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Determination of Ethanol Content in Sudanese Non-Alcoholic Beverages, Sharboat Drink, and Drinking Yogurt by Gas Chromatography.

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ABSTRACT

In this study the determination of ethanol content in two types of non alcoholic beverage (Champion and Veno), two types of Sudanese Sharboot drinks (treated with yeast and not treated with yeast) and drinking yogurt were performed using gas chromatography flame ionization detector (GC-FID).The results show that the ethanol percentage in Sharboat samples which treated with yeast were have higher values in the range between 0.0184-0.194% (v/v) according to the conditions of storage , while in Sharboat samples which not treated with yeast the range were between 0.00017-0.0267 %(v/v). Moreover, in non alcoholic beverage samples the range was between 0.00017-0.0047 % (v/v), while in drinking yogurt samples the range were between 0.00-0.0128 % (v/v). The method was validated in terms of linearity, recovery and repeatability. The coefficient correlation (R^2) was found to be 0.998 and the recoveries percentage were found to be between 96.5-99.2%.

Keywords: Gas Chromatography, Non-Alcoholic Beverage, Sudanese Sharboat Drink, Drinking Yogurt.

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INTRODUCTION

Non-alcoholic beverage in general mean a product contains no, or only small traces of ethanol-based alcohols. In the United Kingdom, alcohol-free beverage contains 0.05 % (v/v) or less, while de-alcoholised contains 0.5 % (v/v) or less. In the United State of America and most of Europe countries, drinks that containing up to 0.5% (v/v)are classified simply as alcohol-free.

In Sudan the consumption of non- alcoholic beverages was increased since last 25 years and consequently the national factories were increased regularly. The types of non alcoholic beverage in Sudan are potable liquids without ethyl alcohol. But due to the constituents of non alcoholic beverage and the bad storage conditions, bacteria fermentation will take places specially in the summer season in which the temperature reach 45 ^{0}C or more. Other drink which may contain small amounts of alcohol is Sharboat drink, which is a famous Sudanese indigenous date juice, it has been used as a general drink for many centuries. Earlier, Sharboat has been used as a common drink in Eid Aladha and sometimes in wedding celebrations. But now days, it is used all over the year in many restaurants. Sharboat is made by boiling the date palm with water for 3-5 hours, and then cooled and finally small amount of ginger, cinnamon, hbhan, grenjal and hibiscus were added in small quantities. Sharboat is considered to be one of the richest foodstuffs drink. It contains monosaccharides, disacchaontainsrides, mineral salts and vitamins. These substances considered as essential elements for the growth of microorganism, especially yeast and hence production of ethanol when sharboat stored at high temperature (equation 1) [1].

Milk product play an important role in the daily live. It is a very complex substance and it contains over 250 chemical compounds, like water-soluble vitamins, lactose sugar, protein, fat and minerals [2].The facts that raw milk will fermented and curdle if not consumed almost immediately, while fermented milk has a sour taste, it can be stored without spoiling for longer periods of time than milk itself [2]. In preparing a cultured milk product, generally the milk is pasteurized, the starter will be added to the milk, in a step known as inoculation. Then the milk will be allowed for a period of time that will help the bacteria to grow and ferment the milk. This period is known as an incubation period, the product is sometimes agitated. Finally, the milk will cooled to stop or to slow down the bacteria growth. Drinking yogurt contains lactose, lactic acid and vitamins B. It is usually low in calories [2]. The fermentation of milk by using harmless bacteria is due to the fermentation of glucose or fructose to produce tow molecules of lactic acid (homolactic acid fermentation) without production of ethanol and gases (equation 2), but further metabolized (heterolactic acid fermentation) results in lactic acid, ethanol and carbon dioxide (equation3) [3].

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \quad (1)$$
$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (2)$$
$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2 \quad (3)$$

Determination of ethanol in alcoholic beverages and non alcoholic beverages can be performed by different techniques such as: oxidation of the distillate [4,5, 6], dichromate oxidation spectrophotometry [4,5,7], enzymatic method [8-10], biosensor [11,12], potentiometry [13], gas chromatography (GC) [4,5,14,15,16,17], capillary electrophoresis [18], high performance liquid chromatography (HPLC) [19,20], modular Raman spectrometry[21], near-infrared (NIR) spectroscopy[22], beer analyzer [5], and flow injection analysis (FIA) [23,24]. For oxidation of the distillate and dichromate oxidation spectrophotometry, more than 5 ml of sample volume is required for analysis. Besides that the reagents used are highly toxic and the method was classified as low stability. Low reproducibility and low accuracy are the disadvantages for enzymatic method, biosensor, and potentiometry [10]. Raman spectrometry and capillary electrophoresis are not popular due to the expensive instruments were required. HPLC method obtains a comparatively low sensitivity [19]. Developed near-infrared (NIR) spectroscopy and beer analyzer both are time consuming methods in establishing calibration curves and they have low accuracy. In addition they can be interfered by other alcohols in alcoholic beverages.

GC is a powerful tool in the analysis of alcoholic or non alcoholic beverage products. Minimum sample preparation, in general will be required because samples are in liquid state in an alcohol or alcohol/water matrix. The flavor compounds tend to be volatile in nature, that fulfillment the main requirements of GC. General detectors, like the flame ionization detector (FID), or more information-rich detectors, such as the mass selective detector (MSD), can be used. In addition, the ability to automate the

analysis makes GC a very practical tool in a QA/QC environment. Due to the above mentioned reasons, quantitative determination of alcohol in different matrices samples was carried out by using GC technique with different detectors [25,26,27,28,29,30,31]. GC is considered to be one of the most appropriate and rapid method for determination of ethanol contents in non alcoholic beverages, Sharboat and yogurt with complicated alcoholic contents and small sample amount.

The aim of this work is to investigate the ethanol percentage (%v/v) in non alcoholic beverage, Sharboat Sudanese drink and the drinking yogurt at different storage temperatures and different period of time using GC. The obtained results will be compared with international limit which define the non- alcoholic beverage.

MATERIALS AND METHODS

Standard solution

Different concentrations of standard solutions of ethanol (0.1, 0.05, 0.01, 0.005, 0.001 and 0.0001% v/v) were prepared using absolute ethanol (99.88%) and de-ionized doubly distilled water (Table 1). These standard solutions were used for recovery tests, standard curve, peak area calculation and investigation of analyte concentrations.

Samples collection and preparation

Two types of fresh non alcoholic beverage (Champion and Veno) were taken directly from the factories (30 bottles of each type) and then were stored at different conditions of temperatures and periods of time as shown in Tables 2 and 3.

Two types of house made fresh Sharboat drink were taken (27 bottles not treated with yeast and 12 bottles treated with yeast), and then were stored at different temperatures and periods of time as shown in Table 4.

Fifteen samples bottles of drinking yogurt were collected and then were stored at different conditions of temperatures and periods of time as shown in Table 5.

The differences in storage interval periods of time were due to the differences in the rate of fermentation and expire dates of the 3 types of samples (non alcoholic beverage, Sharboot drink and drinking yogurt). Before analysis all samples were degassed, and then 200 g of each sample was distilled in rotary evaporator for two hours, after that the distillate was weighted, and then 2 µL of each sample was injected in GC.

Instrumentation and conditions

In this work gas Chromatography type: QP 2010 Shimadzu with FID detector (general column DB-210, temperature: 50-220 ^{0}C (4 ^{0}C at 1min and hold for 10 mins) was used for samples analysis.

RESULTS AND DISCUSSION

Statistical analysis

All results were statistically evaluated by Student *t–test* and ANOVA *test* (*p=0.05*). In addition, Microsoft Excel and Origin software's were also used to assess the significance of the differences between the variables investigated in different samples. The concentration values obtained were expressed as mean value ± standard deviation (*p=0.05*). All statistical analyses were based upon triplicate measurements of all standards and sample solutions.

Method validation:

The method was validated in terms of linearity, recovery, repeatability. The peak area of standard ethanol solutions and the retention time were shown in Table 1 and the standard calibration curve was shown

in Figure 1. The coefficient correlation (R^2) was found to be 0.998 and the recoveries percentage were found to be between 96.5-99.2%.

The percentage of the ethanol in the samples

Table 2 and Figure 2 show the ethanol concentration percentage of Shampion, while Table 3 and Figure 3 show the ethanol concentration percentage of Veno beverage at different storage temperatures. The results show an increase in ethanol percentage with increase in storage time, and the percentage of ethanol is found to be higher for the samples stored at room temperature (25-40 ^{0}C). Also, the results show that the rate of the fermentation of the samples which stored at the direct sun light (temperature more than 45 ^{0}C) were less than the rate of fermentation of the samples which were stored at room temperature (25-40 ^{0}C), this may be due to inhibition of yeast enzymes and yeast destruction or may be due to evaporation of the ethanol by high temperature. For the Sharboat drink, Table 4 and Figure 4 show an increase of ethanol percentage with increase in storage time for both types of samples (treated with yeast and not treated with yeast). But there are remarkable increases in ethanol percentage in the samples that treated with yeast, this indicated that yeast increase the rate of the fermentation process especially at room temperature. Table 5 and Figure 5 also show an increase of ethanol percentage in drinking yogurt samples with increase in storage time. Table 6 and Figure 6 show the maximum percentage of ethanol found in all studied samples. The results show that the Sharboat drink which treated with yeast has a higher percentage of ethanol. This indicates that great differences in ethanol percentage in the Sharboat drink that treated with yeast compared to the other samples. When we compared the obtained results with concentration of ethanol used in definition of non-alcoholic beverage which mentioned before we found that all the obtained result were below the percentage of alcohol used to define the non-alcoholic beverage in the United State of America and most of Europe countries (0.5% v/v). But if we compare the obtained percentage with the maximum level for the free alcoholic percentage of ethanol in United Kingdom (0.05% v/v) we found that all samples contain less percentage of ethanol except the Sharboat drink that treated with yeast.

Table 1: GC peak area of standard ethanol solutions

Standard sample no.	Ethanol concentration % v/v	Peak area
1	0.0001	8.0
2	0.001	105.5
3	0.005	280.5
4	0.01	690.5
5	0.05	2650.0
6	0.1	5710.0

The retention time of standard ethanol is 1.58

Table 2: The concentration percentage of ethanol in Champion beverage

Champion sample no.	Condition of storage	Period of storage days	Ethanol concentration % v/v
1	Before storage	0	1.70×10^{-4}
2	Refrigerator	10	6.60×10^{-4}
3		20	1.10×10^{-3}
4		30	1.50×10^{-3}
5	Room temperature	10	1.30×10^{-3}
6		20	1.80×10^{-3}
7		30	4.20×10^{-3}
8	Sun light	10	3.10×10^{-4}
9		20	4.20×10^{-4}
10		30	4.30×10^{-3}

Table 3: The concentration percentage of ethanol in Veno beverage

Veno sample no.	Condition of storage	Period of storage days	Ethanol concentration % v/v
1	Before storage	0	6.70×10^{-4}
2	Refrigerator	10	9.40×10^{-4}
3		20	1.00×10^{-3}
4		30	1.70×10^{-3}
5	Room temperature	10	2.10×10^{-3}
6		20	2.70×10^{-3}
7		30	4.70×10^{-3}
8	Sun light	10	1.10×10^{-3}
9		20	1.20×10^{-3}
10		30	2.60×10^{-3}

Table 4: The concentration percentage of ethanol in Sharboat drink

Sharboat sample no.	Condition of storage	Period of storage days	Ethanol concentration % v/v
1	Before storage	0	1.70×10^{-4}
2	Refrigerator	4	1.00×10^{-3}
3		8	1.70×10^{-3}
4		12	7.70×10^{-3}
5		16	1.45×10^{-2}
6	Room temperature	4	2.60×10^{-3}
7		8	1.74×10^{-2}
8		12	2.03×10^{-2}
9		16	2.67×10^{-2}
10	Room temperature Which treated with yeast	4	2.62×10^{-2}
11		8	1.94×10^{-1}
12		12	5.11×10^{-2}
13		16	4.84×10^{-2}

Table 5: The concentration percentage of ethanol in drinking yogurt

Drinking yogurt sample no.	Condition of storage	Period of storage days	Ethanol concentration % v/v
1	Before storage	0	0.00
2	Room temperature	4	0.00
3		8	0.00
4		12	4.62×10^{-3}
5		16	1.28×10^{-2}

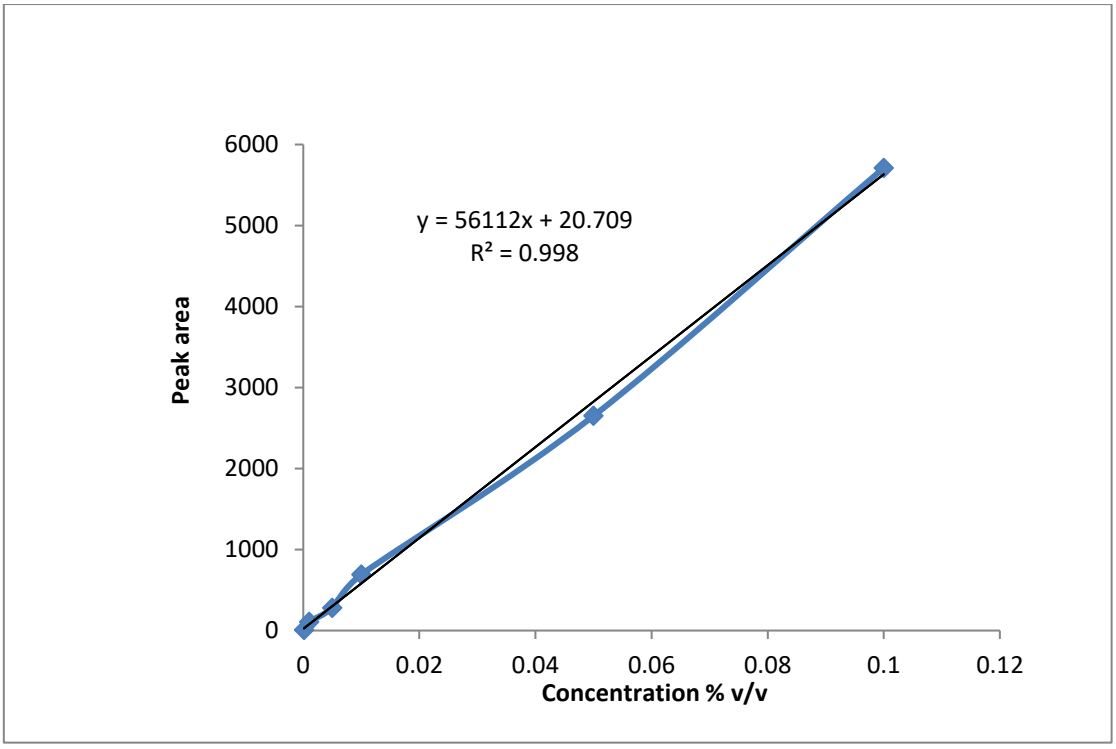

Figure 1: The standard calibration curve of ethanol percentage and peak area

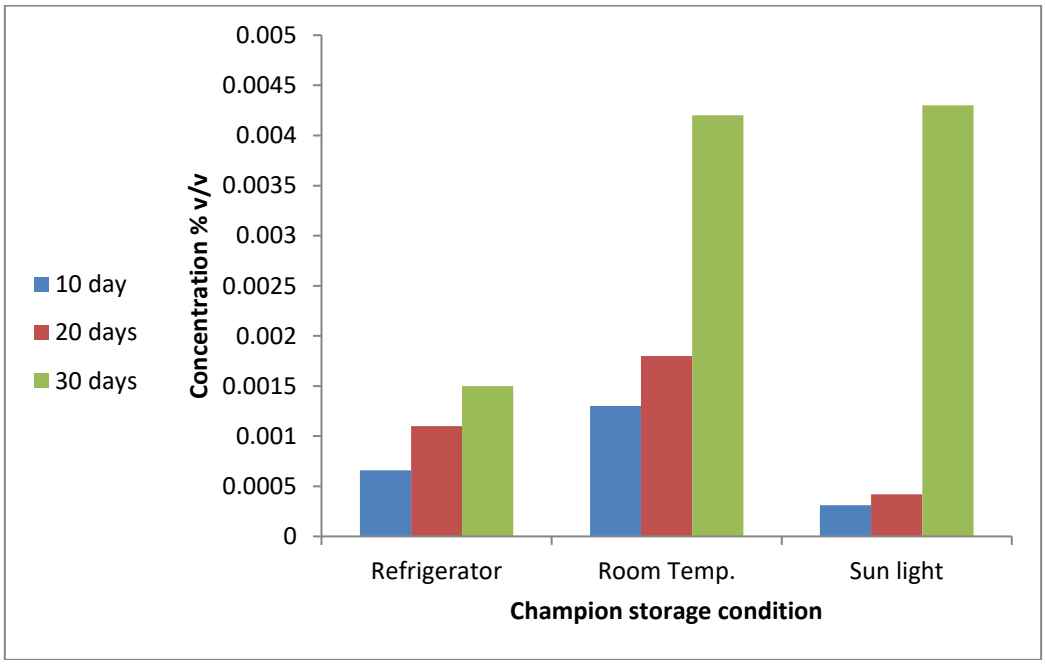

Figure 2: The percentage of ethanol in Champion samples

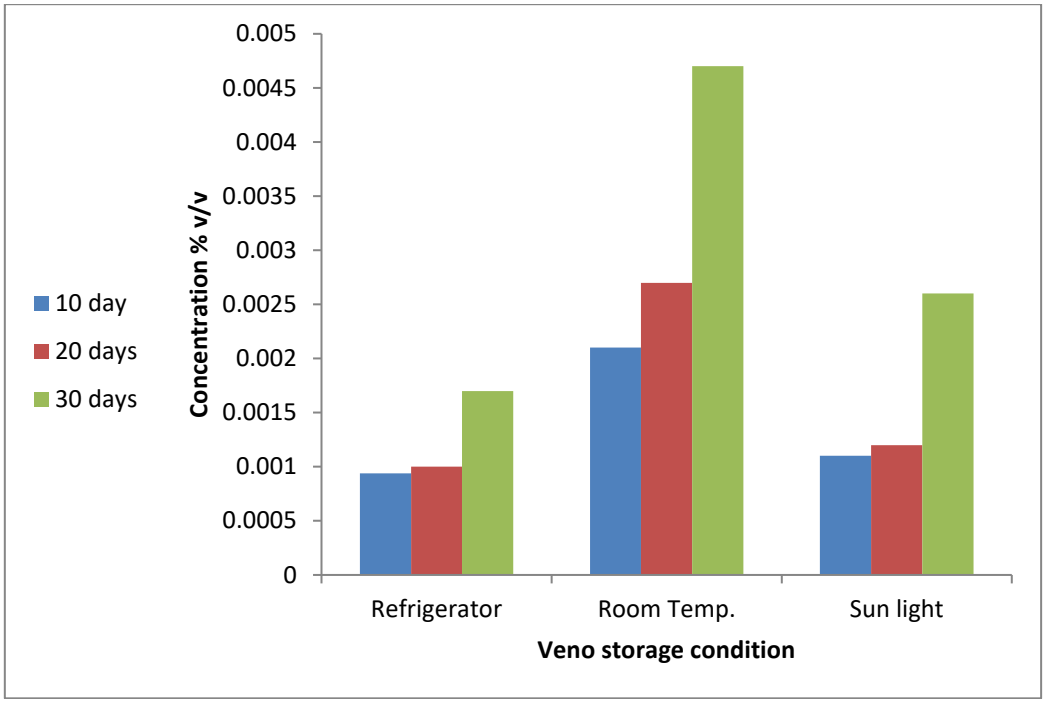

Figure 3: The percentage of ethanol in Veno samples

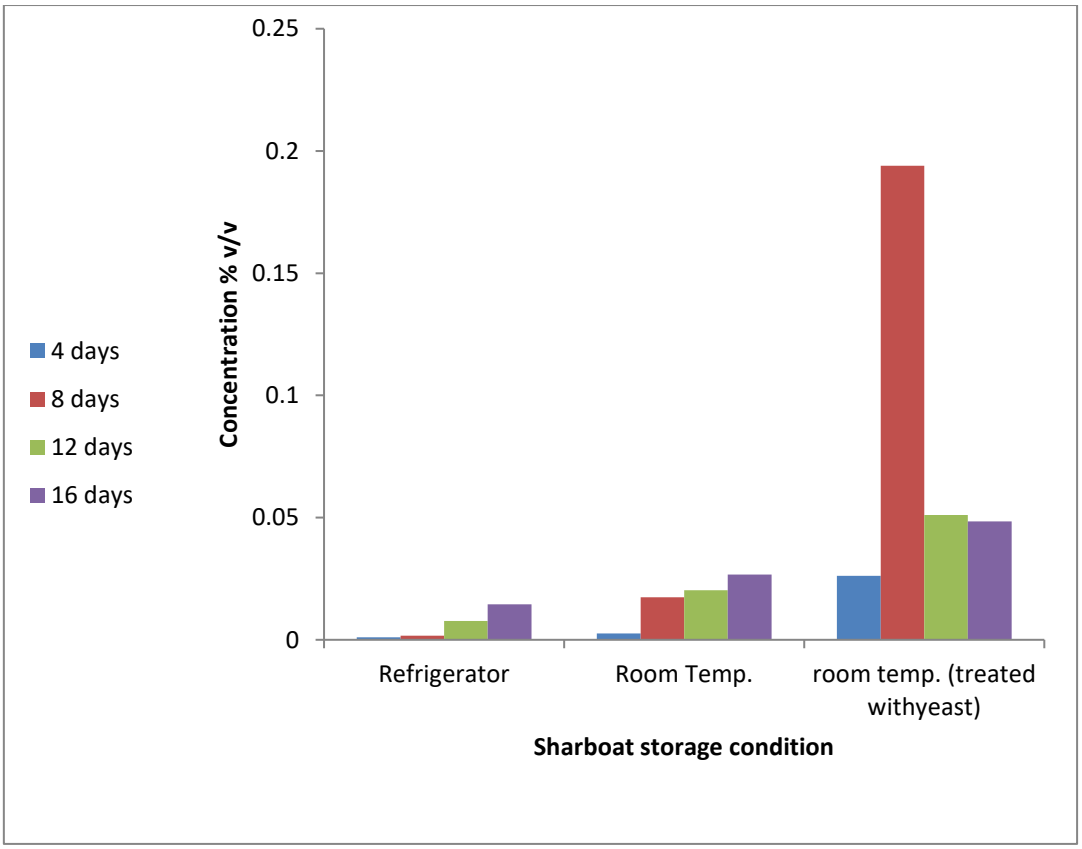

Figure 4: The percentage of ethanol in Sharboat samples

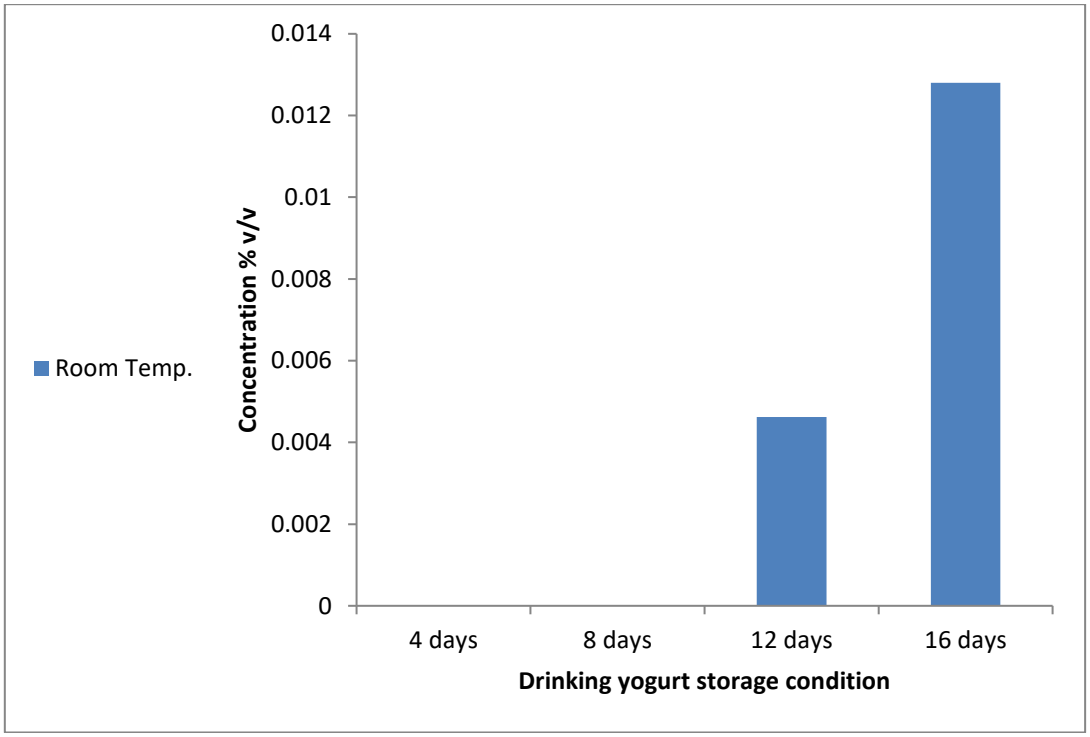

Figure 5: The percentage of ethanol in drinking yogurt samples

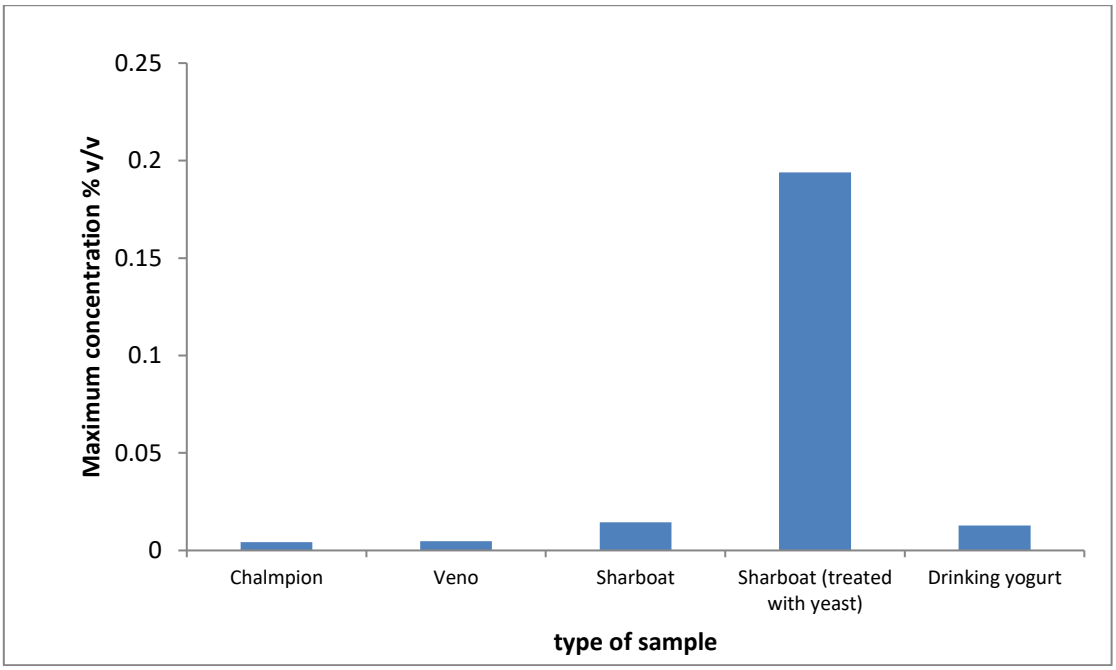

Figure 6: The maximum ethanol percentage in different samples

CONCLUSION

Traces or small amount of ethanol were found in the non-alcoholic beverage, Sharboat Sudanese drink and drinking yogurt, were not exceed 0.0267% (v/v) except the samples of Sharboat that treated with yeast (0.194% v/v). This percentage (0.0267% v/v) is less than the United Kingdom percentage (0.05% v/v) which is the lowest international values to consider the drink as alcohol-free contains. Further study should be carried

out to investigate the slow rate of fermentation of non-alcoholic beverage at the sun light (temperature more than 45 ^{0}C).

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