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RESEARCH ARTICLE

Deterioration of sugarcane molasses during storage and its effect on ethanol fermentation efficiency in distillery factories

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Abstract

Bioethanol is one of the most employed liquid biofuels due to the easy adaptability of this fuel to existing engines and because this is a cleaner fuel with a higher-octane rating than gasoline. Among the widely used substrates for ethanol production is sugarcane molasses. Unsuitable storage of molasses results in deterioration of fermentable sugars consequently ethanol yield quality and efficiency. The present study aimed to shed light on the effect of the storage period on the efficiency of sugarcane molasses and its effect on ethanol fermentation efficiency in distillery factories. The results showed that the total fermentable and non-fermentable sugars involved in sugar cane molasses varied between samples that were collected according to the location source. Also, ethanol yield and fermentation efficiency were different in the fermented molasses media which contained the same concentration of fermentable sugars. About 2.65-11.20% of fermentable sugars in molasses were lost when stored under different temperatures. The loss was increased with the increasing storage temperature. Most of the loss happened in the first two months, especially at high temperatures. The decrease in ethanol yield and fermentation efficiency was affected sharply in the molasses storage at high temperatures than the low ones. From this study, the suitable storage temperature must not exceed 40°C to maintain molasses quality and avoid molasses deterioration by heat in temperatures.

Keywords: *Sugarcane; Molasses; Deterioration; Storage; Ethanol fermentation.*

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Introduction

At present, the world is facing an energy crisis caused by the continuous use of fossil oil. As a result, petroleum oil use has dropped sharply. There is an increased interest in alternative fuels, especially liquid transportation fuels. Bioethanol source of particular interest whose production by microbial fermentation is increasing to replace gasoline. (Ogbonna et al. 2001; Limtong et al. 2007; Dhaliwal et al. 2011). Bioethanol is one of the most employed liquid biofuels due to the easy adaptability of this fuel to existing engines and because this is a cleaner fuel with a higher octane rating than gasoline (Rattanapan et al. 2011).

Among the widely used substrates for ethanol production are the molasses, a byproduct of sugar industries from sugarcane and sugar beet (Goksungur and Zorlu 2001). This is because they are cheap raw materials, readily available, and ready for conversion with limited pretreatments as compared with starchy or cellulosic materials, as all sugars are present in a readily fermentable form (Razmovski and. Vučurović 2011).

Yeasts, particularly Saccharomyces spp., are the most common ethanol producers employed in the industry. Yeast has proven to be more effective for ethanol production (Balat and Balat 2009). Biofuels have great importance as it is directly related to energy consumption. Many research organizations and other energy-related multi-national companies are now trying to focus their attention on the production of biofuels with an increase in its efficiency and reduction of harmful, release products if any (Azenha et al. 2000; Bai et.al. 2008).

High storage temperatures (from 45°C) can promote the decomposition of molasses. In addition, the quantity of molasses stored and the length of the storage period undoubtedly play a part in these events (Abou El-Ela 1984; Kuqkarni, 2007).

The prevention of sudden decomposition reactions is possible provided the storage tanks are properly equipped (circulation devices, ventilation equipment, and remote thermometer system) so that the temperature can be checked and regulated and the heat is drawn off when the temperature rise arouses concern.

Olbrich (1963) stated that the decomposition of molasses may be by two theories. One theory is based on a reaction between lime and reducing sugars. It states that at a suitable temperature, the decomposition accompanied by the formation of foam with the production of carbon dioxide represents a continuation of the reaction between the reducing sugars in the molasses and the unstable organic substances, which have previously been formed in the purification process from the excess of the lime present. The other theory is based on the reaction between amino acids and reducing sugars, which leads via N-glycosides to the melanoidin and which is accompanied by the evolution of gas, especially carbon dioxide. This reaction is known as the MAILLARD reaction. It is the basis of the fact that 15 – 50 % of the total nitrogen content of cane molasses is present in a form that cannot be assimilated by yeasts. However, it is also possible that both theories apply in explaining the phenomena of the decomposition of molasses. This study aims to keep the storage molasses without deterioration and store it in a good state.

Materials and Methods

Sugarcane Molasses

Molasses samples were obtained from different sugar factories (Deshna. Kous. Nag Hammady (N.H) and Edfu), Egyptian Sugar and Integrated Industries Company (ESIIC) at the beginning of mills season 2020/2021، at the Hawandia distillation factory. Separately samples of molasses were taken directly from receiving station (Hawamedia's Transportation Alfairs) by using Nile units at receiving it before mixing all molasses come from ESIIC's factories from Upper Egypt. Samples of molasses were collected in containers of 20 kg each and transported as soon as possible to the National Research Center lab. Different four samples (5 Kg) of molasses from each factory were collected, mixed well, and stored in a jar in a refrigerator until physic-chemical analysis and used for different experiments in this study.

Yeast Strains

Saccharomyces cerevisiae F-514 which is already applied for ethanol production in Egyptian distillation factories supplied by Microbial Chemistry Lab. National Research Centre, Dokki, Giza, Egypt used in this study. This yeast strain was maintained at 4˚C on agar slants. The composition of the agar medium was (g/L): yeast extract 3, malt extract 3, peptone 5, glucose 10, and agar 20. The cultures were maintained by sub-culturing every 20-days and the test tubes were then incubated at 30˚C for 36 h.

Inoculum Preparation

Sterilized 500 ml capacity conical flasks each contained 200 ml of medium containing (g/L) malt extract, 3, yeast extract, 3, peptone, 5, and sucrose, 30 was steam-sterilized at 121°C for 20 minutes, cooled to room temperature, then inoculated with a loop of yeast strain S. cerevisiae F-514 and incubated at 34°C for 24 h. This inoculum was used to inoculate the prepared fermentation vessels at 2% v/v (Fadel et al. 2013).

Fermentation of Molasses Medium

The sugar cane molasses was diluted with water to give the demand sugar amount in fermentation containers, 18%. This diluted molasses was supplemented with 2 g/L diammonium phosphates as a source of nitrogen and phosphorus and 0.5 g/L magnesium sulfate as a source of magnesium and sulfur. Molasses medium was dispensed into 500 ml Erlenmeyer flasks containing 200 mL. The molasses medium was inoculated by 2 % of the prepared inoculum and incubated statically to complete fermentation at 32° C under anaerobic conditions.

Effect of Storage Temperature on Molasses Composition

A mixed sample of molasses collected from the different four sugar factories was introduced in 2 L capacity containers. The containers were divided into five sets, stored at different temperatures of 40, 45, 50, 55, and 60 °C. The samples were withdrawn at intervals of 2 months and subjected to complete fermentation.

Analytical determination

Measuring pH

The pH was measured by using a digital bench pH-meter, model pH-526/sentix – 20/ASDIN/. SIN/ STH/ 650.

Determination of Specific gravity

Specific gravity was measured according to ICUMA (International Commission for Uniform Measurement of Sugar Analysis 2003).

Determination of total solids (Brix solids)

Mean the total applicable solids content of sugarcane molasses. The Brix value was determined using a Brix hydrometer corrected to 20°C.

Determination of total sugar

The total sugars of molasses solution are determined by the volumetric method (Lane and Eynon 1923). The Lane and Eynon method is also described in ICUMSA (2003), which measures reducing sugars by titration. Reducing sugars are those sugars that reduce Fehling reagents. Glucose and fructose reduce Fehling reagents, sucrose does not, so the sucrose was inverted using hydrochloric acid (1N) and the titration was repeated and the total reducing sugars can be calculated.

Determination of non-fermentable sugars

Total non-fermentable sugars are reducing matters that have a reducing effect on Fehling's solution and cannot ferment by S. cerevisiae and are determined by the volumetric method (Lane and Eynon 1923). Diluted 12 g of molasses 500 by boiled distilled water in 500 mL conical flask, cooled, added 0.2g urea, 0.2g di-ammonium phosphate, 25g of fresh yeast, and drop of antifoam, mixed and closed by cotton, and incubated at 32 °C for 24 hours. Transferred quantitatively into 250 ml volumetric flask, added 50 ml of 10 % neutral lead acetate solution diluted to the mark, mixed well, and filtrated. Transferred 150 ml of filtrate into 200 volumetric flasks then added 5 ml of 4% potassium oxalate, cooled to 20 °C, and diluted to the mark with water. Mixed well then filtrated and titrated the filtrate with 10 ml of Fehling's solution its factor is known and determines the volume consumed in titration (V1).

Total non-fermented sugars% g molasses =

Lane - lynon's factor x Fehling's factor x250 x200 x100 /12 x 150 x (V1) x 1000

Determination of ash

Total ash in the molasses sample was determined by the methods described by A.0.A.C (2000).

Determination of ethanol yield

Ethanol percent was determined by Ebulliometer apparatus approved in distillation factory which consists of condenser, thermometer, burner and Calibrate scale. It depends on a constant boiling temperature for two liquids mixture having different boiling temperatures ethanol and water according to Jacobson (2006).

The proper statistical analyses of variance according to Gomez and Gomez, (1984). Mean comparison between treatments and their interactions was determined using Duncan's multiple range tests at a 0.05 probability significance level .

Results and Discussion

Sugar content of the samples collected of molasses

Data presented in Table 1 shows the total fermentable and non-fermentable sugars involved in sugar cane molasses vary between samples collected according to the location source. This fact was reported previously by many investigators (Mohamed 1999). Curtin (1983) reported that the composition of molasses is influenced by many factors such as soil type, ambient temperature, moisture, and season of production. Also, the variety and technology of sugar mills can control the amount of sucrose extracted. Therefore, the sugar content of molasses produced in different countries varies according to the production technology employed (Barzegari et al. 2005). Also, Damtew (2008) reported that changes in the design of centrifuges used to separate sugar and syrup constitute one of the major advancements in the cane sugar industry. Moreover, the sugar manufacturing process of cane molasses will inevitably generate some hazardous substances such as excessive metallic ions, which are toxic to cells (Xu and Xu 2014).

Also, Table 1 shows that the Nag Hamady molasses was the best one containing the highest concentration of total sugar and fermentable sugar but the least one is in non-fermentable sugar than the molasses of the other three Factories (Deshna, Kous and Edfu). The results in Table 2 recorded the physicochemical analysis of a collected sample of molasses as a mixture of the molasses of the four Factories under examination.

Table 1 Physicochemical analysis of some sugar cane molasses samples obtained from different Egyptian sugar factories located in different governorates at the starting of mills season.

Factory	Nagh Hamadi	Kous	Edfu	Deshna	P value
pH	5.4a	5.6a	5.4a	5.5a	0.13 n.s
Specific gravity	1.43a	1.39a	1.41a	1.45a	0.09 n. s
Brix	86.85a	86.91a	85.65a	86.95a	0.23 n. s
T.S. % in molasses	52.57a	48.9b	52.00a	52.22a	0.06 n. s
Non-fermentable Sugars % in molasses	4.20a	4. 40a	4.50a	4.40a	0.08 n. s
T.F.S. % in molasses	48.37a	44.6b	47.50a	47.82a	0.04*
Ash % in molasses	10.63b	12.39a	11.03ab	10.53b	0.03*

- T.F.S: Total fermentable sugar & T.S: Total sugar
- *, **, and n. s: significant at 5%, 1% level, and not significant, respectively.
- Different letters following the data within each row mean significant difference at $p < 0.05$

Table 2 Physiochemical analysis of the collected sample.

Parameter	Results
pH	5.3
Specific gravity	1.42
Brix	86.6
T.S % molasses	51.5
Non-Fermentable Sugar	4.4
T.F.S % molasses	47.1
Ash %	11.1

T.S: Total Sugar & T.F.S: Total fermentable sugar.

Ethanol yield

Data presented in Table 3 shows that ethanol yield and fermentation efficiency, as well as residual sugars, were different in the fermented molasses media which contained the same concentration of fermentable sugars (18 %). This may be attributed to the inhibitors and deleterious substances in molasses such as heavy metals (Abd El-Rahman 2010), microbial contamination (Fadel et al. 2018), and other yeast inhibitors such as furfural, hydroxyl methyl furfural (HMF), and organic acids (Bronn 1985; Godbole 2002) involved in sugar cane molasses.

Table 3 Fermentation of sugar cane molasses (18 % w/v invert sugar produced by different Egyptian sugar factories by S. cerevisiae F-514 at 32 °C for 48h.

Sugar Factory	R. S	Ethanol % v/v	F. E	Ethanol L/ton molasses
Nagh Hamadi	2.86b	9.25a	84.10a	248.0a
Kous	3.53a	8.84a	80.37bc	208.7c
Edfu	3.43a	8.90a	80.90bc	234.8b
Deshna	2.94b	9.20a	83.65a	244.4a
Collected Sample	3.28a	8.99a	81.70b	235.0b
P value	0.05*	0.14	0.04*	0.02*

- F. E: Fermentation efficiency &R. S: Residual sugar % molasses
- Different letters following the data within each row mean significant difference at $p < 0.05$

Effect of storage temperature on total fermentable sugars involved in sugar cane molasses of collected sample

Data presented in Tables 4 a&b show that storage temperature was an important factor to keep the quality of sugar cane molasses to be invested in economic ethanol production. The data reveal that molasses loss about 2.65-11.20% from fermentable sugars when stored under different temperatures for 6 months. The loss increased with the increasing storage temperature. Data also indicates that the most of loss happened in the first two months, especially in high temperatures. The obtained result can be discussed in light of the two theories of the decomposition of molasses reported by Olbrich (1963).

One theory is based on a reaction between lime and reducing sugars. It states that at a suitable temperature, the decomposition by the reaction between the reducing sugars in the molasses and the unstable organic substances, which have been formed in the purification process from the excess lime present. The other theory is based on the reaction between amino acids and reducing sugars, which leads via N-glycosides to the melanoidins. It is the basis of the fact that 15-50% of the total nitrogen content of cane molasses is present in a form that cannot be assimilated by yeasts.

Decomposition of destruction is two milder forms of decomposition phenomena that proceed gradually and lead either to loss of sugar because of the destruction of saccharose or which involve particularly the inverted sugar content of the molasses. The loss of reducing sugars is ordinarily connected with a concurrent increase of non-fermentable reducing substances.

The reaction of reducing sugars and amino acids and the metabolic processes of osmophilic organisms may participate in the gradual decrease of the sugar content and invert sugar while the molasses is in storage (Fadel et al. 2016). The extent of the deterioration is related to numerous circumstances that surround the particular case (Fadel et al. 2013). The proven hydrolysis of saccharose and the demonstrated loss of total sugar in laboratory studies show that the gradual alterations in the composition of molasses while in storage are not due necessarily to purely chemical reactions alone, rather these changes result at least in part from the activity of micro-organisms. The loss of saccharose, reducing sugars and total sugar is invariably accompanied by an increase in the non-fermentable reducing substrate (Fadel et al. 2016). Ordinarily, the increase in the latter is most rapid during the first two months of storage Chen and Chou (1993).

Under extreme conditions of heating, molasses may decompose completely. From time to time instances occur, particularly on tropical sugar estates, which result in the complete destruction of large quantities of cane molasses. When molasses is heated the decomposition that occurs is exothermic and therefore heat is generated. If the heat generated by the decomposition cannot escape from the sides of the storage tank fast enough then the temperature increases still further and the rate of reaction becomes faster until in the end molasses may either be ejected from the tank with the formation of steam or in extreme cases will catch alight to leave charred molasses.

Although it is not possible to be precise about the level of heat required to start destructive decomposition, at any temperature over 50°C there is always the possibility of thermal decomposition. Earlier it was mentioned that the products produced by the reaction of reducing sugars and amino acids may play some part in thermal decomposition but whatever the mechanisms of decomposition it is most important to keep the temperature of cane molasses below 50°C for safe storage. It is a useful precaution to fit molasses storage tanks with thermometers so that a temperature rise may be seen if it occurs and then action may be taken before a complete disaster happens.

Tables 4a&b Effect of storage temperature on total fermentable sugars (4a) and reduction present of fermentable sugars (4b) involved in sugar cane molasses of collected sample.

Table 4a Total fermentable sugars %.

Storage period Month	Temperature °C					
	40	45	50	55	60	Mean
0	47.10a	47.10a	47.10a	47.10a	47.10a	47.10a
2	46.65a	45.70ab	44.60b	43.96bc	42.60c	45.03b
4	46.15a	45.55ab	44.45b	43.70bc	42.00c	44.67b
6	45.85a	45.45ab	44.30b	43.55bc	41.80c	44.47b
Mean	46.44a	45.95a	45.11b	44.58b	43.38c	

Table 4b Reduction % of fermentable sugars.

Storage period month	Temperature °C					
	40	45	50	55	60	Mean
0	0.00i	0.00i	0.00i	0.00i	0.00i	0.00C
2	0.95h	2.97f	5.30d	6.80cd	9.50b	4.41b
4	2.00g	3.33e	5.60d	7.20c	10.80a	5.16ab
6	2.65f	3.50e	5.91d	7.50c	11.20a	5.57a
Mean	1.40d	2.45c	4.20bc	5.38b	7.88a	

Effect of storage temperature on ethanol yield and fermentation efficiency of sugar cane molasses of collected sample

Data presented in Tables 5 a,b&c show that ethanol yield and fermentation efficiency were affected negatively by increasing both the storage period as well as increasing the temperature of storage, and this attributed to the decrease in the fermentable sugars (Table 4a&b). The data revealed that the decrease in ethanol yield and fermentation efficiency was affected sharply in the molasses storage at high temperatures than in low temperatures and the decreasing not linear with the decrease in fermentable sugar resulting from the deterioration of molasses storage temperature. These results are in harmony with other studies (Grancelli La 1978; Huretas & Serrales 2003). The obtained results indicate that not only fermentable sugars are the principal factor affecting ethanol yield and fermentation efficiency. The obtained data may be discussed on the basis of scientific data as the alterations in the composition of molasses during storage such as the formation of decomposer substances as well as organic acids and both hinder the activity of yeast to ferment the fermentable sugars as it present in the fermentation medium.

Tables 5a,b&c Effect of storage temperature on ethanol yield and fermentation efficiency of sugar cane molasses of collected sample fermented by S. cerevisiae F-514 for 48 h at 32°C

Table 5a Ethanol % v/v.

Storage period month	Temperature °C					
	40	45	50	55	60	Mean
0	8.99a	8.99a	8.99a	8.99a	8.99a	8.99a
2	8.73a	8.65a	8.35ab	7.83b	7.57b	8.31ab
4	8.68a	8.48a	8.20ab	7.60b	6.95c	8.10b
6	8.62a	8.43ab	8.11ab	7.46b	6.84c	8.01b
Mean	8.76a	8.64a	8.41ab	7.97b	7.59b	

Table 5b Fermentation efficiency %.

Store perid monh	Temperature °C					
	40	45	50	55	60	Mean
0	81.70a	81.70a	81.70a	81.70a	81.70a	81.70a
2	79.40b	78.70b	75.90e	71.20g	68.80g	75.57b
4	78.90b	77.10d	74.60ed	69.10g	63.20i	73.63c
6	78.40c	76.70d	73.70d	67.80h	62.20i	72.87c
Mean	79.60a	78.55ab	76.48b	72.45c	68.98d	

Table 5c % Ethanol Reduction.

Storage period month	Temperature °C					
	40	45	50	55	60	Mean
0	0.00i	0.00i	0.00i	0.00i	0.00i	0.00d
2	2.30h	3.00g	5.60f	10.50d	12.90b	6.10c
4	2.60h	4.60fg	7.10ef	12.60c	18.50a	8.00ab
6	3.30g	5.00f	8.00e	13.90b	19.50a	8.83a
Mean	2.05de	3.15d	5.18c	9.25b	12.73a	

F. S.: Fermentable sugar & F. E.: Fermentation efficiency.

Effect of storage temperature on the ethanol production from sugar cane molasses (L/ton) fermented by S. cerevisiae F-514 for 48 h. at 32°C.

Table 6 summarized the results obtained from the fermentation of the same concentration of molasses of the collected sample after storage for a different period at various temperatures. The results reported that the suitable storage temperature must not exceed 40°C to maintain molasses quality and avoid molasses deterioration by heat in temperatures. Similar results were obtained by several researchers (Saha et al. 1961; Baikow 1982). The data show that molasses can be used firstly by firstly as it produces from sugar factories to shorten the storage time, especially that produced in Upper Egypt.

Table 6 Effect of storage temperature on the ethanol production from sugar cane molasses (L/ton) fermented by S. cerevisiae F-514 for 48 h. at 32°C.

Storage Period (month)	Ethanol Production liter/ ton molasses					Mean
	40°C	45°C	50°C	55°C	60°C	
0	235a	235a	235a	235a	235a	235.0A
2	228b	226b	218.6c	205e	188g	215.60B
4	227b	222bc	214.6d	199f	182h	211.93BC
6	225.6b	220c	212.3de	195fg	179h	209.6BC
Mean	228.9A	225.8A	220.1B	208.5C	196.0D	

Conclusion

Among the widely used substrates for ethanol production are the molasses, the wastes byproduct of sugar industries from sugarcane and sugar beet. This is because they are cheap raw materials, readily available, and ready for conversion with limited pretreatments, as all sugars are present in a readily fermentable form. High storage temperatures (from 45°C) in addition to the quantity of molasses stored and the length of the storage period undoubtedly play a role in the decomposition of molasses. Thought it must be store in proper equipment, under suitable temperature and not for long periods.

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EKB

الملخص العربي

تدهور حاله مولاس قصب السكر اثناء التخزين وتأثيره على كفاءة تخمير الايثانول في مصانع التقطير

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[٢] قسم كيمياء الكائنات الدقيقة - المركز القومي للبحوث - مصر.
[٣] شركة السكر والصناعات التكاملية المصرية - مصر.

في الوقت الحاضر يواجه العالم نقص في الطاقة نتيجة الاستخدام المفرط في زيت البترول مما يهدد بنفاذه ولذلك اتجه العالم إلى استخدام طاقة بديله نظيفه وهي استخدام الكحول الاثيلى الذي يتميز باحتوائه على عدد مرتفع من الاوكتان. ومن أهم المواد التي تستخدم في صناعة الكحول هي مولاس القصب وذلك لأحتوائه على سكريات قابلة للتخمير بواسطة خميرة الخباز وكذلك لإحتوائه على مغذيات مفيده لفطر الخميرة. ومن أهم المشاكل التي تواجه هذه الصناعة التدهور الحاد في مواصفات المولاس نتيجه تخزينه بطريقه غير صحيحة وفي درجة حرارة مرتفعة مما يؤدى إلى تدهور في إنتاج الكحول وارتفاع فى تكلفه المنتج ونخلص من ذلك إلى أهمية تخزين المولاس بطريقة صحيحه وكيفية تخزينه للحفاظ عليه من التدهور وبالتالى الحصول على نتائج جيدة من الكحول مما يقلل تكلفه انتاجه.