Determination of the alcohol content in kombucha with Alcolyzer 3001 Beer

Relevant for: producers of kombucha, taxation offices

Kombucha, a tea-based fermented beverage that may contain small amounts of alcohol, has been enjoying increased popularity due to its refreshing taste and possible positive health effects on the human organism. The amount of alcohol, however, plays an important role for the wellbeing and enjoyment of the consumer and also for legal aspects.

Alcolyzer 3001 Beer gives reliable information on the alcohol content of kombucha.

1 Kombucha: a composition of tea, sugar and a symbiotic culture of bacteria and yeast

Kombucha is a fermented effervescent drink that may contain small amounts of alcohol. Kombucha is made from tea by adding sugar and a symbiotic culture of bacteria and yeast. During the fermentation process, the sugar is converted into CO_2 and ethanol. Subsequently, most of the ethanol is transformed into acids by the bacteria leading to a final product that possibly contains a small amount of alcohol.

Generally, kombucha is referred to as a soft drink. Depending on country-specific regulations, soft drinks may contain up to 0.5 percent alcohol by volume (% v/v). For example, in the United States a kombucha product is considered an alcoholic beverage if it contains 0.5 % v/v or more alcohol and therefore is subject to TTB (Alcohol and Tobacco Tax and Trade Bureau) regulations.

Most kombucha products do not exceed this amount, but there are also kombucha beverages with more than 0.5 % v/v alcohol available on the market. These beverages are called “hard kombucha”. Fermentation in a packaged kombucha product below 0.5 % v/v may continue, e.g. when stored without cooling. In such a case, the alcohol content may increase to amounts above 0.5 % v/v, and the product is no longer considered a soft drink, but an alcoholic beverage, which is subject to different laws and regulations. This makes an accurate alcohol determination necessary.

2 Alcohol measurement in kombucha

Head space gas chromatography is a recommended test method for accurate ethanol testing by Kombucha Brewers International (https://kombuchabrewers.org). It was the goal of this study to investigate whether also other analytical methods are as suitable for reliable measurements of the alcohol content in kombucha beverages.

For that purpose, 14 different kombucha samples were acquired, all of them soft drinks with less than 0.5 % v/v alcohol.

Four different methods were used to determine the alcohol content in these kombucha products.

The alcohol content was determined using

1. head space gas chromatography,
2. a Beer Analyzing System,
3. distillation followed by density measurement, and
4. a PBA 5001 Beer (Packaged Beverage Analyzer for Beer).

The first two of these four determination methods were carried out by a field test partner of Anton Paar. Distillation plus density and PBA 5001 Beer measurements were done exclusively by Anton Paar. The results of all four methods were compared to each other.

3 Analytical methods and procedures

3.1 Sample preparation

For reliable comparison between the four methods, a sufficient amount of exactly the same composition had

to be provided for all analyses. To avoid possible influences of variations on the alcohol results caused by bottle fermentation, four bottles each of the 14 selected kombucha samples were combined in one big bottle. The contents were mixed, and the bottle carefully closed and stored in an ice bath.

Prior to analysis, each homogenized sample was divided into four equal portions and transferred to smaller packages for analysis.

3.2 Head space gas chromatography

Chromatographic analyses were performed using a Perkin Elmer Clarus 500 and Turbomatrix 110 Head space Unit. Note: There are many different suppliers of chromatographs on the market. Operating such a system requires skilled personnel.

3.3 Beer Analyzing System

Sample investigations have furthermore been carried out using Beer Analyzing System (shown in the standard configuration in **Figure 1**). The setup consisted of a DMA 5001 density meter and an Alcolyzer 3001 Beer.

Figure 1: Beer Analyzing System, consisting of DMA 5001, Alcolyzer 3001 Beer and Xsample 520 sample changer

As this system is not pressurized, degassing the sample is required prior to analysis. The samples were filtered prior to analysis.

Tip: For this kind of samples, folded paper filters (e.g. Rotilabo Type 113) 5 µm - 8 µm are recommended.

3.4 Distillation and density measurement

The distillation was performed according to the TTB (Alcohol and Tobacco Tax and Trade Bureau of the U.S. Department of the Treasury) proofing regulations using two Erlenmeyer flasks with attached distilling column, condenser and adapter tip. The distillate was collected in two 100 mL volumetric flasks which were placed in an ice bath. The density of the distillate was then analyzed using a DMA 4500 M density meter. The alcohol content (in % v/v) was calculated according to the Ethanol AOAC Association of Official Agricultural Chemists 60 °F.

Tip: According to TTB's procedure, the sample is not neutralized prior to distillation. However, omitting neutralization may have a falsifying effect on the alcohol result: if there are volatile components in the sample, they are transferred to the distillate thus increasing the density. An increased density pretends a lower alcohol concentration. For that reason, neutralizing the sample is compulsory in case of volatile components in the sample. If the composition is not exactly known, all samples should be neutralized in any case.

Also, the International Organisation of Vine and Wine (OIV) gives valuable information ("Alcoholic strength by volume" oiv-ma-as312-01a) on the complex and elaborate distillation procedure.

3.5 Packaged Beverage Analyzer for Beer

A Packaged Beverage Analyzer for Beer (PBA 5001 Beer) was used for analyzing these 14 kombucha samples.

Figure 2: PBA 5001 Beer, consisting of DMA 5001, CarboQC ME and PFD Piercing and Filling Device

Tip: For samples that are colder than 15 °C (59 °F), a sample conditioner between DMA 5001 and Alcolyzer 3001 Beer ensures sample pre-heating in real time, allows quicker measurement results and avoids condensation on the instruments' measuring cells.

4 Results and discussion

All results obtained with the four different analysis methods are summarized in **Table 1**.

To graphically display the agreements between the different methods and the agreement with the expected alcohol content (i.e. below 0.5 %v/v), the data summarized in **Table 1** are presented in **Figure 3**. It can be seen at a glance from **Figure 3** that not all samples are within the allowed limit.

All four analysis methods deliver results that are close to each other. This confirms that the actual alcohol content of the samples 5, 6, 7, 8, 9, 13 and 14 was above the allowed level.

The Beer Analyzing System and PBA 5001 Beer are based on an optical measurement principle. Therefore, only transparent samples can be analyzed. Strongly turbid samples (samples 5, 6, 14) might have a detrimental effect on the determination. For that reason, highly turbid samples have to be filtered prior to analysis. Also, samples with pulp or solid particles have to be filtered before being investigated with an Beer Analyzing System and a PBA 5001 Beer Packaged Beverage Analyzer for Beer.

Table 1: Obtained ethanol (EtOH) results with four different methods

Sample	EtOH [% v/v] (with head space chromatography)	EtOH [% v/v] (with Alcolyzer Beer Analyzing System, not pressurized)	EtOH [% v/v] (by means of distillation and density measurement)	EtOH [% v/v] (with PBA 5001 Beer; pressurized)
1	0.18	0.12	0.16	0.14
2	0.32	0.27	0.31	0.28
3	0.13	0.13	0.06	0.16
4	0.21	0.18	0.16	0.20
5*)	1.44	1.32	1.24	1.37
6*)	1.44	1.34	1.45	1.33
7	0.68	0.63	0.55	0.63
8	0.74	0.68	0.61	0.70
9	0.93	0.91	0.86	0.91
10	0.31	0.30	0.26	0.30
11	0.08	0.06	0.01	0.06
12	0.01	0.01	0.01	0.01
13	1.25	1.22	1.19	1.22
14*)	1.23	1.14	1.04	1.17

*) sample was slightly turbid

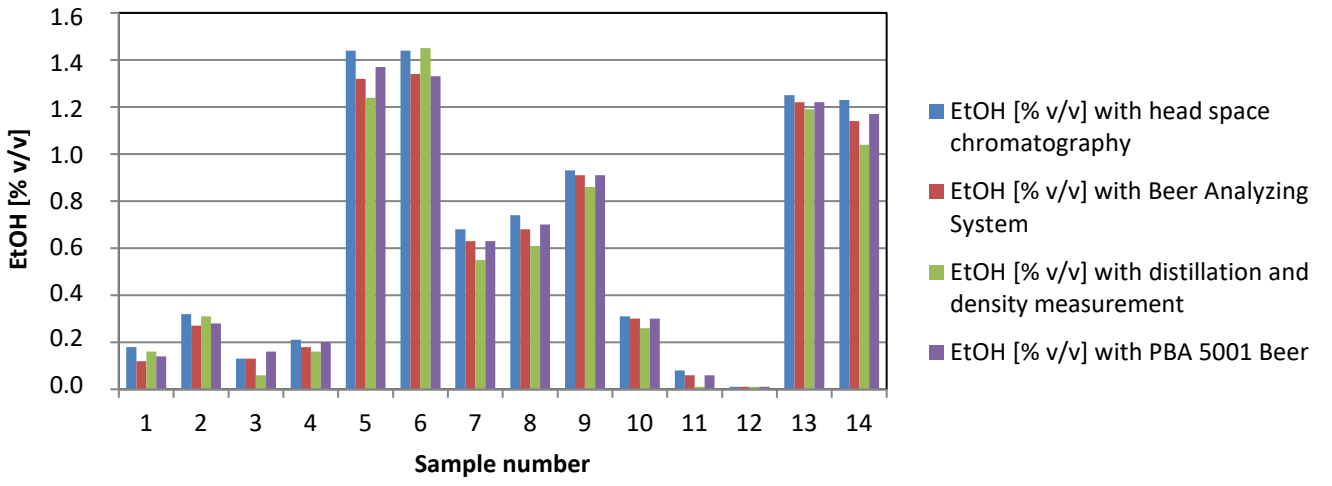

Figure 3: Obtained results with four different analytical methods

5 Ready for the future

Beer Analyzing System as well as PBA 5001 Beer offer options for modular extension should the need for accurate measurement of additional parameters arise. This is valid for kombucha below 0.5 % v/v of alcohol, for hard kombucha with an alcohol content above 0.5 % v/v, and also for other beverages such as beer.

The Beer Analyzing System can be complemented with a pH 3101 measuring module for the simultaneous determination of the pH value.

Also a PBA 5001 Beer can be combined with a pH measuring module and, as an addendum to CarboQC ME, an Option O2 for the simultaneous measurement of oxygen in the sample. This paves the way for comprehensive quality control of numerous beverage types.

In case the sample to be investigated is beer, there is even the additional option for color determination for Alcolyzer 3001 Beer. Beer Analyzing System as well as PBA 5001 Beer can be upgraded with an option color for the simultaneous measurement of beer color (complies with EBC Analytica 8.5 color of wort: spectrophotometric method (IM) – 2000 and ASBC Method of analysis – BEER 10 Color).

6 Summary

The results indicate that all four analytical methods are suitable for alcohol determination in kombucha. It could also be shown that not all samples contain alcohol below 0.5 % v/v of alcohol.

Comparison of four analysis methods showed that Anton Paar Beer Analyzing Systems and PBA 5001 Beer systems provide a fast and reliable way of alcohol determination on kombucha samples. The pressurized as well as the non-pressurized system allow the simultaneous determination of other important parameters in the sample such as pH. In case of the pressurized PBA 5001 Beer system, CarboQC ME can be combined with an Option O_2. In that case, also carbon dioxide and oxygen can be measured - in as little as 3 minutes without sample preparation.

Thus, both systems represent a suitable alternative to head space gas chromatography and the time-consuming distillation followed by density measurement.

An additional advantage manifests itself in the fact that - in contrast to head space gas chromatography - kombucha samples with alcohol contents ranging from zero up to 12 % v/v of alcohol can be measured with Beer Analyzing Systems and PBA 5001 Beer systems using the same adjustment.

Both, Beer Analyzing Systems and PBA 5001 Beer act environmentally friendly as no chemicals are required for performing the measurements.

7 Recommendation

Kombucha samples are commonly stored at low temperatures to avoid progressing fermentation. With a Beer Analyzing System the kombucha samples have to be degassed which requires them to be warmed up in order to be able to drive CO_2 out. A PBA 5001 Beer in combination with a sample conditioner can even accommodate cold samples below 15 °C (59 °F) without the need of degassing and is therefore considered the best suitable solution for this application.

Neutralizing any sample will hold back volatile acids. Even if the levels of volatile acids in the sample are extremely low, Calcium Hydroxide may be routinely added to all samples prior to distillation to avoid any falsified alcohol results.

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