Volume 6 Issue 4 (2018) 240-242 ISSN 2347-3258
International Journal of Advance Research and Innovation

Preservative Effect of Proteolytic Enzymes on Cane Molasses Storage

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Article Info:
Article history
Received 18 August 2018
Received in revised form:
28 August 2018
Accepted 2 September 2018
Available online 15 December 2018

Keywords: Molasses, Proteolytic enzymes, Lactobacillus, Fermentation, Total reducing sugar

Abstract

Molasses is an intermediate product obtained during sugar process. Usually, it has more than 85^0 Brix i.e. only 15% moisture. It contains more than 45% fermentable sugars. Basis composition, it is impossible to microorganism which could survive in this environment for too long. But microorganisms like *Lactobacillus spp*. and Gram –ve Coccobacilli possibly Pseudomonas spp. are able to survive in this medium and deteriorate the molasses. Therefore molasses preservation requires specific solutions which can stop deterioration and maintain total reducing sugar (TRS) value in molasses to produce alcohol. Therefore, two groups of proteolytic enzymes were developed which prevents the deterioration of molasses by preventing the microbial growth at 20ppm but also preserve the 3 – 5% of TRS content during storage of molasses up to six months or till application continued.

1. Introduction

Molasses is the byproduct obtained during the preparation of sugar crystals in sugar industries. It is being utilized as a vital source to produce alcohol. The storage of molasses is an important feature of distilleries process and is done throughout the year.

Temperatures play critical role to maintain the qualities and composition of molasses during storage. During the span of high temperature in summer it is very difficult to manage the molasses quality as earlier (during storage time) due to the milliard reaction (color and sugar composition changes due to conversion of fermentable sugar into un-fermentable sugar) and microbial contaminations.

Now a day, it is very difficult that the microbes contaminate to the molasses due to the high percentage of sugar and low moisture content during storage. Basis industrial reports few microorganisms are known in the categories of aerobic and anaerobic bacteria (Gram +ve bacteria i.e. Bacillus spp., *Lactobacillus spp*. and Gram –ve Coccobacilli possibly *Pseudomonas spp*.) for the deterioration of molasses during storage period and responsible for lowering down the reducing sugar and increase the volatile acid. These microbes are not only reduce the total reducing sugar (TRS) but also reduce the fermentation efficiency, resulting lowering down the percent alcohol recovery during distillation. In addition to that these microbes are responsible for the excess CO_2 generation and foaming in tanks during storage.

On the other side, primarily milliard reaction is also known cause for deterioration of molasses due to the chemical reaction of organic constitutes (sugars and non-sugars compounds) and inorganic constitutes (nitrogenous compounds) during storage. Still the complete mechanisms of milliard reaction is not well understood in molasses deterioration.

Ultimately, Millard reaction and microbial contamination are the main cause of deterioration of molasses during storage subsequently low recovery of present alcohol in distilleries.

At plant level, the reduction in alcohol recovery is the main cause of economic losses to the industries. To minimize this significant losses during alcohol recovery, industries are using many products which are composed of dangerous chemicals and biocides. Somehow they are capable to reduce the deterioration of molasses and improve the alcohol recovery but somewhat at the cost of high risk.

Keeping all these views in mind, the *Catalysts Biotechnologies Pvt. Ltd*. has been developed two separate blends of proteolytic enzymes (Proteolytic enzyme A and Proteolytic enzyme B) which are not only ecofriendly, economic but also reduced the deterioration of molasses and improve molasses qualities by minimizing the risk level during application. Ultimately enhance the recovery of percent alcohol during fermentation. Various plant trials of proteolytic enzyme groups have been conducted throughout the India to observe their effectiveness on molasses preservation at lab scale as well as at plant level which is further elucidated in this article.

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2. Material and Methods

2.1 Application of Proteolytic enzyme

Analyzed TRS, UFS and FS at 0 days then dosed both proteolytic enzymes A and B at 20ppm in storage tank and mixed properly with the help of circulation pumps. Continuously allowed proper cooling of storage tank by spraying of water on the outer wall. After 15 days dosed 20 ppm of only proteolytic enzyme B in the same test storage tank and mixed properly with the help of pumps. After 30 days again dosed 20 ppm of both proteolytic enzyme A and proteolytic enzyme B in the same storage tank and mixed properly with the help of pumps. Continued same process in similar way till the molasses stored in tanks. Recorded date and time when enzyme dosed and collected sample for analysis.

All parameters such as TRS, UFS and FS of molasses were tested when enzyme dosed and samples were collected on every 0, 15, 30 and 45 days onward of stored molasses in test and control tank.

Observed the product efficiency by taking control molasses tank (without application of proteolytic enzyme) against test molasses tank and conducted analysis.

2.2 Dosage pattern to be followed:

a. Proteolytic enzyme A- 20 g / Ton of molasses @ 20 ppm (to be dosed once in every month till storage).
b. Proteolytic enzyme B- 20 g / Ton of molasses @ 20 ppm (to be dosed twice in every month till storage).

Notes**:** For maximum output of this product, four circulation pumps were installed with tank at every 90 degree angle; which were collected the molasses from center of the tank bottom and poured on top near wall of the tank at same side. Alternate of this circulation system; Minimum 2 circulation pumps were used at every 180 degree angle; which were collected the molasses from center and poured on top of the tank.

3. Results and Discussions

During storage, molasses qualities maintenance is a challenge to alcohol industries. Industries are using many products to prevent deterioration of molasses to get the maximum yield of alcohol. Maximum products are being used by industries are biocides but these product are not provide complete solution to prevent the deterioration of molasses during storage. Basis these observations, two innovative groups proteolytic enzymes A and B were introduced which is amalgamation of inimitable enzymes developed by *Catalysts Biotechnologies Pvt. Ltd.* provides complete solution for the molasses preservation by preventing deterioration of reducing sugar and control the volatile acid formation in stored molasses. *Catalyst Biotechnologies* claims that these groups of proteolytic enzyme maintained the TRS value up to 180 days (6 months) or till application continued.

Application of proteolytic enzymes A and B in Plant Trial 1, showed 0.45% difference in TRS value up to 60 day (table 1), similarly Fig. 1 also indicate the pattern of prevention of deterioration in TRS value up to 60 days (2 months) during storage. Basis of Plant Trial 1, another Plant Trial 2 was conducted and observation was taken up to 180 day. The proteolytic enzyme showed 0.57 % difference in TRS values (table 2), similarly Fig. 2 also represented the pattern of prevention of deterioration in stored molasses. Plant Trial 2 showed that the proteolytic enzyme maintained the TRS value up to 180 days (6 months) during storage of molasses.

Table 1: Plant trial 1 - Effect of proteolytic enzymes (A & B) on TRS of stored molasses over the period.

TRS	Reading	Percent Difference against Initial TRS
Initial TRS taken as 100%	R0	0.00
	R1	0.00
	R2	+ 0.31
	R3	+ 0.39
	R4	+ 0.39
	R5	+ 0.45

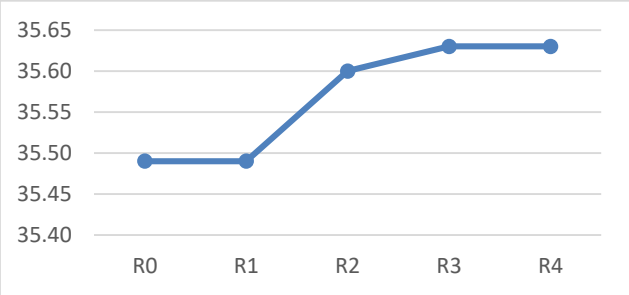

Fig.1: Effectiveness of proteolytic enzymes (A & B) on TRS value in stored molasses.

Table 2: Plant trial 2 - Effect of proteolytic enzymes (A & B) on TRS of stored molasses over the period.

TRS	Reading	Percent Difference against Initial TRS
Initial TRS taken as 100%	R0	0.00
	R1	+ 0.23
	R2	+ 0.55
	R3	+ 0.55
	R4	+ 0.57
	R5	+ 0.57

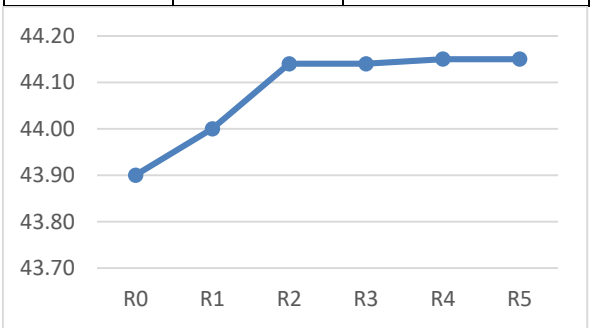

Fig.2: Effectiveness of proteolytic enzymes (A &B) on TRS value in stored molasses.

Table 3: Plant trial 3 - The effect of proteolytic enzymes (A & B) on TRS of molasses storage in Control as well as Treated tank.

TRS	Reading	Control (% Difference against Initial TRS)	Treatment (% Difference against Initial TRS)	Actual % Difference in TRS	Actual Difference in TRS Unit
Initial TRS taken as – 100 %	R1	0.00	0.00	0.00	0.00
	R2	-1.66	0.83	+ 2.53	1.08
	R3	-2.86	0.83	+ 3.80	1.60
	R4	-3.25	1.27	+ 4.67	1.96
	R5	-3.44	1.29	+ 4.90	2.05

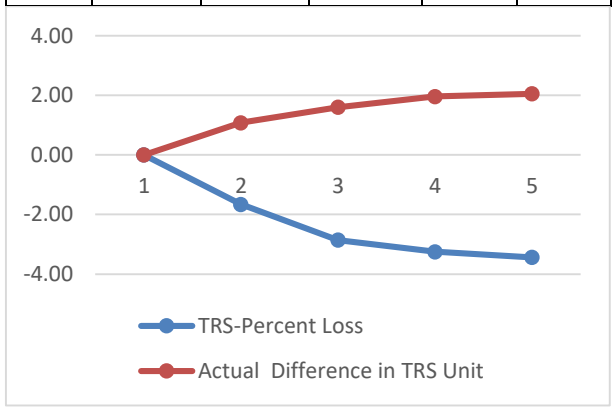

Fig. 3: Comparative study of TRS deterioration prevention of treated and control molasses during storage in plant trail 3.

Table 4: Plant trial 4 - The effect of proteolytic enzyme (A & B) on molasses storage in Control as well as Treated tank.

TRS	Reading	Control (% Difference against Initial TRS)	Treatment (% Difference against Initial TRS)	Actual % Difference in TRS	Actual Difference in TRS Unit
Initial TRS taken as – 100 %	R1	0.00	0.00	+ 0.00	0.00
	R2	-1.96	0.37	+ 2.38	0.95
	R3	-2.72	3.24	+ 6.12	2.43
	R4	-3.48	3.65	+ 7.39	2.91
	R5	-3.53	3.50	+ 7.29	2.87

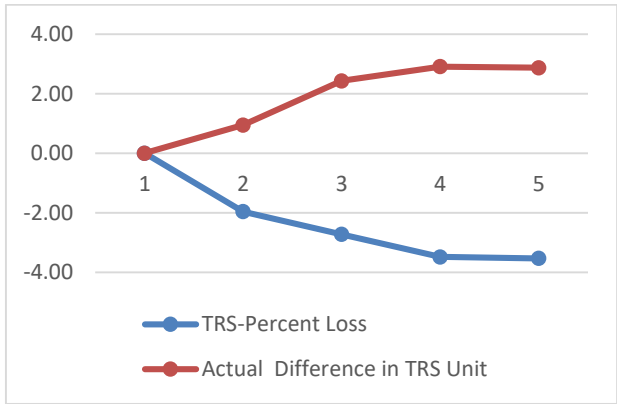

Fig. 4: Comparative study of TRS deterioration prevention of treated and control molasses during storage in plant trail 4.

Basis encouraging observation from plant trial 1 and 2, further Plant Trial 3 & 4 were conducted along with comparative studies to observed actual percent enhancement in treated molasses with Proteolytic enzyme (at 20ppm) against control. Plant trial 3 showed that the 2.05unit difference in TRS of molasses against control while -3.4 % of TRS loss was observed in control molasses during the same period (Table 3 & Fig. 3). Similarly in other Plant trial 4 was

conducted on different place and molasses, there was another significant improvement in result was observed. Plant trail 4 showed 2.87unit difference in TRS of molasses against control stored molasses while -3.5 % TRS loss was observed in untreated molasses with same condition and period (Table 4 & Fig. 4). Above studies, clearly indicated that the plant trials showed significant enhancement in TRS value of molasses during storage. The mechanism behind the TRS enhancement during application of proteolytic enzyme is further need to be elucidated.

4.Conclusions

The groups of proteolytic enzyme (A & B) are a combination of inimitable enzymes, which are not only significantly prevent the deterioration of molasses by preventing the microbial growth at 20ppm but also enhance the TRS content during the storage of molasses up to six months or till application continued.

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