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To cite this article: Anwar Borai, Shahad Sebah, Albandari Alshargi, Fatma Albarzan, Ali Al-Ghamdi, Sami Al-Ghamdi, Sultanah Boraie, Suhad Bahijri, Ali S. Al-Shareef, Abdulfattah Al-Amri & Gordon Ferns (2021) Ethanol content of a traditional Saudi beverage Sobia, International Journal of Food Properties, 24:1, 1790-1798, DOI: [10.1080/10942912.2021.2005089](https://doi.org/10.1080/10942912.2021.2005089)

To link to this article: <https://doi.org/10.1080/10942912.2021.2005089>



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Published online: 08 Dec 2021.



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Ethanol content of a traditional Saudi beverage Sobia

Anwar Borai^a, Shahad Sebah^a, Albandari Alshargi^a, Fatma Albarzan^a, Ali Al-Ghamdi^a, Sami Al-Ghamdi^b, Sultanah Boraie^a, Suhad Bahijri^c, Ali S. Al-Shareef^a, Abdulfattah Al-Amri^a, and Gordon Ferns^d

^aKing Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, Faculty of Medicine & Applied Medical Sciences, King Abdulaziz Medical City, Jeddah, Saudi Arabia; ^bToxicology Regional Laboratory, Jeddah, Saudi Arabia; ^cDepartment of Clinical Biochemistry, Faculty of Medicine, and Food, Nutrition and Lifestyle Research Unit, King Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia; ^dDivision of Medical Education, Brighton and Sussex Medical School, Mayfield House, Falmer, Brighton, UK

ABSTRACT

Sobia is a traditional locally made fermented beverage consumed in Saudi Arabia and made by the fermentation of wheat. We aimed to measure ethanol concentration in Sobia beverage stored under different conditions. Forty-eight freshly made Sobia samples were purchased from different vendors. Twenty-four samples were stored at room temperature (RT: 21–25°C), and another 24 samples at a cold temperature (CT: 2–8°C). Ethanol was measured in RT samples on 0, 1, 2, 3, 4 and 7 days, and on 0, 4, 7, and 14 days in CT samples. The mean \pm SE ethanol at zero-time for all 48 samples was 27.2 ± 7.2 mg/dL. Ethanol concentration increased to 121.8 ± 33.5 mg/dL on day 14 at CT, compared to 514.0 ± 91.9 mg/dL after one week at RT. Sobia can be categorized as Halal beverage, and can be consumed within two weeks when stored at a CT.

ARTICLE HISTORY

Received 25 June 2021
Revised 4 November 2021
Accepted 5 November 2021

KEYWORDS



Sobia; Saudi Arabia; Alcohol; Ethanol

INTRODUCTION

Fermented beverages are widely consumed around the world. They are usually produced under controlled conditions, during which the effects of microorganisms (MOs) such as yeasts that contain fermenting enzymes lead to specific biochemical changes^[1] that is temperature dependent. The health and economic benefits of the fermented beverages are well documented.^[2,3] However, fermentation by yeasts such as *Saccharomyces cerevisiae* may lead to the production of ethanol because they convert sugar into ethanol and CO₂, and many fermented beverages are therefore alcoholic.

In African countries, cereals are the most common foods, and fermented cereals are important ingredients for traditional beverages. Examples of alcoholic beverages and the major MOs involved in fermentation are as follows: Marissa in Sudan (*Lactobacillus spp.* and acetic acid bacteria) and Kishk in Egypt (*Lactobacillus spp.* and yeasts). Other nonalcoholic fermented beverages such as Koko-kenkey in Ghana (*Lactobacillus spp.* and yeasts), and Kisra in Sudan for which the MOs involved in fermentation are unknown.^[4]

The tropical weather in the region of the Asian-Pacific is suitable for the cultivation of rice and this is fermented in the Southeast and Far Eastern countries. Examples of alcoholic beverages and their major MOs involved in fermentations are as follows: Shaosinghjiu in China (*S. cerevisiae*), Sakee in Japan (*S. sake*); Chongju in Korea (*Lactic acid bacteria* and *S. cerevisiae*); and Brembali in Indonesia (*Mucor indicus* and *Candida*).^[4]

CONTACT Anwar Borai  anwareeka@gmail.com  King Abdullah International Medical Research Center (KAIMRC), King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City, Jeddah 21423 Saudi Arabia

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Sobia is a traditional locally made fermented beverage consumed in Saudi Arabia. Sobia is produced by the fermentation of wheat, and is prepared by fermenting bread with cardamom, cinnamon, sugar, and baker's yeast. All ingredients are mixed and soaked in water, placed into a tightly closed container, and kept between 30°C and 40°C for at least 24 hours. The liquid is then filtered before being served as a cold beverage (Figure 1). It is commonly left unpasteurized. Sobia is sold and mostly consumed during the holy month of Ramadan, but it is available at any time of the year. Sobia without additives is

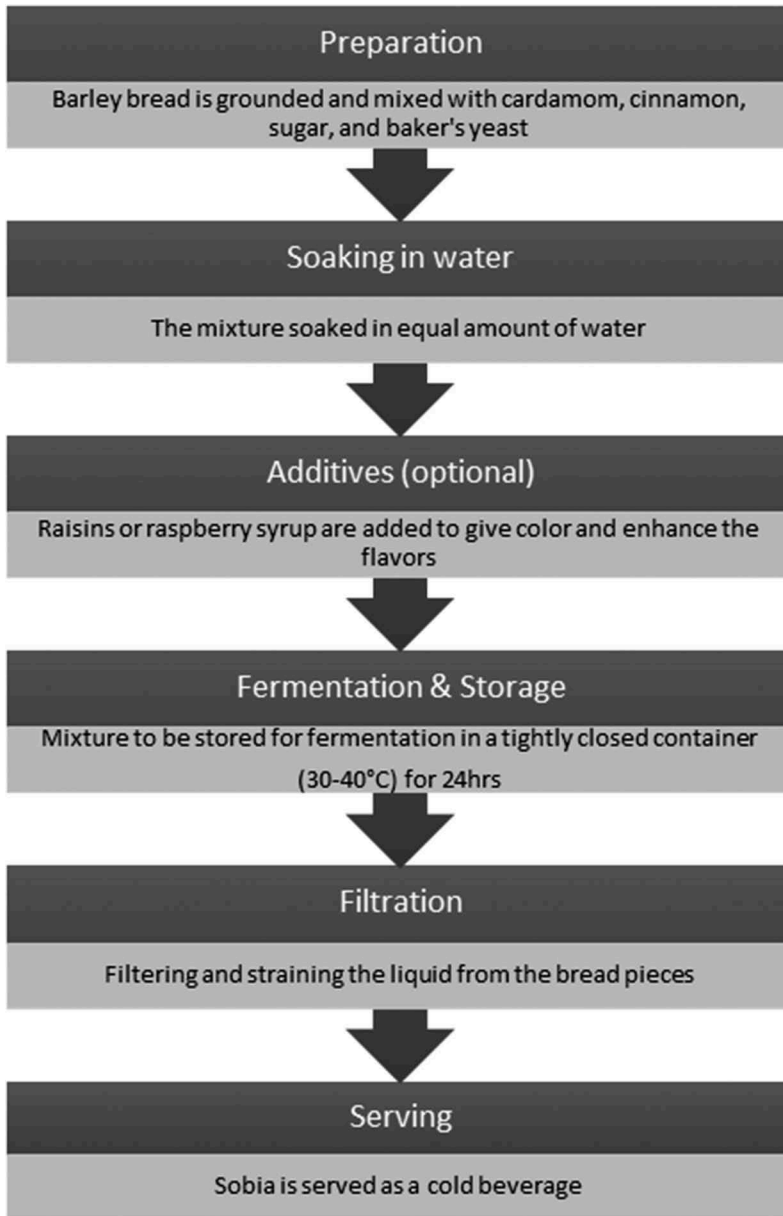


Figure 1. Diagram of preparation of Sobia beverage.

white in color, but different flavors and colors can be added, such as raisins, or raspberry syrup.^[5] Reaching the desirable taste of Sobia requires manipulation in temperature, acidity, added flavors etc., which are also factors that regulate the activity of MOs during the fermentation process.^[4]

Sobia is usually sold either in sealed bottles made by local distributors, or in open containers by independent street vendors. It is generally acknowledged that freshly made Sobia should be consumed within two to three days if it is stored in the fridge. However, Sobia is often manufactured, and stored under conditions that are not strictly regulated. For decades, there has been a strong connection between Sobia and its preparation as a traditional home-made product by families living in different cities in the western region of Saudi Arabia, that is, Makkah, Jeddah, and Madinah. Nowadays, Sobia has become more popular and has started to be available in other regions of Saudi Arabia.

Recently, there have been ongoing debates on social media regarding the alcoholic content of Sobia. Alcohol in foods and drinks is prohibited under Islamic Shariaa law, and under the perspective of Islamic law, the issue of alcoholic content of Sobia is sensitive and requires clarification. For this reason, the Saudi Drug and Food authority has reported recently that the small amount of alcohol present in Sobia is generated from self-fermented fruits and the amount of the alcohol is due to fermentation from the added sugar.^[6] This statement was essentially based on an early study reporting that Sobia has low levels of ethanol compared to other alcoholic beverages.^[7] However, this statement has not been adequately supported by scientific data, especially with respect to the effect of storage, and the debate has continued. Indeed, no studies have been carried out on the effect of storage at different temperatures on the alcoholic content of this beverage.

Recently, we have investigated the types and counts of MOs in Sobia (unpublished data), and we have found that the dominant MOs present at RT and CT were *Lactobacillus* spp., *Candida* spp., *Saccharomyces cerevisiae*, and *Klebsiella* spp. Furthermore, some pathogenic *E. coli* were detected, possibly due to the poor hygienic conditions during the preparation. We found that the predominance of MOs is contingent upon the temperature and method of storage. However, our results on the microbial content of Sobia have not been completed, yet the outcomes remain compatible with previous studies.^[5,8] Therefore, we thought that the difference in the types and counts of MOs under different storage conditions is likely to affect the composition of the beverage, including its alcohol content. This study aimed to investigate the content of ethanol in freshly prepared Sobia and how its concentration, changes over time under different storage conditions.

Methods and Materials

Samples collection and preparation

Forty-eight freshly made Sobia samples (1 liter each) were purchased from licensed stores (24 samples) named as bottled samples (BS) and another 24 samples were purchased from street vendors (SV). Sobia from BS are available throughout the year while Sobia from SV are regularly home-made and sold in the streets only during the holy month of Ramadan. Samples were selected randomly from different sectors in Jeddah city. Sobia should be sold and served as a cold beverage, and the temperature was 2–8°C for all samples when purchased from both shops and street vendors,

Each sample was transferred to a labeled and closed sterile container. Twenty-four samples (12 BS +12 SV) were stored in the fridge (2–8°C) and the other 24 samples were stored at RT (21–25°C) as shown in [Figure 2](#). The reason for this diversity in samples collection is to ensure a representative unbiased distribution of Sobia samples available in the market.

A sterile syringe was used to transfer 10 mL aliquots from each sample container to a sterile labeled tube for analysis at different time intervals, taking care to avoid contaminating samples during storage and samples analysis. All samples were tested at different time intervals for

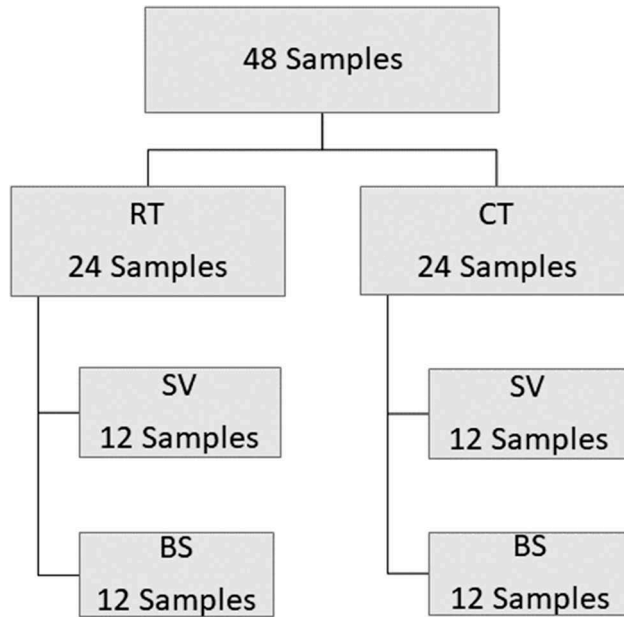


Figure 2. Protocol for Sobia samples ($n = 48$) collected in the study and equally divided between cold temperature (CT; 2–8°C) and room temperature (RT; 21–25°C) and equally subdivided between street vendor (SV) and bottled samples (BS).

ethanol content. Samples stored at RT were tested on six different days starting from purchasing day (zero-time), day 1, day 2, day 3, day 4, and day 7. Samples stored in the fridge were tested at zero-time, day 4, day 7, and day 14. The selection of these time intervals under each storage condition was based on the likely maximum storage period by consumers. The study was performed in the department of pathology and laboratory medicine, King Abdulaziz Medical City - Jeddah, Saudi Arabia.

Ethanol determination

Ethanol was estimated based on the principle of enzyme multiplied immunoassay technique (EMIT) plus Ethyl Alcohol assay. The latter is an enzymatic reaction using alcohol dehydrogenase enzyme and the reduction of NAD^+ to NADH, so that the increase in absorbance at 340 nm is proportional to the alcohol concentration in the sample. Ethanol was quantified accurately throughout the range of 3–600 mg/dL without dilution. The assay quantifies ethanol concentration up to a concentration of 600 mg/dL (0.60%). Samples containing >600 mg/dL of ethanol were diluted with negative ethanol calibrator. Within and total run precision were determined using low and high controls with the minimum and maximum coefficient of variations (CV) 1.39 to 1.78, respectively. Ethanol assay was conducted using a Syvia V-Twin analyzer (Siemens Healthcare Diagnostics Inc).

The V-Twin Alcohol method is dedicated to measuring ethanol in biological fluids but not alcohol in food or drink. Therefore, the method was validated by Headspace gas chromatography using a flame ionization detection (HS-GC-FID) and a Shimadzo Nexis-2030 and Headspace HS-20.

After pretreatment, 20 Sobia samples were prepared by mixing 200 μL of the internal standard solution with 1 mL of Sobia in a 20-mL headspace vial which was sealed immediately with a crimper prior to analysis. A control standard was prepared by mixing ethanol in HPLC-grade water at concentration of 25 and 80 mg/dL and column Rtx-BAC1, 30 m \times 0.32 mm \times 18 μm . Helium gas was used as a carrier and Flame Ionization as a detector.

Statistical analysis

All analyses were performed using SPSS v.25. Repeated measures one-way ANOVA was used to compare means of variables across different time intervals. Paired t-test was used to compare different means. Spearman's correlation method was used for the purpose of association between different methods. Data was expressed as mean \pm SE or otherwise as indicated. A $p < .05$ indicated statistical significance.

RESULTS

Ethanol measurement at zero-time

The mean (\pm SE) ethanol concentration at zero-time for the total 48 samples was 27.2 ± 7.2 mg/dL (0.03%). Mean values at zero-time of each 24 samples stored at RT and CT were not significantly different to each other (Tables 1 and 2). The mean concentration of ethanol from the 24 samples from SV (25.3 ± 3.3 mg/dL) was significantly higher than those 24 samples obtained from BS (7.9 ± 1.2 mg/dL) and $p < .001$.

Ethanol measurement at different time intervals

The ethanol (alcohol) concentration in Sobia on different days ranged between 3.0 mg/dL and 1435.4 mg/dL at RT, and 3.0 mg/dL to 607.6 mg/dL at CT. Ethanol concentration increased at a faster rate over time in samples stored at RT compared to those stored at CT as shown in Table 1 and Table 2. Indeed, ethanol concentration almost doubled during the first four days when stored at RT. Moreover, compared to zero-time, ethanol concentration increased ~ 29 times after one week of storage at RT, while at CT the concentration increased ~ 4 times after two weeks of storage. At RT, the means of ethanol concentration on days 1, 2, 3, 4, and 7 were significantly higher compared to the mean at zero-time ($p < .001$ for all). At CT, the means on days 4, 7, and 14 were significantly higher compared to the mean of zero-time ($p < .05$, $p < .01$, and $p < .001$ respectively). Repeated ANOVA shows significant changes in ethanol mean values over storage time for both conditions RT ($p < .001$; Eta = 0.532) and CT ($p < .01$; Eta = 0.333). Eta squared being the effect size of Sobia storage on ethanol variable. For the

Table 1. Ethanol concentration (mean \pm SE) at RT (21–25°C) measured on different time intervals.

	Zero-Time	Day 1	Day 2	Day 3	Day 4	Day 7	Repeated ANOVA	
							p	Eta
Ethanol \pm SE (mg/dl)	17.2 ± 3.4	$38.4 \pm 8.8^{***}$	$63.3 \pm 16.8^{***}$	$145.2 \pm 35.8^{***}$	$251.0 \pm 58.9^{***}$	$514.0 \pm 91.9^{***}$	<0.001	0.532

*** $p < 0.001$; mean compared to the zero-time. Abbreviations: ANOVA, analysis of variance; Eta – partial Eta squared, i.e., the percentage of variance that is accounted for by the effect of storage.

Table 2. Ethanol concentration (mean \pm SE) at CT (2–8°C) measured on different time intervals.

	Zero-Time	Day 4	Day 7	Day 14	Repeated ANOVA	
					p	Eta
Ethanol \pm SE (mg/dl)	24.6 ± 9.1	$37.4 \pm 13.9^*$	$52.0 \pm 18.1^{**}$	$121.8 \pm 33.5^{***}$	<0.01	0.333

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; mean compared to the zero-time. Abbreviations: ANOVA, analysis of variance; Eta – partial Eta squared, i.e., the percentage of variance that is accounted for by the effect of storage.

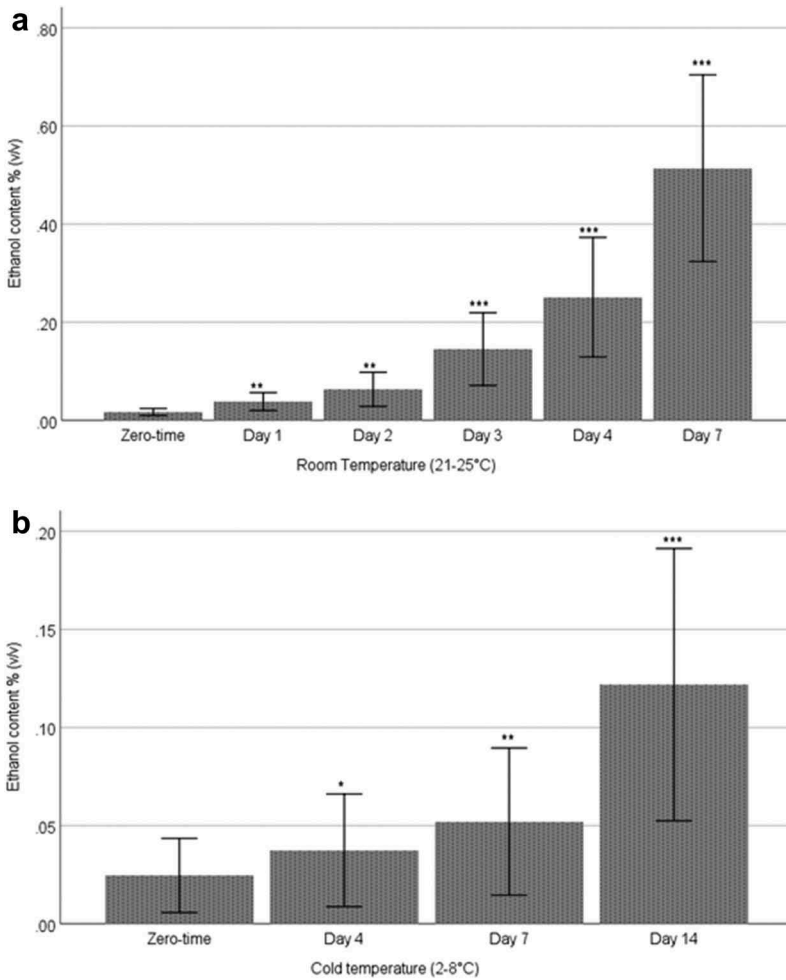


Figure 3. Percentage mean of ethanol concentration on different days of storage at (a) room temperature and (b) cold temperature. Bars represent 95% Confidence Interval. ** $p < .01$, *** $p < .001$; mean compared to the zero-time.

purpose of interchanging between ethanol weight/volume and ethanol content % (v/v), ethanol concentrations were converted from mg/dL to alcohol percent (% ethanol) as shown in **Figure 3 (a)** for RT and **Figure 3 (b)** CT, respectively.

Our data shows that ethanol in the zero-time samples is lower in BS compared to those obtained from SV. This is expected as Sobia from BS are prepared in licensed stores and under strict supervision and regulations of the municipal services of the city, that is, BS must be labeled with the preparation and expiry date while those from SV are not labeled.

Storage at either RT or CT was associated with increasing ethanol concentrations in Sobia. The ethanol concentration exceeded the 1% limit in some samples after 2 days when kept at RT. However, storing the beverage in the fridge (CT) for up to 7 days maintained its ethanol concentration within permissible limits. The lower amount of ethanol produced at CT could be due to the slow growth rate of yeast and bacteria compared to that produced at RT

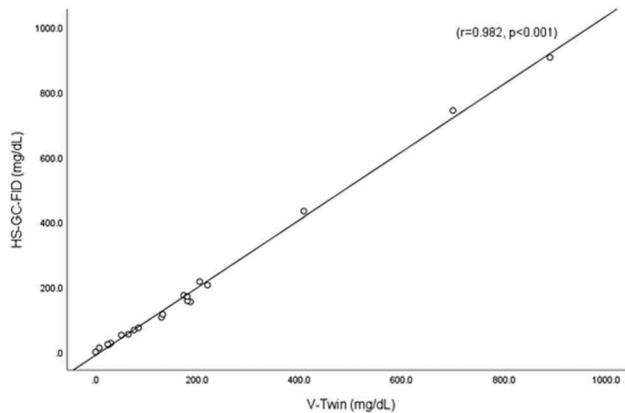


Figure 4. Correlation between the two methanol methods (V-Twin and HS-GC-FID).

Ethanol Methods Correlation

For the purpose of validating the measurement of ethanol using the V-Twin (EMIT), the CLSI-EP15-A2 guidelines were used.^[9] Twenty Sobia samples with different concentrations of ethanol were measured using the V-Twin method and run in parallel with ethanol reference methodology using HS-GC-FID analyzer. Using the paired t-test, the mean (\pm SE) concentrations for V-Twin (188.0 ± 51.7 mg/dL) and HS-GC-FID (186.0 ± 54.0 mg/dL) were not statistically significant ($p = .61$). The results between both methods were highly correlated ($r = 0.982$, $p < .001$) (Figure 4).

DISCUSSION

We have investigated the presence of ethanol in Sobia at the time of purchase, and the changes in its concentration during storage at different temperatures in a large sample of freshly prepared Sobia samples. One of the major features of Halal food or beverage is that they should not contain alcohol (ethanol). However, alcohol is allowed if the amount is not sufficient to cause intoxication.^[10] To issue a certificate for Halal food or drink in Muslim countries, the percentage of alcohol present should not exceed 0.5% in most of the countries and 1.0% in other countries. For example, in Indonesia it is at 1%, and in Singapore it is at 0.5%.^[11] In Malaysia, the permissible alcohol is either below 0.5% or 1.0% depending on the specific conditions of alcohol content if the intention is to produce alcohol or not.^[12] Additionally, the product must be prepared under strict conditions so that the alcohol is naturally present and does not cause intoxication.^[13] Therefore, according to our results Sobia when freshly prepared can be considered Halal, as it contains negligible amounts of alcohol.

Previous studies show that changing the temperature affects the metabolism of yeast and consequently the fermentation rate.^[14,15] However, the high amount of ethanol produced has an inhibitory effect on all MOs, and fermentation at very high temperature, that is, 35°C, will eventually yields a low amount of ethanol.^[15,16] These two conditions were not tested in our study, as lower amounts of ethanol and temperatures were applied. In general, the alcohol contents in both freshly collected and stored Sobia had lower reported ethanol concentration compared to other fermented beverages such as Bouza (3.8–4.2%) and Merissa in Sudan (1–2.5%).^[4]

Our main outcomes are similar to a previous study which was dedicated to investigating the presence of alcohol in the fermented beverage of Kombucha tea. It is a traditional beverage in Indonesia and made up of tea and sugar.^[17] The alcohol content of Kombucha tea found to be 0.06%. Therefore, the result of alcohol has met the Islamic regulations and declared to be Halal drink.

In 2003. Gassem et al., investigated the physico-chemical properties of Sobia on 14 samples. There were significant differences in chemical composition between the samples. Sobia samples were higher in carbohydrate (9.95%–17.22%) compared to those obtained by other nonalcoholic beverages including melon seed and mawe.^[18,19] The carbohydrates present were mainly sucrose, glucose and fructose. No fat was detected in any of the Sobia samples and high-water content with low protein and ash were found.^[5] However, Gassem et al. study had some limitations including the low number of samples and defects in the protocol of samples collection.

Our study shows that the alcohol levels in the samples obtained from SVs are higher than those from BS. This could be due to strict adherence to the process of Sobia prepared in the stores compared to the SVs. Usually, Sobia samples prepared by the stored (BS) are tightly closed and labeled with the preparation and expiry dates while those obtained from SV's are not. The exact date of samples prepared by SVs depends on their honesty. This will be reflected on the fermentation process and consequently on the level of ethanol.

Our study has some limitations. The first limitation is that the exact time of Sobia preparation when purchased was based on the integrity of the vendors, as sobia might have been prepared one day or more before. Furthermore, due to the small number of samples, the effect of temperature and storage factors on Sobia samples collected from SV and BS was not investigated separately.

The importance of the study is that it is the first, and only, study investigating this sensitive issue which has been causing heated discussions among Saudis. It proved that Sobia is Halal, and that alcohol in Sobia is considered to be a by-product during the preparation procedure and the quantity does not exceed 0.5% when kept at CT for up to 14 days. Hence, drinking Sobia is permissible. However, if Sobia is kept at RT, which is not common, then the quantity of alcohol may exceed the allowable limit.

CONCLUSION

From the results of this study, we can conclude that Sobia contains alcohol, but it is considered as a natural product from sugar fermentation by MOs. The amount of alcohol present depends on temperature and length of stay after preparation. Sobia can be considered a Halal product and the alcohol contents are within the permissible limits for the Muslim community. For optimum storage conditions, Sobia should be consumed within two weeks of storage at cold temperature (fridge).

Ethical approval

The study was approved by the Research Ethics Committee at King Abdullah International Medical Research Center, Jeddah, Saudi Arabia (IRB # RJ20/071/J).

Data availability statement

The data that support the findings of this study are available from the corresponding author, Anwar Borai, upon reasonable request.

Acknowledgments

The authors acknowledge support received from King Abdullah International Medical Research Center (KAIMRC) of King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Jeddah, Saudi Arabia. The authors are grateful to laboratory staff in the department of Pathology, King Abdulaziz Medical City and from the Regional Toxicology Center, Jeddah for their collaboration and technical support. All authors declare no competing interests. The study was approved by the Research Ethics Committee (RJ20/071/J) at King Abdullah International Medical Research Center, Jeddah, Saudi Arabia.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Gordon Ferns  <http://orcid.org/0000-0002-0957-8349>

Author contributions

AB researched literature and conceived the study. Analytical work was carried out by SS, AA, FA, AG, and SG. SA, SB, AA, AA, and GF reviewed and corrected all different drafts of the manuscript. All authors reviewed and approved the final version of the manuscript.

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