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Measurement of Ethanol in Raw Unpasteurised Kombucha using the Megazyme K-ETOH Test Kit

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INTRODUCTION:

Kombucha is a fermented, lightly effervescent sweetened black or green tea drink. It is marketed as a functional beverage based on its reported health benefits. Kombucha is produced by fermenting tea using a "symbiotic 'colony' of bacteria and yeast" (SCOBY).

Kombucha is marketed as a "non-alcoholic beverage". It is sold in a raw and unpasteurised form. If kept at temperatures above 4°C, there is a possibility that it will continue to ferment and produce ethanol. This possibility of continued fermentation may lead to an increase in ethanol content from levels below 0.5% "alcohol by volume" (AbV) at time of production to higher levels at the time of consumption. Thus there is a potential for levels rising to greater than the 0.5% AbV required to pass as non-alcoholic beverage."

AIM:

Evaluation of K-ETOH for the measurement of ethanol in five different commerical Kombucha preparations.

METHOD:

Approximately 2 mL of each Kombucha solution was filtered using a 0.2 micron filter to remove live microorganisms present in the sample.

The solution was mixed using a vortex mixer for approx. 30 sec to remove any residual gas.

The samples were diluted using distilled water to a concentration suitable for analysis using the Megazyme Ethanol assay kit (K-ETOH). A 1:200 dilution was used in this experiment.

The samples were analysed using the K-ETOH method, however the reaction was lengthened to 10 min in order to ensure the reaction had fully reached completion (see Figure 2, page 3).

The absorbance values were entered into the K-ETOH *MegaCalc*™ to calculate the ethanol concentration in both g/L and % w/v.

The experimental results are outlined in Table 1 & Figure 1(page 2 & 3).

Sample	Change in Abs at 340 nm	Ethanol obtained % w/v	Ethanol expected % w/v	Alcohol (Ethanol) by volume (AbV) % v/v
Sigma Chemical Company, Ethanol standard. Cat. no. 459836	0	0	0	0.0000
	0.0515	0.097	0.1	0.1229
	0.1095	0.2055	0.2	0.2605
	0.541	1.006	1	1.2750
	0.795	1.494	1.5	1.8935
	1.0555	1.984	2	2.5146
Megazyme Ethanol standard (K-ETOH Kit) Cat. no. K-ETOH	0	0	0	0.0000
	0.0545	0.1025	0.1	0.1299
	0.107	0.2015	0.2	0.2554
	0.529	0.99425	1	1.2601
	0.7925	1.489	1.5	1.8872
	1.056	1.985	2	2.5158
Kombucha A	0.312	0.5864	< 0.5	0.7432
Kombucha B	0.4925	0.9256	< 0.5	1.1731
Kombucha C	0.7975	1.4988	< 0.5	1.8996
Kombucha D	0.455	0.8551	< 0.5	1.0838
Kombucha E	0.5905	1.1098	< 0.5	1.4066

Table 1. Ethanol values determined using the Megazyme ethanol kit (K-ETOH) and calculated using the Megazyme *MegaCalc*™ Excel calculation device.

$$\% \text{ AbV} = \frac{\% \text{ Ethanol w/v (g/100mL)}}{0.789 \text{ (density of Ethanol at 20°C)}}$$

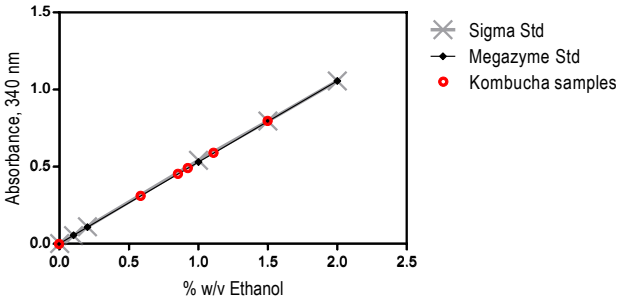

Figure 1. Graph showing the relationship between absorbance at 340 nm and ethanol concentration (% w/v) for Kombucha samples and ethanol standards.

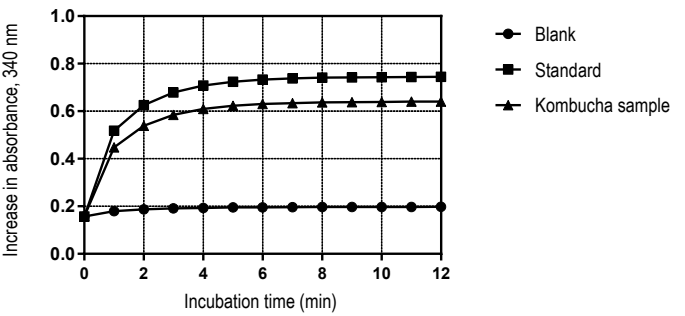

Figure 2. Graph showing the increase in absorbance at 340 nm for Kombucha sample, Megazyme standard (5 µg) and reaction blank on incubation with alcohol dehydrogenase and aldehyde dehydrogenase in the presence of NAD^+.

RELIABILITY OF METHOD:

The experiments were performed using the ethanol standard from the Megazyme K-ETOH kit and Sigma Ethanol Standard Cat. no. 459836. Two different Konbucha samples were analysed before and after the addition of a known amount of ethanol (spiking experiments), and the recovery of added ethanol determined.

The recovery of the added ethanol was quantitative (99.7-102.2 %), demonstrating lack of interference of components in the Kombucha samples with the analyses (data not shown).

CONCLUSION:

In this study, all of the five commercially available Kombucha products analysed had an ethanol content above 0.5 % AbV, the legally allowed level to pass as a "non-alcoholic beverage". These samples were micro-filtered and analysed immediately the commercial bottles were opened, to avoid the possibility of continued fermentation on opening.

Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com