The effect of storage temperature and duration on the microbial growth in Sobia; a Saudi Arabian traditional drink

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Abstract

Sobia is a popular fermented traditional beverage in Saudi Arabia. None of the previous studies have reported on the microbial content of Sobia, stored under different conditions. Sobia samples (48) were divided into two equal sets. The first set was stored at room temperature (RT=25°C), while the second was stored at cold temperature (CT=2-8°C). All samples were examined for the presence of micro-organisms (MOs), and pH was measured on different days. Samples of Sobia stored at RT were analyzed on days 0, 1, 2, and 7, while those stored at CT were examined on days 0, 7, and 14. The presence percentage of each MO in the 24 samples stored at RT at the different four days were, respectively: *Lactobacillus* spp. (50%, 83%, 88% and 100%), *Candida* spp. (42%, 71%, 79% and 83%), *Saccharomyces cerevisiae* (17%, 21%, 25% and 25%), and *Klebsiella* spp. (63%, 50%, 38% and 25%). The MOs present at the greatest quantities in the samples stored at CT at the three different days were, respectively: *Lactobacillus* spp. (58%, 92%, and 96%), *Candida* ssp. (29%, 29%, and 54%), *Klebsiella* spp. (71%, 54%, and 46%), and *Saccharomyces cerevisiae* (17%, 33%, and 33%). On day 7 of storage, the pH (mean ± SD) fell in the samples stored at RT from baseline values of 4.20±0.80 to 3.28±0.55 ($p<0.001$), while in CT samples it decreased from baseline reading of 4.20±0.83 to 3.50±0.55 ($p<0.001$) after 2 weeks of storage. Sobia, a fermented beverage in Saudi Arabia, may be contaminated with different pathogenic MOs. Differences in temperature of storage and duration are associated with changes in MO content and Sobia storage in the cold condition can minimize the risk of any potential infection.

Keywords: Sobia, contamination, Saudi Arabia, traditional drink, micro-organism, storage

1. Introduction

Fermented beverages are produced world-wide. Fermentation is defined as a metabolic process which involves yeast and/or bacteria to convert carbohydrate into alcohol or acid. Acid fermentation is normally carried out by lactic acid bacteria, with cereal fermented beverages being examples of popular products. These beverages

may have beneficial health effects because of the high content of vitamins in their ingredients (Blandio et al., 2003; Basinskiene et al., 2016). The presence of lactic acid bacteria causes a reduction in the pH of the fermented beverage, making it more acidic, which results in a characteristic tangy, highly palatable taste (Morcos et al., 1993).
Several countries around the world have their own traditional, locally made fermented foods and beverages. Examples of the best-known fermented non-alcoholic beverages are Shalgam juice (turnip juice) in Turkey, Mawe (from maize) in South Africa, Mahewu (magou) from maize in east Africa, and Ayran, a cold savory yogurt-based beverage, popular in the middle east, central Asia, and eastern Europe with various names (Blandio et al., 2003; El-Said, 2019).

Sobia is a traditional fermented non-alcoholic beverage produced mainly in Saudi Arabia from ground barley and/or bread in addition to cardamom and cinnamon, with the addition of baker's yeast. The mixture is kept in a tightly-closed container and stored in a warm place (30–40°C) for a minimum of one day, before filtering and serving it as a cold drink. Usually, it is sold during the holy month of Ramadan, and it has different colors and flavors due to the variability of its ingredients (Blandio et al., 2003). The red color imparted to some preparations of Sobia is usually because of the addition of raisins or raspberry syrup, while the white-colored Sobia has no other additives (Al-Mukhtar, 2011). Sobia is categorized as a street food; hence it has no specifically indicated expiry date. However, it is commonly known among consumers that once bought, it should be kept in the fridge for no more than 3 to 4 days. Otherwise, its taste and odor will deteriorate. For a longer period of storage, Sobia could be frozen. A common belief among consumers is that Sobia is a good source of energy. It is also claimed that Sobia is beneficial for reducing the levels of blood cholesterol as well as controlling hypertension (Alain News, 2020). However, there have been many articles in the media warning against the street-sold Sobia, suggesting that it may be prepared without following proper hygienic and food safety procedures, and hence, can be a potential source of microbial contamination leading to serious ill health consequences (Dawood, 2012). Despite the importance of investigating Sobia microbial contamination and food safety issues, only two previous studies were conducted to investigate possible contamination (Gassem, 2002; El-Said, 2019) with another study investigating its physicochemical properties (Gassem, 2003) This study is a continuation of our previous research on Sobia. Previously, we investigated ethanol content in Sobia beverage under different storage conditions (Borai et al., 2021). Our conclusion was that Sobia can be considered as a Halal beverage with a minimal amount of alcohol present.

Indeed, no previous study has investigated the possible risk of increased microbial load and acidity when Sobia is exposed to different storage conditions. Therefore, our study aimed to investigate the presence of different micro-organisms (MOs) in freshly prepared Sobia, as well as the effect of different storage conditions at different time intervals on MOs and acidity of the stored beverage.

2. Materials and methods

2.1 Samples collection & preparation

The study was carried out between May and December of 2019 in the department of clinical laboratory sciences, King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), King Abdulaziz Medical City- Jeddah. A cross sectional design was used to collect 48 different samples.
Freshly made Sobia samples (sold in one-liter plastic bags) were collected randomly from local venues. By asking the vendors, samples suspected to have been prepared >24hrs prior to collection time were excluded. Twenty-four samples were bought from shops located in different areas of the city of Jeddah, and another 24 samples were bought from street Sobia vendors positioned in various locations. All samples were accepted and collected on the same day.

Sobia samples were aseptically transferred from their original bags to sterilized one-liter containers, which were tightly closed and labelled. Samples were then equally divided into two groups so that each group contained 12 samples from shops and 12 from street vendors. The first group of samples was stored at Room Temperature (RT, 25°C), while the second group was stored in Cold Temperature (CT, 2-8°C) in the regular fridge. During the process of testing, samples' aspiration was done by sterile syringes and transferred into sterile testing tubes. All samples were handled and processed aseptically to avoid any source of contamination that may interfere with the results.
Samples were examined on different days based on their storage conditions. Samples at RT were examined on four different days starting on the day of purchase (1st reading), then one day later (2nd reading), after 2 days (3rd reading), and finally after one week (4th reading). Samples at CT storage were examined on three different days for a total of

two weeks starting on the day of purchase (1st reading), then after 7 days (2nd reading), and finally after 14 days (3rd reading). However, both RT and CT baseline (1st readings) were done at the same time after collection (0 time). Each of the 48 samples was cultured individually at each temperature and time interval.

2.2. Culturing micro-organisms and determination of pH

For culturing micro-organisms, 10µL of the sample were streaked on different mediums (Blood, MacConkey, and Sabouraud agars) obtained from Becton Dickenson Prepared Pated Media and then incubated at 37°C in a 5% CO_2 incubator to enhance the growth of micro-organisms (MOs). The plates were examined after 24hrs, and colony forming units (CFU) were counted to express the number of MOs per 1mL of the original sample. The CFU was estimated semi-quantitatively as 100, <100k, 100k, and >100k, as the target was to identify the presence of pathogenic MOs but not the exact quantification.

The identification of MOs was performed using Vitek-MS™- Mass spectrometry microbial identification system (bioMérieux, France) according to manufacturer instructions. Vitek-MS is an automated microbial identification system that uses the Matrix-Assisted Laser Desorption Ionization Time-of-Flight "MALDI-TOF" technology. The VITEKTM-MS system can identify each spectrum of MO in a form of a series of peaks that are identified by mass and intensity. Technically, the prepared target slide of the sample is introduced to a high-vacuum environment where the contents of the sample are ionized by a laser burst. The released cloud of proteins is then accelerated by an electric charge. Time of Flight is recorded by using a specific formula and proteins in each sample are detected in a spectrum (Nori et al., 2013).

The final percentage of each MO represents the number of samples with detected MO over the total number of samples in each condition (24 samples) multiplied by 100. pH was measured using Siemens analyzer (V-Twin system) based on indicator's principle. The change in pH or percentage difference (Diff%) was calculated by subtracting the baseline (first reading) pH from the current day of measurement multiplied by 100.

2.3. Statistical analysis

Statistical data analysis was performed by using the software of JMP and SPSS v.25. A separate data collection sheet was used to record data at each time interval during the three weeks. The microbiology data collection sheet contained each sample; number, colony-forming unit (CFU), microbial count, and the percentage of each MO. Paired t-test, unpaired t-test, and analysis of variance (ANOVA) were used to compare changes over time in the mean pH of stored samples at RT and CT.

3. Results

3.1. Micro-organisms and their predominance

The types of MOs isolated at RT (25°C) and their percent representation on the four different days are shown in Table 1. At the 2nd and 3rd time points, the number of MOs increased to 64 but it decreased in the 4th time point to 56. Klebsiella spp. was present in the highest percentage of samples at baseline, followed by Lactobacillus spp. and Candida spp. However, the percentage representation of Lactobacillus and Candida species, and to a lesser extent that of Saccharomyces cerevisiae, increased with the increase of incubation time. In the 4th time point, Lactobacillus spp. was detected in all the cultured sample after one week of incubation at RT; while Klebsiella spp. showed a gradual decrease in percentage until it becomes 25% after one week. The percentage of samples positive for the other MOs was low, with E. coli and Nisseria subflava disappearing after 24h of storage, and others in later samples.

Table 1: Readings of different micro-organisms and their percentages in each time interval at RT (25°C).

		1st Reading (Baseline)			2nd Reading (24hr)			3rd Reading (48hr)			4th Reading (7 days)		
Type of MO	Name of MO	No. of MO	CFU	%MO	No. of MO	CFU	%MO	No. of MO	CFU	%MO	No. of MO	CFU	%MO
Lactic acid bacteria	*Lactobacillus* spp.	58	>100k (64%), <100k (24%), 100k (5%), 100 (7%)	50%	64	>100k (69%), 100k (5%), <100k (26%)	83%	64	>100k (66%), <100k (34%)	88%	56	>100k (79)%, <10 (7)%, 10k (14)%	100%
Yeast	*Candida* spp.			42%			71%			79%			83%
	Saccharomyces cerevisiae			17%			21%			25%			25%
Coliforms	*Klebsiella*			63%			50%			38%			25%
	AHS			17%			13%			8%			
	CNSA			4%						4%			
	Enterobacter cloacae			17%			13%			9%			
	Bacillus			21%			13%						
	Nisseria subflava			4%									
	E.coli			4%									

Abbreviations: MOs, micro-organisms; CFU, colony-forming unit; AHS, Alpha hemolytic Streptococcus; CNSA, Coagulase-negative Staphylococcus Aureus; E. coli, Escherichia coli.

The types of organisms isolated at CT (2-8°C) and their percent presence on the three different days is shown in Table 2. The total number of MOs in the 1st reading was 59. The number decreased to 52 in the 2nd time point, then increased to 58 in the 3rd time point. Similar to what was noted in the fresh samples stored at RT, Klebsiella spp. had the highest predominance in these fresh samples, followed by Lactobacillus spp. and Candida spp. However, the percentage representation of both Lactobacillus spp. and Saccharomyces cerevisiae increased after 7 days, then remained stable, while that of Candida spp. continued to increase in the sample taken 14 days later. On the other hand, that of Klebsiella showed a gradual decrease over time in a manner similar to that noted for samples at RT. Similarly, the percentages representation of other MOs was low in the fresh samples, and disappeared in later samples. The changing trend over time of the percentages representation of the most predominant MOs at both RT and CT storage conditions respectively is summarized in Figures 1 and 2.

3.2 pH measurement

Changes in pH measurements over the study period in both conditions (RT and CT) are presented in Table 3 as (mean ± SD). The mean pH of fresh samples destinied to be stored at RT or CT was acidic (4.20 ± 0.80 & 4.20 ± 0.83 respectively), with no statistically significant difference (p=0.93) between the two means. The pH readings gradually decreased but with a different rate between RT and CT kept samples (Table 3).

In samples kept at RT, the maximum decrease of pH was from the basline 4.20 ± 0.80 to 3.28 ± 0.55 (4th reading) with a difference in percentage of -21.90% and p<0.001. The repeated analysis of variance (ANOVA) across different reading intervals at RT was significant (p<0.001).

The mean pH of samples kept at CT showed a significant decrease after 7 days (3.83 ± 0.81) compared to the baseline (4.20 ± 0.83; p<0.001) with a difference in percentage of -8.81%. However, the maximum decrease of pH was from the baseline to 3.50 ± 0.55 (3rd reading) with a difference in percentage of -16.67%. The repeated ANOVA across different reading intervals at CT was significant (p=0.004).

4. Discussion

Our study showed that the three most commonly detected MOs in fresh (baseline) Sobia samples were the coliform of Klebsiella spp., the yeast of Candida spp., and the lactic acid producing bacteria of Lactobacillus spp. The distribution of MOs was similar to that obtained in a previous study (Gassem, 2002) which was conducted on 14 Sobia samples collected from Makkah and Riyadh, and it reported that Klebsiella spp., Candida spp., and Lactobacillus spp. were the most predominant MOs. However, this later study reported higher counts with more variability in MOs than our study. The noted difference could be due to either the use of different analytical techniques in the two studies, less hygienic conditions followed during Sobia preparation, or samples transportation as reported in the study.

A more recent study was conducted on 10 Sobia samples bought from Makkah city vendors to investigate their microbiological content, assess the antibiotic sensitivity pattern, and study the effect of flush pasteurization on present MOs (El-Said, 2019). The study showed the presence of 50 coliforms including E. coli, Enterobacter cloacae, Klebsiella pneumoniae, Cronobacter sakazakii, Pseudomonas fluorescens, and Streptococcus parasanguinis with the first three being most predominant. In addition, yeast and lactic acid producing bacteria, mostly identified as Lactococcus spp. were detected. Yeast was the most predominant in the tested samples, followed by lactic acid producing bacteria, with the coliforms showing lower counts. This is in contrast to our results, since coliforms were the most predominant MOs in our samples, with Klebsiella presenting the highest percentage, and E. coli the lowest. Even though exact comparison between our study and El- Said study could not be performed due to differences in methods reporting MOs counts and prevalence, the apparent presence of higher count of E.coli; an indicator of possible fecal contamination; in samples bought from Makkah indicates poorer hygienic conditions during the preparation of the beverage (El-Said, 2019). The lower presence of coliforms in our study compared to the previous two studies could also be due to more recent strict regulations applied by the Municipality of Jeddah city regarding Sobia preparation.

Table 2: Readings of different micro-organisms and their percentages in each time interval at CT (2-8°C).

		1st Reading (Baseline)			2nd Reading (7 days)			3rd Reading (14 days)		
Type of MO	Name of MO	No. of MO	CFU	%MO	No. of MO	CFU	%MO	No. of MO	CFU	%MO
Lactic acid bacteria	Lactobacillus spp.	59	>100k (69%) 100 (5%) 100k (7%) <100k (19%)	58%	52	>100k (63%) <10 (4%) 10 (31%) 10-100 (2%)	92%	58	>100k (45%) <100 (17%) <10 (3%) 10 (35%)	96%
Yeast	Candida ssp.			29%			29%			54%
	Saccharomyces cerevisiae			17%			33%			33%
	Cryptococcus laurentii			4%			8%			8%
Coliforms	Klebsiella			71%			54%			46%
	AHS			17%						
	CNSA			4%						
	Bacillus			17%						
	Enterobacter cloacae			17%						
	Nisseria subflava			4%						
	E.coli			4%						

Abbreviations: MOs, micro-organisms; CFU, colony-forming unit; AHS, Alpha hemolytic Streptococcus; CNSA, Coagulase-negative Staphylococcus Aureus; E. coli, Escherichia coli

Fresh Sobia samples were found to be acidic. Our mean reported pH for the fresh (baseline) samples was within the range of 3.37 to 5.53 as reported previously (Gassem, 2003). This can be explained by the presence of lactic acid produced by Lactobacillus spp. The pH decreased upon storage in our study, showing a more rapid decrease in samples at RT. Indeed, the mean pH of samples stored at RT for 7 days was significantly different to the mean of samples stored at CT for the same period. On day 7 of CT storage, the number of total MOs, %MO Lactobacillus spp. and %MO Candida spp. were less than the number at RT. This decrease in numbers indicates that the growth of MOs is inhibited by the storage at CT.

Table 3: pH measurements (mean ± SD) at different time intervals in each of the storage condition RT and CT.
*The mean value is significantly different ($p<0.001$) when compared to the baseline value. ^The p value of repeated measures ANOVA across different pH reading intervals. Diff. %, indicates the change in percentage between the current pH reading and the baseline reading (1^{st}).

RT	1^{st}Reading (Baseline)	2^{nd} reading (24hr)	Diff.%	3^{rd} reading (48hr)	Diff.%	4^{th} reading (7 days)	Diff.%	ANOVA p-value^
pH	4.20 ± 0.80	3.89 ± 0.72*	-7.38%	3.35 ± 0.62*	-20.24%	3.28 ± 0.55*	-21.90%	<0.001

CT	1^{st}Reading (Baseline)	2^{nd} reading (7 days)	Diff.%	3^{rd} reading (14 days)	Diff.%	ANOVA p-value^
pH	4.20 ± 0.83	3.83 ± 0.81*	-8.81%	3.5 ± 0.55*	-16.67%	0.004

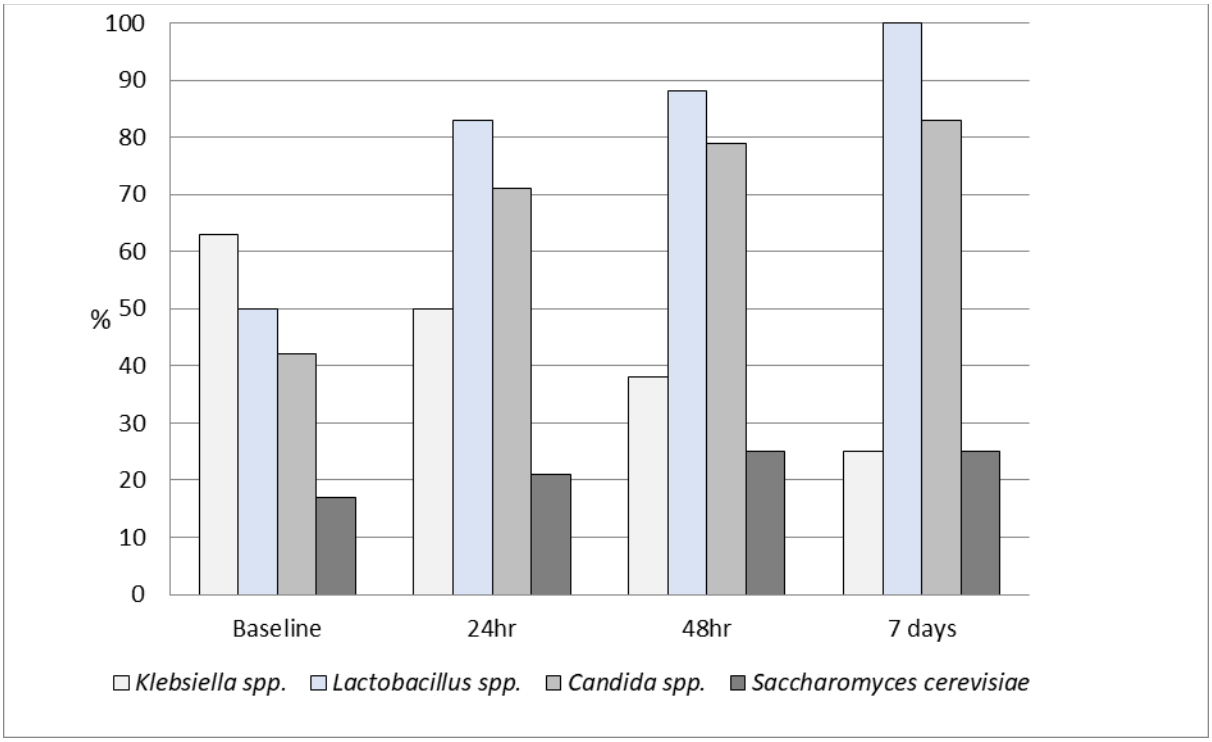

Figure 1: Trend percentages for the most frequent micro-organisms at Room Temperature.

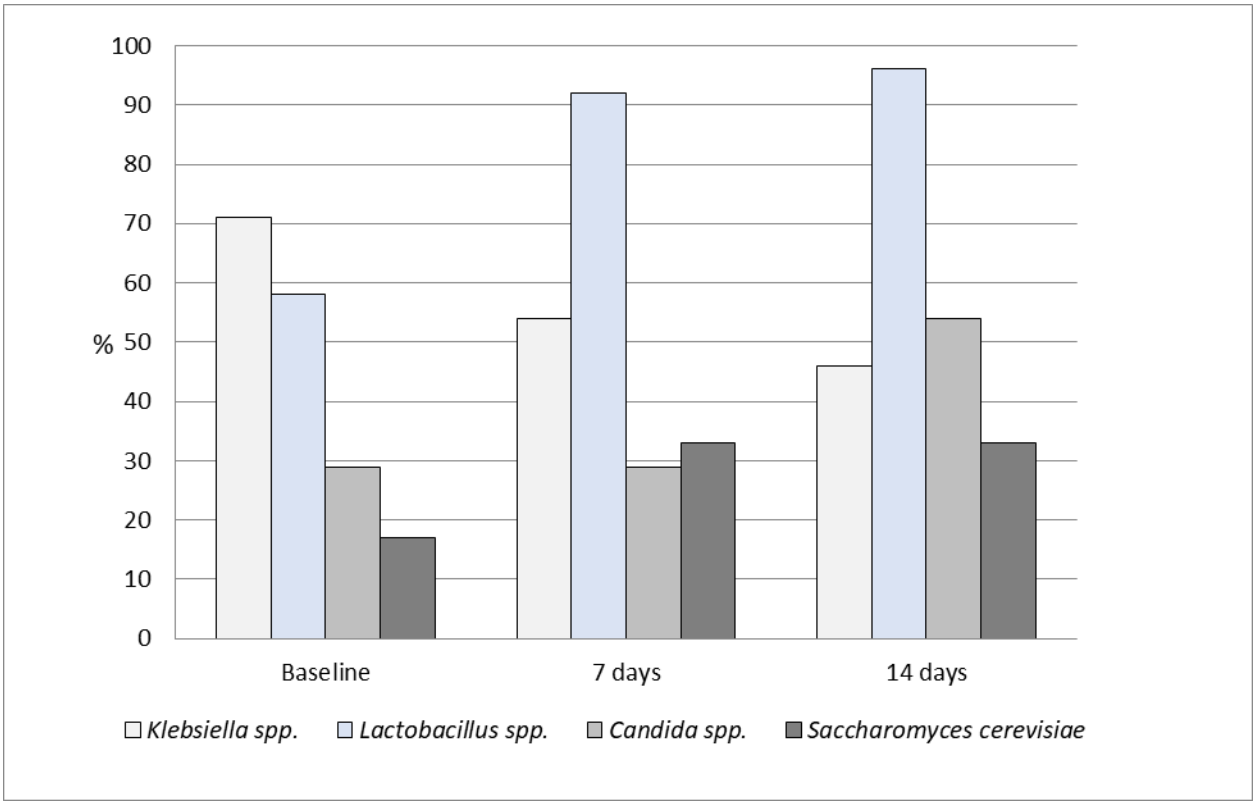

Figure 2: Trend percentages for the most frequent micro-organisms at Cold Temperature.

Along with the decrease in pH there was a marked increase in the percentage count of Lactobacillus spp, accompanied by a concomitant decrease in the percentage of coliforms, and a disappearance of some types including E. coli and Nisseria Subflava at both storage conditions. This observation could be explained by the production of hydrogen peroxide by Lactobacillus during the process of fermentation, which has an antibiotic effect, thus inhibiting the growth of other gram-positive and negative bacteria (Daly, 1991). Another possible product of longer fermentation period is alcohol, which is reported to have an inhibitory effect on Gram-negative bacteria while, Saccharomyces, and Lactobacillus are alcohol tolerant (Dinh et al., 2008). Sobia is supposed to be alcohol free. The level of alcohol (ethanol) in freshly prepared Sobia had been investigated earlier by our research group. It was reported that Sobia had low ethanol level when freshly prepared (<0.03%) and the levels may increase with storage temperature and length of stay after preparation, exceeding the permissible level of alcohol of 0.5% in halal drink (Borai et al., 2021).

Most of the isolated MOs from Sobia in our study are not considered a common cause of food poisoning bacteria but may cause serious infection under certain conditions. For example some strains of lactic acid bacteria have been reported to have the potential to be opportunistic pathogens (Aguirre & Collins, 1993). In some other cases the presence of Candida, Klebsiella and Nisseria species in food and drink were reported to cause serious clinical conditions (Costa et al., 2010; Daisuke et al., 2012; Kiddy et al., 1987). Furthermore, although MOs isolated in our study were mostly not common causes of food poisoning, several previous reports implicated Klebesiella as a rare cause of gastroenteritis (Gregg, 2016). In addition, gastrointestinal colonization by multi-drug resistant Klebesiella was linked to contaminated food (Hartantyo et al., 2020). Moreover, the presence of E. coli is considered an indication of fecal contamination in the collected Sobia samples. This can cause gastroenteritis and other serious diseases due to enterotoxins and metabolic end products released by these bacteria (Gözde, 2019). Although the reported cases in the above mentioned previous studies were limited, contamination of drinks and foods could have clinically significant consequences.

Similar to what has been found in our study and that of Gassem and El- Said, the presence of coliforms in traditional beverages and foods has been reported earlier in different countries including the Nigerian beverage (burukutu), the Turkish (boza) and the Sudanese (kisra) (Mohammed et al., 1991; Altay et al., 2013; Olaniyi et al., 2018). As a precaution to avoid adverse health effects in 2015 the world health organization issued useful recommendations about street food safety in order to prevent foodborne illnesses (WHO, 2015). These recommendations should be adapted locally to avoid Sobia contamination and prevent the potential risk of acquiring enteric infections. In addition to this, as a further precaution, thermal pasteurization could be performed as an additional step following preparation since it has been shown to be an effective method to eradicate MOs from beverages (Nile, 2015) and Sobia in particular (El-Said, 2019).

Our study has several points of strengths. The first of these is that it is based on higher sample size, collected randomly from both street vendors and regular shops following enforcement of new food safety regulations in our region. Furthermore, it is the first study investigating the storage effects on Sobia samples at different temperatures and at different time intervals. The main limitation to our study is that 25°C was considered RT. This might not be the case in real life, specifically in the hot summer of Saudi Arabia. Furthermore, the time of production of the purchased samples depended on the integrity of the vendors, hence, our freshly prepared samples might have been prepared at an earlier time than reported. Another limitation is that our sample contained both white and colored Sobia, but the difference between them was not investigated and no antibiotic sensitivity was performed for the isolated strains.

5. Conclusion

In conclusion, our study showed that Sobia sold at both street vendors and shops contain a significant number of micro-organisms. Although none of the isolated bacteria and yeast in our study has been directly implicated as a cause of food poisoning, their presence raise many concerns about the risks to enteric infection. Sobia storage at different conditions is associated with varying degrees of acidity and micro-organism content but storage in the cold condition can decrease the risk of enteric infection by minimizing the growth micro-organisms.

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