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Monitoring of ethanol content in non-alcoholic beer stored in different packages under different storage temperatures

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ABSTRACT

Non-alcoholic beer is one of the most popular soft drink in Islamic countries. Inevitably this valuable product experiences prolonged periods of storage prior to consumption. In the recent project, four famous Iranian non-alcoholic beer brands (A, B, C and D) were stored for 6 month in three types of packaging materials (PET, glass and tin can bottles) at two temperature conditions (4 and 24℃). Ethanol production during storage periods in all beer brands samples was determined. Results obtained showed that an increase in temperature during storage leads to ethanol production, significantly. Moreover storage periods had significant effect on enhancing ethanol concentration in beers. Furthermore, experimental data showed that the PET packaging materials influenced ethanol concentration in beers, impressively. In general, all three factors had significant effect on ethanol production in non-alcoholic beers however maximum ethanol concentration (0.240 w/v %) in beers did not exceed of allowed amount (0.5 % V/V) in non-alcoholic beers.

Keywords: *non-alcoholic beer; ethanol; storage temperature; packaging material.*

1. INTRODUCTION

Beer is one of the most popular drink in the world. According to food and agricultural organization (FAO), beer was the most consumed alcoholic drink in the world in 2005 [1, 2]. Scientific researchers have been demonstrated beneficial effects of beer on human health; includes reduce cancer risk and carcinogenic cells grow, improves absorption of vitamins, inhibits osteoporosis [3-5].

Despite its useful properties, have been shown that consistent consumption of beer has an adverse effect on pregnancy women, professional athletes, and cardiovascular and hepatitis patients [6-10]. On the other hand, prohibition of alcoholic beverage consumption in Islamic countries and also in united states between 1919-1933, as sale and consumption of alcohol and alcoholic drinks was denied, which increased the production of reduced or alcohol free beers (AFBs) in the world [11, 12]. The alcohol reduced beer or AFBs also contain health promoting components with simultaneous low energy intake effect and absence of adverse health effects of alcoholic beverages. However the sales of AFBs did not meet the initial optimistic expectations, nowadays it is incredible growing phase of the non-alcoholic beer manufacture and market worldwide [5, 11].

Several methods have been developed for production of reduced alcohol or AFBs. These methods include dilution method, restricted fermentation, and dealcoholization (vacuum distillation and dialysis). In Islamic countries, restricted fermentation is the most common method in non-alcoholic beer production. The method of the non-alcoholic beer in which production based on alterations in technology or use of special yeast (that produces very low amounts of ethanol) [5, 11, 13].

Although the restricted fermentation is applied for production of non-alcoholic beer, ethanol concentration may increase during processing or storage which is known as beer spoilage. This phenomenon may be due to the contamination of raw materials or processed product with wild strains yeasts or other ethanol producer microorganisms which grow during storage and enhance the ethanol concentration in the final product [14-16].

Prior to being marketing, manufactured beers may remain in store for a certain time. The product shelf life depends on many variables including the actual beverage itself, the production process, the storage time and temperature, and of course, the packaging, which is capable of prolonging or, if not chosen carefully, also influencing the shelf life. It has been shown that storage temperature has an important role in fermentation process. Elevated temperatures usually encountered in several geographical regions during storage and transportation may adversely affect beers quality [15, 17, 18]. Temperature rises to a certain levels (below 35 ℃) in a fermentation medium, improves microorganisms metabolites production. However, some mutant strains of *Saccharomyces cerevisiae* are able to grow and produce ethanol at a maximum temperature of about 36 ℃ [19]. Chiang et al, have been determined that the maximum ethanol production with S*accharomyces cerevisiea* was about 35 ℃ and ethanol production decreased as the temperature elevated above 35℃ [20]. In addition yeasts belonging to the genus *Kluyveromyces* are capable of growth and alcohol production at above 40 ℃ which has been categorized as thermophilic yeasts. It is believed that 20-35 ℃ is the ideal range for fermentation process. In this temperature range yeast grow and ethanol production is at the maximum level [21, 22]. So existence of such yeasts in beers and improper storage temperature (above 10 ℃) can develop new and undesired components such as alcohol in AFBs in a way that makes the final product a spoiled and unusable product.

Furthermore storage of beers for a long time in improper condition may alter its initial properties. Ethanol production increases in mediums contain glucose (like non-alcoholic beers), as long as ethanol concentration exceeds concentrations of 7% and above, changes the polarity degree of cytoplasm and cell membrane and inhibiting growth and metabolite production in microorganism. Since in Islamic countries, the ethanol contents must not exceed 0.5%, so contamination of these products by wild strains yeast may increase the ethanol contents if store for a long time in insufficient conditions [5, 15, 19, 23]. Tange et al demonstrated that ethanol productivity of yeast increased as the fermentation time increased (until 1 month of fermentation) [24]. In addition to storage duration and temperature, packaging material is also a key factor that affects beers quality.

Different beverage packaging materials were shown to affect the chemical properties of beverage. Commonly AFBs as other kinds of beers are usually packed in glass bottles. However glass is a chemical inertness material and is impermeable to gases, but its weight and fracture problems during transportation considered as glass disadvantages. Indeed the idea of using polymer materials for beer and wine came up in 1970. The first material was tested for packaging these kinds of beverages was polyvinyl chloride (PVC). Migration of PVC plasticizers in to beverage and its carcinogenicity were important factors that prevented the use of PVC as a packaging material. In 1980, poly ethylene terephthalate (PET) was introduced as an appropriate material for food packaging. PET is a clear, inert material and is relatively resistance to gases permeation. Its light weight and ease of transportation make PET as the most popular alternative material to glass. Aluminum cans are also other materials are used for beer packaging. It’s a good barrier to gases and its moldable properties make it suitable for beer packaging [15, 25, 26].

Achieving stable AFBs remain a major challenge, taking into account what happens during storage and marketing that are mostly out of control of the brewer. All of the studies showed separately the influence of temperature, time and packaging material on ethanol production during storage of alcoholic beers. To the best of our knowledge no report is available for monitoring ethanol contents in AFBs. Therefore, the aim of the present study is to assess the combined effect of storage conditions (time and temperature) and packaging materials on ethanol production in four famous and commercial brands of alcohol free beers in Iran

2. MATERIALS AND METHODS

2.1. Sample collection.

Eight pasteurized (at 74 ℃ for 15 minutes) bottled AFBs samples were used in the recent study. Samples were bottled and stored in glass, PET and aluminums can of 300 mL volume. Specimens were produced from four popular brands (A, B, C, and D) in Tehran province, Iran. All samples were kept at 7 ℃ for 2 days before they were transferred in to their temperature control units. Samples were stored in a dark place at different temperature (4 and 24℃) for a period of up to 9 month. These storage conditions were chosen to stimulate the real conditions in market place or a house where AFBs are kept. This product is usually consumed in the first 9 month from the production date and is rarely subjected to more storage time. Ethanol concentration in each sample was analyzed in the interval of 1 month until 5th month and then every 2 month was detected.

2.2. Ethanol analysis.

After cooling and degassing (with ultrasound equipment), ethanol levels in all packed samples were measured with a digital beer Alcolyzer (Anton Paar, Graz, Austria) [27].

2.3. Statistical analysis.

Analysis of variance (ANOVA) was performed to determine the significant differences between the ethanol concentrations of samples. If significant differences existed among means, a multiple t-test (for multiple comparison) was applied. Analysis of variance also was also applied to compare the effect of packaging materials on ethanol concentration over the time. An alpha level of 0.05 (α<0.05) was used to determine significance. The values were statistically different, were indicated by different superscripts. Charts were plotted using Microsoft Excel software (version 2013).

3. RESULTS

3.1. Effect of storage temperature on ethanol contents.

Ethanol concentrations were changed in AFBs due to the different storage temperature. Results revealed the significant effect of temperature on ethanol concentration of treatments during storage periods. However, ethanol produced in P-A, P-B, P-D, C-A, C-D, and G-D treatments stored for 9 month, but in all other stored samples, ethanol did not produce until the end of storage time (α<0.05). These results demonstrate the beer contamination with ethanol producing organisms (such as *Saccharomyces cerevisiae*) during storage or imperfect pasteurization process after packaging [28, 29]. As shown in Table 1, at the end of storage period, maximum ethanol production (0.122 % v/v) was detected in P-D sample stored at 24 ℃ (α<0.05). Results showed that ethanol volume in samples stored at 24 ℃ was higher than refrigerated samples. The results obtained in the recent research are in accordance with Briggs et al [28]. They expressed that the ideal temperature for a yeast fermentation is about 25-35 ℃ , such that fermentation at higher temperature or at refrigerated conditions would be decrease. Temperature rises up to the ideal growth temperature, enhances the yeast growth rate and its metabolites production. This phenomenon may be a consequence of changing in saturation level or transport activity of organism cell wall which increase accumulation of substrate (glucose) concentration inside cells, subsequently [21, 30]. Hosseini Joobeh et al, have stated that temperature is the major factor effects on the ethanol production, so that in a fermentation process minimum ethanol contents are produced at 0℃. At low temperature (<20 ℃) yeast cell owns low tolerance to ethanol and shows a decline in specific growth rates.

Moreover, due to the reduction of oxygen solubility at high temperature (more than 35 ℃), yeast metabolites decrease and causes reduction in ethanol fermentation [28, 31].

3.2. Effect of storage time on ethanol contents.

After 9 month of storage, in most samples ethanol did not produce except in P-A, P-B, P-D, C-A, C-D, and G-D, specially, when stored at 24 ˚C. Moreover, as it was shown in Table 1, time of storage significantly affected the ethanol concentration in these samples. There were significant increase in ethanol productions along with increase in the time of storage (in P-A, P-D, C-A treatments). Yeast activities in anaerobic conditions cause ethanol synthesis from fermentative sugars to exist in the beer during storage periods [28]. Experimental data revealed that after 9 month of storage the maximum ethanol concentration obtained in P-D (0.027 and 0.122 % v/v at temperature of 4 and 24℃, respectively). During storage, the fermentative organism has opportunity to use more sugar, so produce more ethanol in its medium culture [32]. In addition, the ethanol amount in C-A sample was found to decline after 3 month of storage (at 4 ℃). These phenomenon may indicate that viable cells originally used glucose switched from fermentative metabolism (in which glycolysis process forms ethanol) to respiratory metabolism in which ethanol produced in the first three month of storage was consumed in glyoxylate, tricarboxylic acid cycles and electron transport chain in mitochondria [26, 33, 34]. Results in Table. 1 also indicated that ethanol concentration in C-D and G-D treatments remained constant after 9 month storage at 24 ˚C, since the ethanol producer organisms were not active because of their low tolerance to ethanol [30, 35]. In general, in all treatment, ethanol concentration did not exceed 0.5 %v/v.

3.3. Effect of packaging material on ethanol content.

Effect of packaging materials on ethanol production during storage of different beers is presented in Table .1. The maximum ethanol concentration determined in the beer packed in PET. As can be inferred from data shown in Table. 1 the influence of the container material on ethanol content over storage time was significant for PET bottles (more especially in D brand). Since all three packaging material was not available for each one of experimented brands, so its impossible to compare the effect of packaging material on ethanol concentration in one brand individually. But also, by considering effect of materials individually in all treatment can be conclude that beers in PET contained more ethanol compared with the other ones. This fact could be attributed to more permeability of plastic materials to gases (especially oxygen) in comparison with impermeability properties of glass and metal. Bhunia et al, stated that gases permeability of plastic materials increase as well as temperature rise [36]. The more permeability to gases causes more fermentation process in a medium. Results indicated the changes in ethanol concentration in beers in the respect of time of storage in three types of packaging materials. Therefore the nature of packaging material might not be the main reason for ethanol production in AFBs. However, suitability of a PET material for nonalcoholic beer packaging considering monomer migration from packaging material requires to be examined, since monomer migration in to the beer could be a health hazard.

Table 1. Ethanol contents (% v/v) in treatments during 9 month storage (4 or 24℃)*.

Treatment	Ethanol (% v/v)																
	4℃ (month)									24℃ (month)							
	0	1	2	3	4	5	7	9		0	1	2	3	4	5	7	9
P-A	0.016^{A}_{d}	0.016^{A}_{d}	0.016^{A}_{d}	0.030^{A}_{b}	0.010^{A}_{e}	0.016^{A}_{d}	0.016^{A}_{d}	0.016^{B}_{d}		0.020^{A}_{c}	0.016^{A}_{d}	0.020^{A}_{c}	0.035^{A}_{a}	0.013^{A}_{d}	0.035^{A}_{a}	0.020^{Bc}	0.033^{Ca}
P-B	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	0.003^{Ca}	$0.003C^{a}$
P-C	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND
P-D	ND	ND	ND	ND	ND	0.002^{Be}	0.015^{A}_{d}	0.027^{A}_{c}		ND	ND	ND	ND	ND	ND	0.055^{A}_{b}	0.122^{Aa}
C-A	0.015^{A}_{b}	0.015^{A}_{b}	0.015^{A}_{b}	0.015^{B}_{b}	ND	ND	ND	ND		ND	ND	0.010^{A}_{b}	0.010^{B}_{b}	0.010^{A}_{b}	0.010^{B}_{b}	0.015^{B}_{b}	0.020^{Ba}
C-B	-**	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
C-C	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
C-D	ND	ND	ND	ND	ND	0.003^{Bc}	0.003^{Bc}	0.033^{A}_{a}		ND	ND	ND	ND	ND	ND	0.016^{B}_{b}	0.016^{BC}_{b}
G-A	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	–
G-B	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
G-C	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND
G-D	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	0.005^{Ca}	0.005^{Ca}

*Means in the same row and column with different small and capital letters (respectively) are significantly different (α<0.05).

**This type of packaging is not available by special brand.

ND: Not detected

Abbreviation symbols in each treatments include packaging materials (P: PET, C:Can, and G: Glass) and beer brands (A-D) name, respectively

4. CONCLUSIONS

Unnecessary lose of quality of alcohol free beers are found in choosing improper packaging materials or storage condition. The results of this study demonstrated the problem of beers storage under inadequate conditions leading to produce ethanol and loss of quality of alcohol free beers. Experimental results determined that beers stored at cold temperature (4℃) contained less ethanol than beers were placed at ambient temperature (24℃). So that the maximum ethanol obtained in refrigerated condition was less than beers placed at 24℃. Furthermore, a combination of an increase in storage time and temperature showed significant positive effect on ethanol concentrations. Clearly, based on the ethanol production in some treatments stored at different temperature and time, the existence of ethanol producer organisms such as yeasts are the likely cause of ethanol detection in beers due to contamination during storage or improper pasteurization process. In addition to storage condition, packaging material influenced ethanol concentrations in D brand such a way that PET packaged beers contained maximum ethanol concentration among all's. Accordingly, knowledge of variations in ethanol production as a consequence effect of storage time and temperature and packaging materials in alcohol free beers is valuable for beers producers. By selection of proper storage conditions and transportation and the application of suitable packaging materials, each alcohol free beer producers can then aim at a consistent beer quality.

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