the total) did the actual concentration differ from the declared concentration by as much as 5%, whereas as many as 31 samples within group A were compliant with BJCP recommendations. Group B included 38 samples, of which only 21 were characterized by the alcohol volume appropriate to their style. The greatest number of all beer samples (as many as 46 specimens) were those with a maximum relative error value up to 20%. The last two groups (D and E), with the highest degree of non-compliance (as high as greater than 30% of the relative error value), included 16 and 6 samples, respectively (Fig. 3A).

This results indicate the need of given the increasing caution to monitor the compliance of beers with international standards in order to avoid abuse and falsification. It is caused especially by the fact that consumers interested in craft beers are attracted by their authenticity and the long tradition of their production in a specific style.

3.2.2. Soft drinks

Of all samples of non-alcoholic beers and one cider, the actual concentration exceeded the declared value only in the case of sample no. 143. The remaining samples were within the concentration ranges declared by the producers and in accordance with EU standards. The results are presented in Supplementary material (Table S3).

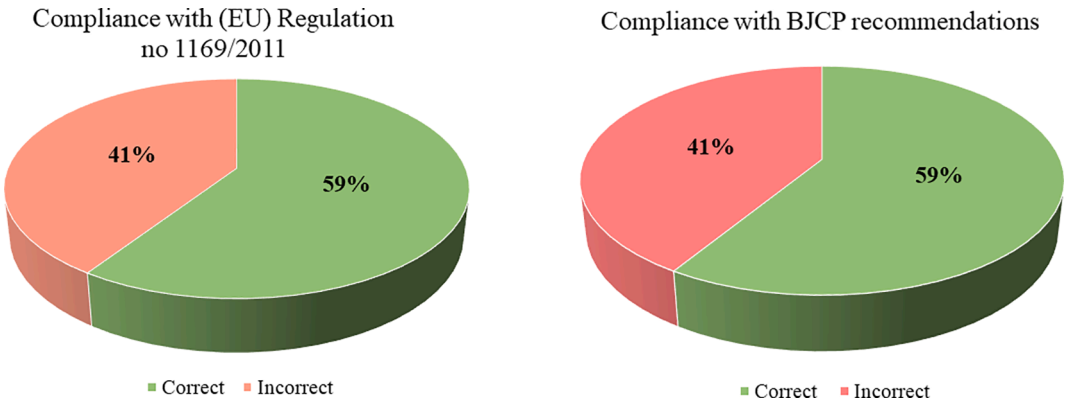

Fig. 2. Diagrams illustrating the percentage share of correct and incorrect beer samples in relation to (EU) Regulation no 1169/2011 of 25 October 2011 on the provision of food information to consumers and recommendations of The Beer Judging Certification Program (BJCP).

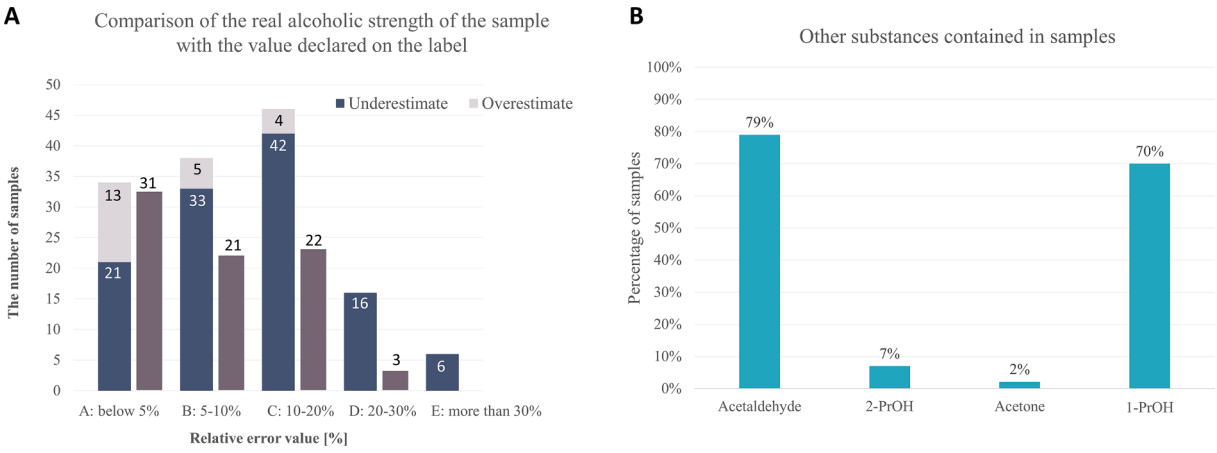

Fig. 3. A) Beers categorized into five groups (A-E) according to the relative error value of the determined concentration in relation to the declared value. Blue parts of bars represent number of samples with underestimated ethanol concentration declared on the label, while light grey parts represent overestimated value. Additionally, the number of samples fulfilling the standards recommended by BJCP for their style was specified (dark grey bars in the chart); B) Percentage of alcoholic beverages containing traces of acetaldehyde, 2-propanol (2-PrOH), acetone and 1-propanol (1-PrOH), qualitatively determined by analysis and comparison of spectra from two columns with different polarity and two flame ionization detectors.

Due to the presence of different small peaks in the chromatograms of the tested samples, we began to analyze the chromatograms obtained from two columns with different polarities and two flame ionization detectors. First, we determined the retention times of available standards: acetaldehyde, 2-propanol, acetone, 1-propanol, and acetonitrile (Fig. 4). This enabled us to qualitatively identify traces of some of these substances contained in the samples; S/N above 10 was set as the cutoff. As a result, it was shown that as many as 79% of all samples contained acetaldehyde, while 70% contained 1-propanol. Content of 2-propanol and acetone was relevant in only 13 samples (Fig. 3B). Additionally, of all soft drinks tested, only two samples contained acetaldehyde and 1-propanol.

The processes leading to the formation of 1-propanol are pH-, and temperature-dependent transformations in industrial vats (García, García, & Díaz, 1994), whereas the formation of acetaldehyde is closely related to oxidation processes and the aging of alcoholic beverages. Moreover, research on the toxicity of acetaldehyde to the human body has shown that it is necessary to monitor its concentrations in alcoholic beverages due to certain carcinogenic effects on cells, especially in ALDH-deficient parts of the population (Paiano et al., 2014). Therefore, the potential for detection of these substances, as well as of ethanol, provides an opportunity not only to improve existing technological processes in the production of alcoholic beverages but also to reduce the negative health effects associated with the accumulation of harmful fusel compounds.

3.2.3. *Wines*

Of all examined samples, only three failed to meet EU standards. For the others, actual concentrations deviated slightly from those declared. Moreover, after analyzing the chromatograms from two columns with different polarities, no low-molecular volatile contaminants (fusel alcohols) except 1-propanol were found. Table S4 in Supplementary material presents the results of the analysis of wine samples.

4. Conclusion

The headspace gas chromatography (HS-GC-FID/FID) method for the determination of ethanol in beers, wines and soft drinks has been evaluated and fully validated. The described technique is sensitive and precise with a sufficiently wide range (0.01–20.0%), therefore it can be applied for quality control of beverages in accordance to both, EU standards and BJCP recommendation. Furthermore, our studies have shown that most alcoholic beverages contained other volatile substances (acetaldehyde, 1-propanol, 2-propanol and acetone). For this reason, the use 1-PrOH and 2-PrOH as the internal standards for ethanol determination, may lead to underestimation of analysis results. The *tert*-butanol used in this research does not spontaneously form as a result of the beverages ageing and can be successfully applied as new internal standard in gas chromatographic methods.

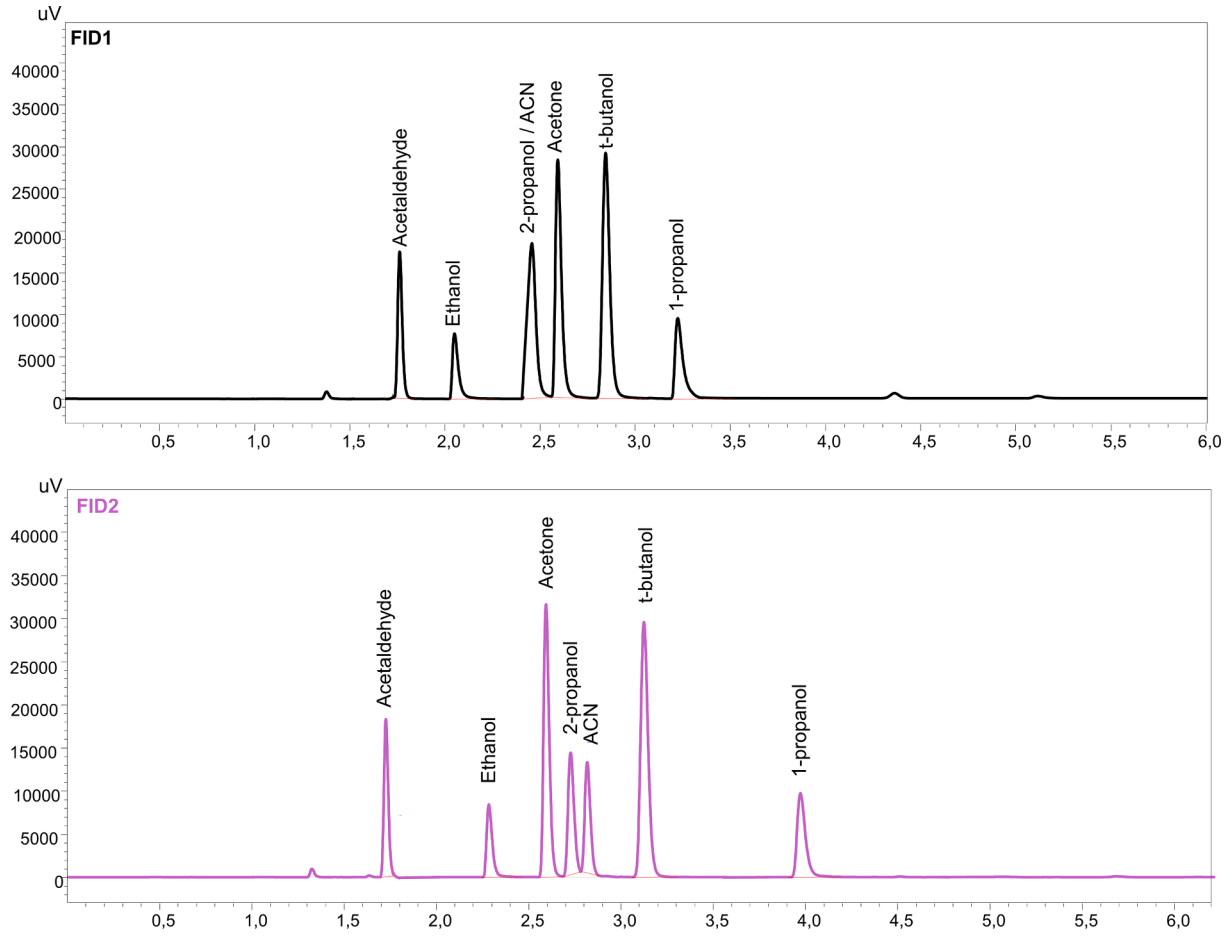

Fig. 4. Chromatograms of other volatile standards from two columns and two flame ionization detectors. Black line is illustrating result from FID1 and violet line from FID2. The above chromatograms show that one peak from first column ZEBRON-BAC1 (Rt = 2.475) comes from two substances: acetonitrile (ACN) and 2-propanol and can only be distinguished when analysing the response of these substances from the second column ZEBRON-BAC2 and FID2.

5. Additional application

On the chromatograms, beyond the peaks of the identified substances, there are also signals deriving from unknown chemical compounds. The authors intend to continue research designed to extend the current method with more chemical compounds in order to create a complex instrument enabling quantitative analysis of the greatest possible number of alcoholic beverages. Exemplary chromatograms are presented in Supplementary material (Fig. S1).

CRediT authorship contribution statement

Olga Wachełko: Validation, Writing - original draft. **Paweł Szpot:** Conceptualization, Methodology, Supervision. **Marcin Zawadzki:** Writing - review & editing, Resources.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128924.

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