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# Moringa extracts used in sugarcane juice treatment and effects on ethanolic fermentation

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**The objective of this study was to evaluate the effects of sugarcane juice treatment using *Moringa oleifera* leaf and seeds extracts on ethanolic fermentation. The experiment was arranged in a split plot statistical design, with four replications. Main treatments were three sedimentation agents (synthetic polyelectrolyte, moringa leaf and seed extracts) and control while the secondary treatments were two sugarcane varieties (RB867515 and CTC4). Extracted sugarcane juice was clarified by simple defecation with pH adjusted to 6.0. The flocculating agents were added in a decanter before the limed juice. After then, the juice was standardized to 16° Brix at pH 4.5, and musts were inoculated with yeast *Saccharomyces cerevisiae* strain, FT858. At the end of the fermentation process, wines were recovered by centrifugation. In all experimental stages, extracted juice, clarified juice and wine were chemically and technologically characterized. The use of moringa leaf and seed extracts as sedimentation adjuvants did not increase the sedimentation speed of impurities. However, there was a high sludge compaction, which was essential for maintenance of yeast and bud population at the beginning of fermentation, and yeast budding rate in the end. The use of different sedimentation agents as adjuvants in juice treatment did not affect wine quality and ethanol yield.**

**Key words:** Juice clarification, *Moringa oleifera* Lamarck, simple defecation, polyelectrolyte, flocculating agents, *saccharomyces cerevisiae.*

## INTRODUCTION

Ethanol is one of the mostly produced biofuels in the world, with the United States and Brazil being the major producers. The estimated production for the season, 2014/2015, is approximately 28.37 billion liters only in Brazil (CONAB, 2014). In this country, ethanol is produced from sugarcane. After extraction, cane juice is treated to remove soluble and insoluble impurities, and submitted to a fermentation process by yeast inoculation, which metabolizes sugars and produces the ethanol that is recovered via distillation process.

Juice treatment is essential to remove some materials and yeast inhibitory compounds, such as dirt, bagasse, acids and phenolic compounds. This process begins with sieving extracted juice to remove some bagasse, followed

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by a dirt removal through hydrocyclone equipment. After physical treatment, calcium hydroxide is added to the juice until pH 6.0 and juice is heated to 100-105°C. A chemical reaction between added calcium and the phosphate present in the juice occurs, and insoluble compounds such as calcium phosphate precipitate and adsorbed biomolecules like acids and phenolic compounds are formed. Juice is placed in a decanter and without stirring for 1 to 3 h to remove these precipitates (Steindl, 2010).

In order to accelerate particle sedimentation, synthetic acrylamide-based polyelectrolytes are used in the clarification process, because they react with the calcium phosphate precipitates to form flakes with higher mass and density which accelerate precipitation (Doherty, 2009).

However, acrylamide has carcinogenic and neurotoxic action in animals and humans (WHO, 2002). Thus, some countries as the United States restrict the use of this product to the maximum of 5 mg/L of juice in sugar production (FDA, 2014). It is also important to know that the clarified juice by this treatment could contain significant amounts of acrylamide. In the fermentation process, the yeasts may absorb this molecule and, when destined to animal food after ethanol production process, may be toxic. This product is sold in Brazil at an average price of $6.00/Kg, and the amount used is approximately 1 kg per 150-250 tons of processed cane (Hassuani, 2012). Considering these problems, the search for molecules to replace synthetic polyelectrolytes in juice treatment has intensified. Among the alternative products, leaf and seed extracts of moringa (*M. oleifera* Lamarck) are considered as they are widely used as flocculating agents in water treatment (Sarpong and Richardson, 2010).

The seed contain a protein (MO2.1) that has a coagulant action. This protein presents molecular weight about 6-30 kDa, and isoeletric point between 10 and 11. It is formed by glutamate, arginine, proline, glycine, valine, serine, among others amino acids (Gassenschmidt et al., 1995). It should be noted that the coagulation/flocculation mechanism of moringa protein is similar to the polyelectrolyte, once positive charges are presents in molecule that adsorb particles of the medium (Borba, 2001). Costa et al. (2014) observed that moringa leaf and seed extracts also had flocculation effects when used to treat sugarcane juice for sugar production. The objective of this study was to evaluate the flocculation effects of moringa leaf and seed extracts treatment on ethanol production during sugarcane juice fermentation.

## MATERIALS AND METHODS

The experiment was conducted at the Sugar and Ethanol Technology Laboratory of the Department of Technology, UNESP, Jaboticabal-SP, Brazil, in 2013/2014 season. The juice of two sugarcane varieties; RB867515 (fourth ratoon harvested in July, 2013) and CTC4 (second ratoon harvested in August, 2013), both ripening between July and September (mid-season), was extracted. The sugarcane plants were collected from commercial sugarcane fields in Jaboticabal-SP, Brazil during the practical industrialization period. The experiment was arranged in a split-plot statistical design, with four replications. Main treatments comprised different sedimentation adjuvants; and secondary treatments were represented by different varieties.

Sugarcane stalks were manually harvested without trash burn and processed using a laboratory scale cane crusher. The raw material was analyzed for total soluble solids (Brix), sucrose content (Pol), purity, reducing sugars (RS), total reducing sugars (TRS), cane fiber, pH, total acidity, soluble ashes (CTC, 2005) and total phenolic compounds (TPC) (Folin and Ciocalteu, 1927).

The extracted juice was filtered in a 60 mm-mesh filter and submitted to a clarification unit by simple defecation process, through a pH adjustment to 6.0 with $Ca(OH)_2$ 6°Bé, and heating until ebullition. Then 1 L of heated-juice was kept for 20 min in a laboratory decanting system that contained the flocculating adjuvants. Sedimentation speed and sludge volume were evaluated (CTC, 2005). The clarified juice was removed using a siphon.

The sedimentation adjuvants used were: conventional synthetic polymer (Flomex 9034), moringa leaf extract (Ghasi et al., 2000) and moringa seed extract (Bhatia et al., 2007) at the doses of 1.5, 5 and 100 mg/L, respectively. Untreated clarified juice was used as control. These concentrations were established in previous assays, where the concentrations from 1 - 500 mg/L of extracts and 1 - 5 mg/L of polymer were tested. Analysis of Brix, pH, soluble ashes and total acidity were performed in clarified juice to characterize the treatments.

For musts preparation, the clarified juice was standardized to 16° Brix at pH 4.5 with $H_2SO_4$ 5 mol/L. Total reducing sugars (TRS), total phenolic compounds (TPC) and total acidity were analyzed.

The yeast strain, FT858, was then inoculated at 10% of cell concentration (m/v), in 250 ml of must. First, 100 ml of must was added and kept for 1 h, and then another 150 ml was added. Yeast cell, bud viability and yeast budding rates were analyzed (Lee et al., 1981) after 1 h of the second must feeding when Brix was ≤ 1 (end of the fermentation process).

Wines were centrifuged at 2500 x *g*, at 30°C and total residual reducing sugars (TRRS), total acidity (CTC, 2005), glycerol (Copersucar, 2001) and alcohol content (Ebuliometter) were analyzed. The results were submitted to analysis of variance (ANOVA) using F test and the means were compared by Tukey test at 5% of probability.

## RESULTS AND DISCUSSION

### Characterization of flocculating agents

Table 1 shows the results of Brix, pH, soluble ashes and total acidity of the three flocculating agents studied. The total soluble solids values obtained from moringa leaf and seed extracts treatments differed from water (0°). These results were similar to that of Costa (2013), who observed Brix values of 1.3 and 1.5 of moringa seeds and leaf, respectively.

The pH values of the flocculating agents were 6.0 for the extracts and 6.5 for synthetic polymer. These results do not corroborate with those of Costa (2013), who obtained values of 3.3 and 7.2 for moringa seed and leaf extracts, respectively. This difference could indicate that time of season influence the acid quantity in the moringa seed. Considering the polymer, the difference could be the water quality used.

### Extracted juice characterization

Table 2 shows the results of Brix, Pol, Purity, RS and

**Table 1.** Results for Brix, pH, Soluble Ashes and Total Acidity of sugarcane juice treated with moringa leaf, seed extracts and synthetic polymer.

| Extract | Brix | pH | Soluble ashes (%) | Total acidity (g/L $H_2SO_4$) |
|---|---|---|---|---|
| Seed | 1.5 | 5.80 | 0.09 | 0.17 |
| Leaf | 1.9 | 5.15 | 0.43 | 0.89 |
| Polymer | 0.2 | 6.50 | 0.01 | - |

**Table 2.** ANOVA results for Brix, sucrose content (Pol), purity, reducing sugars (RS) and total reducing sugars (TRS) of sugarcane varieties RB867515 and CTC4, in 2013/2014 Season.

| Varieties (V) | Brix | Pol | Purity (%) | RS (%) | TRS (%) |
|---|---|---|---|---|---|
| RB867515 | 18.23B | 15.22B | 83.5A | 0.84A | 14.87B |
| CTC4 | 21.33A | 18.57A | 87.0B | 0.29B | 18.73A |
| LSD | 0.52 | 0.02 | 0.02 | 0.03 | 0.21 |
| F test | 270.28** | 168337.50** | 480533.99** | 1743.06** | 2496.6** |
| CV (%) | 1.17 | 0.06 | 0.01 | 2.87 | 0.56 |

Means followed by the same letter are not significantly different according to Tukey test at 0.05 of probability. ns, not significant; *significant at 0.05 **; significant at 0.01.

**Table 3.** ANOVA results for fiber, total acidity, soluble ashes, turbidity and total phenolic Compounds (TPC) of sugarcane varieties RB867515 and CTC4, in 2013/2014 season.

| Varieties (V) | Fiber (%) | Acidity (g/L) | Soluble ashes (%) | Turbidity (NTU) | TPC (mg/L) |
|---|---|---|---|---|---|
| RB867515 | 11.54A | 0.98A | 0.60A | 661A | 553B |
| CTC4 | 11.15B | 0.77B | 0.42B | 658A | 658A |
| LSD | 0.02 | 0.06 | 0.09 | 158.42 | 2.01 |
| F test | 2281.50** | 68.74** | 28.43** | 0.00ns | 20823.21** |
| CV (%) | 0.09 | 3.48 | 7.98 | 10.59 | 0.15 |

Means followed by the same letter are not significantly different according Tukey test at 0.05 of probability. ns , not significant; *significant at 0.05 **; significant at 0.01**.

TRS obtained in extracted juice from sugarcane varieties, RB867515 and CTC4. Both sugarcane varieties were in their practical industrialization period, as Pol was higher than 14%, RS lower than 1% and TRS higher than 14%, according to the earlier reports of Ripoli and Ripoli (2009). The variety, CTC4, contained higher sugar levels than RB867515.

The fiber content was 11.3%, pH 5.0, total acidity of 0.7 g/L of $H_2SO_4$, 0.5% of soluble ashes and TPC about 600 mg/L (Table 3). These data are in accordance with the sugar content with regards to the ripening stage of both varieties.

## Juice clarification process

Figure 1 shows the values obtained for sedimentation speed (Figure 1A) and sludge volume (Figure 1B) when three sedimentation adjuvants were added in the juice from two sugarcane varieties. The use of synthetic polyelectrolyte promoted higher flake sedimentation speed for both varieties. These results are similar to that of Costa et al. (2014), who evaluated moringa leaf extract and synthetic polymer for cane juice clarification with pH 7.0 in sugar production, and found values of 0.5 and 3.5 cm/min, respectively.

Despite the fact that the sedimentation speed was higher when the synthetic polymer was used, the sludge volume after 20 min was similar to treatments in which moringa extracts were used (Figure 1B). These values were lower than those obtained by Thai et al. (2012), who used synthetic polymer as flocculating agent in sugar production and observed sludge formation of about 200 ml. Nevertheless, when these adjuvants were not used in the juice of the variety RB867515, there was low impurities precipitation, which resulted in high sludge volume (Figure 1B), as also observed by Costa et al. (2014).

## Must preparation

After treatment, clarified juices were subjected to an

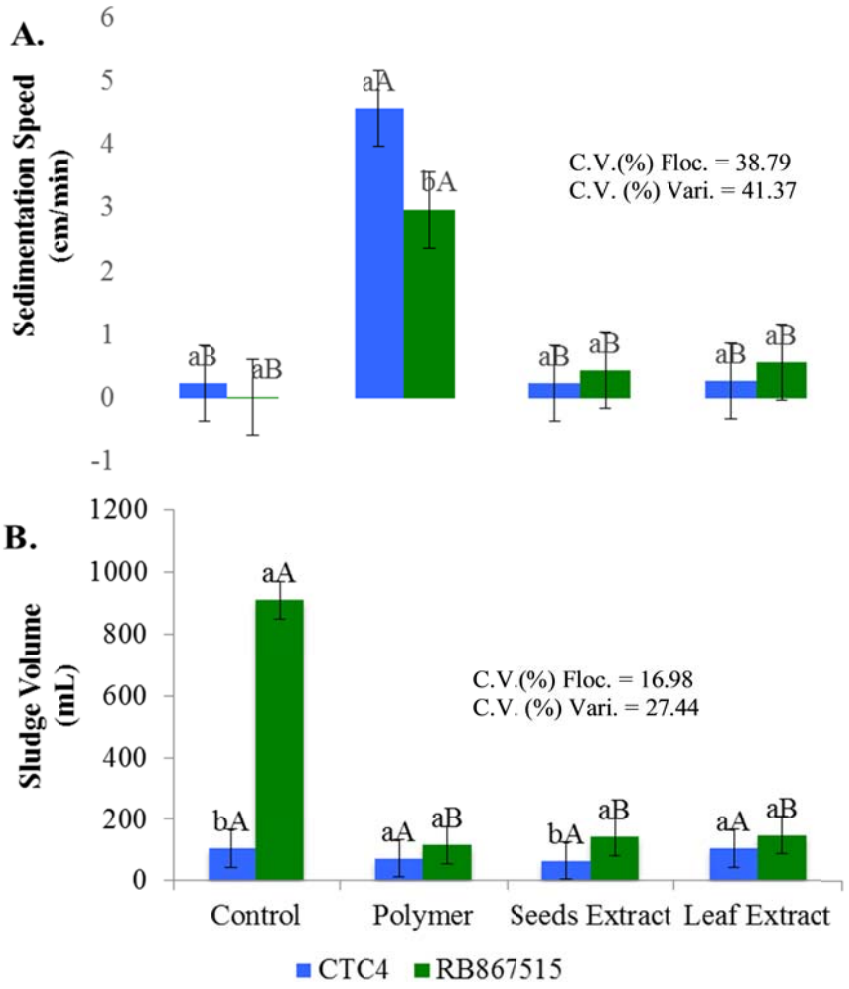


**Figure 1.** Interaction between flocculating agents and sugarcane varieties for: **A)** sedimentation speed; and **B)** sludge volume, during juice clarification. Jaboticabal-SP, Brazil; Season 2013/2014. Lower case letters compare varieties and upper case letters compare flocculating agents.

adjustment soluble solid; pH, TRS, TPC and total acidity were analyzed in musts. The TRS values were between 12.8 and 13.7%. No significant reductions in must TRS was found when the different flocculating agents were used in the clarification process. The same behavior also was verified when musts from the two sugarcane varieties were compared. This was expected since the juice clarification process does not remove sugars (Rein, 2012). The use of sedimentation adjuvants in juice clarification did not reduce the TPC values which were between 368 and 401 mg/L. Nevertheless, there was a reduction in the concentration of these biomolecules in relation to extracted juice. This result was similar to the observations of Costa et al. (2014). The TPC in clarification process are important in the ethanol industry, since these compounds act as yeast inhibitors during fermentation reducing yeast cell viability and conse-quently ethanol yield (Ravaneli et al., 2011).

Musts prepared from the variety RB867515 had higher amount of TPC (504 mg/L) than that of CTC4 (262 mg/L). These values were different from those obtained in the extracted juice (Table 3). Considering that the sugarcane ripening directly impacts in juice clarification process (Ripoli and Ripoli, 2009), especially in TPC removal (Mutton et al., 2010), probably the CTC4 cane quality allowed higher removal of these molecules during juice treatment.

Synthetic polymer caused significant reductions ($p < 0.05$) in must acidity in relation to control treatment. However, all the treatments had total acidity between 1.0 and 1.2 g/L of $H_2SO_4$ (Table 4). These results are important, since acids can act as yeast inhibitors during fermentation as earlier found by Camolez and Mutton (2005). The cane juice obtained from CTC4 variety had lower acidity values. The increase in must acidity in relation to extracted juice occurs probably because the

**Table 4.** Total acidity in musts and total acidity, total residual reducing sugars (TRRS), glycerol and alcohol content in wines obtained during fermentation of the sugarcane varieties of sugarcane varieties RB867515 and CTC4, in 2013/2014 Season.

| Flocculating agent (F) | Total acidity (g/L $H_2SO_4$) | Total acidity (g/L $H_2SO_4$) | TRRS (%) | Glycerol (%) | Alcohol content (%) |
|---|---|---|---|---|---|
| Control | 1.16A | 2.37A | 0.12A | 1.95A | 8.6A |
| Polymer | 1.08B | 2.20A | 0.10A | 1.98A | 8.7A |
| Seed | 1.10AB | 2.31A | 0.16A | 2.03A | 8.8A |
| Leaf | 1.15AB | 2.26A | 0.14A | 1.94A | 8.9A |
| LSD | 0.07 | 0.24 | 0.08 | 0.26 | 0.64 |
| F test | 4.28* | 1.29ns | 1.32ns | 0.40ns | 0.75ns |
| CV (%) | 4.45 | 7.71 | 42.87 | 9.32 | 5.23 |
| **Varieties (V)** | | | | | |
| RB867515 | 1.35A | 2.46A | 0.22A | 1.81B | 8.5B |
| CTC4 | 0.89B | 2.11B | 0.04B | 2.14A | 9.0A |
| LSD | 0.11 | 0.23 | 0.07 | 0.21 | 0.28 |
| F test | 101.12** | 13.02* | 36.18** | 13.70* | 13.80** |
| CV (%) | 11.48 | 12.11 | 64.20 | 12.79 | 3.74 |
| F test F x V | 4.77ns | 1.74ns | 1.98ns | 0.82ns | 1.24ns |

Means followed by the same letter are not significantly different according to Tukey test at 0.05 of probability. Ns, not significant; *significant at 0.05 **significant at 0.01**. FxV, interaction between flocculating agentes (main treatments) and sugarcane varieties (secondary treatments).

pH was adjusted to 4.5 with sulphuric acid.

## Fermentation process

The yeast cell, bud viability and budding rate were 85, 98 and 15%, respectively, which were considered adequate for fermentation according to Amorim (2005). There was a reduction of 15% in yeasts cell viability in the beginning of the fermentation in RB867515 and untreated control. In other treatments, yeast viability remained between 85 and 90% (Figure 2A). Yeast bud viability was also reduced in this same treatment (Figure 2B). When no sedimentation adjuvant is used in cane juice clarification process, probably a higher amount of impurities remain in the juice as calcium phosphate compounds, which are inhibitory to yeasts during fermentation. As a consequence, cell viability and stored trehalose (reserve carbohydrate used by yeast in stress condition) are negatively affected (Walker, 1998; Steindl, 2010). However, these treatments had no negative impact on yeast budding rate, which remained between 10.3 and 13.4% during fermentation.

There were no significant differences ($p > 0.05$) amongst treatments in terms of yeast cell viability. The means ranged from 84 to 88%. The same behavior was observed in yeast bud viability, which was higher than 96% (Figure 2C). These results indicate that the yeast FT858 was adapted to the substrate, and was not affected by treatments. Yeast budding rate in the RB867515 untreated control was significantly (50%) lower than treated musts, which had 30% budding rate. This rate was the triple of that found in the beginning of the fermentation process. However, this behavior was already expected, because in the end of fermentation, there is low sugar concentration, which favors yeast glycolytic pathway, resulting in high energy production (ATP) and biomass (Venturini Filho et al., 2013).

## Wine characterization

Table 4 shows the results obtained for Brix, total acidity, glycerol and alcohol content in wines. The use of different sedimentation adjuvants did not directly affect wine characteristics. However, when wines from two sugarcane varieties were compared, musts from the variety CTC4 showed lower means of total acidity and total residual reducing sugars, which were about 2.11 g/L and 0.04%, respectively. These results are similar to Moreira et al. (2013), who studied the ethanolic fermentation using the yeast CAT-1, and found 2.02 g/L of total acidity and 0.04% of TRRS. Acid production by yeasts during fermentation process is undesired, because sugar is spent to produce this metabolite, at the cost of lower ethanol yield (Camolez and Mutton, 2005). The low TRRS observed in this study indicate high sugar assimilation by yeasts during fermentation. Low ethanol and glycerol yield were observed in wines obtained from sugarcane CTC4 fermentation, with means of 9 and 2.14%, respectively. These results were higher than those obtained by Ferrari (2013), who observed values between 6.8 and 7.8 for ethanol and 0.23 to 0.4% for

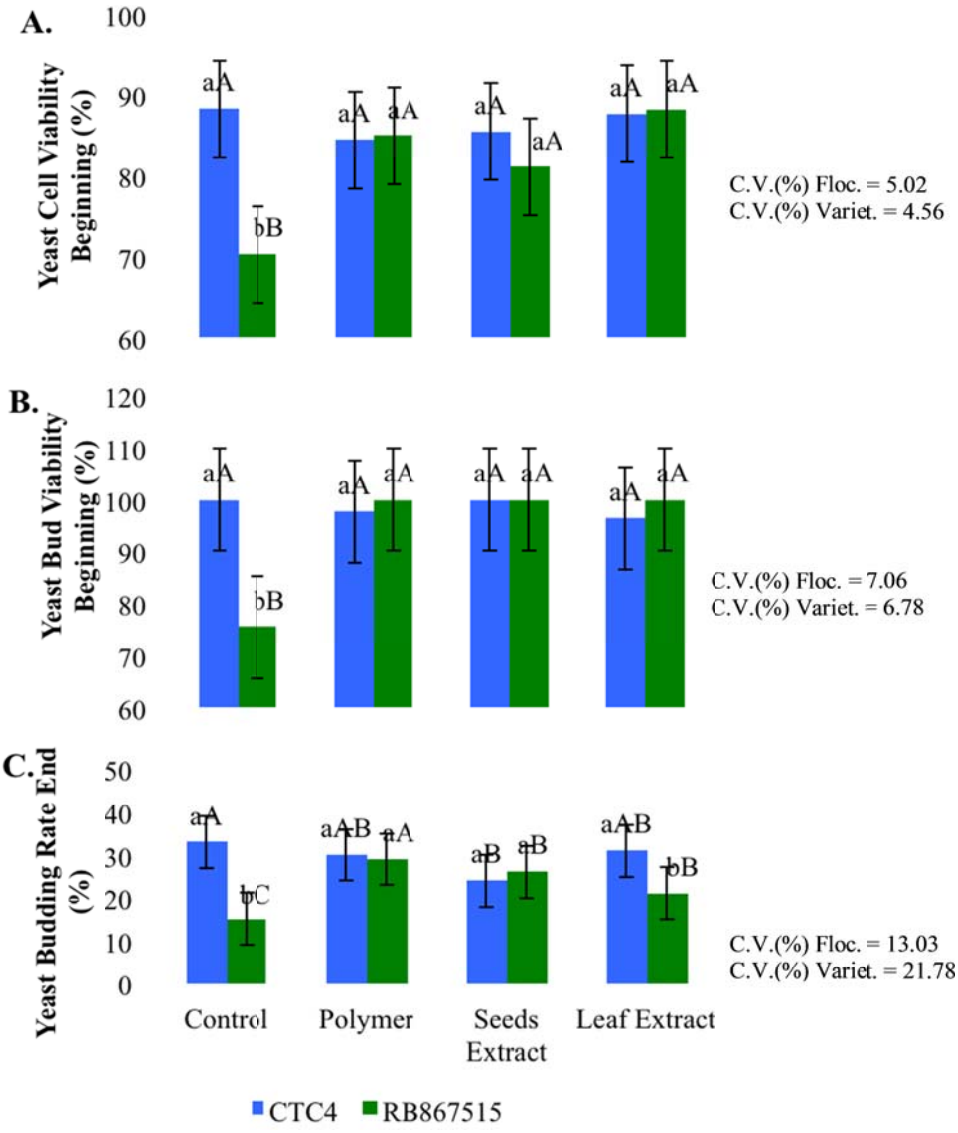


**Figure 2**. Interaction between flocculating agents and sugarcane varieties for: **A,** yeast FT858 cell viability in beggining of fermentation; **B,** yeast FT858 budding rate in beggining of fermentation; **C,** yeast FT858 budding rate in end of fermentation in Jaboticabal-SP, Brazil (Season 2013/2014). Lower case letters compare varieties and upper case letters compare flocculating agents.

glycerol production in industrial scale. These compounds are obtained from the same yeast metabolic pathway, and their production is inversely proportional (Nevoigt and Stahl, 1997; Wang et al., 2001; Ferrari, 2013). In this study, wines from the variety CTC4 presented higher ethanol and glycerol levels than those from RB867515, probably as a result of higher TRRS in wines. Also, it is important to emphasize that yeasts always produce glycerol during ethanolic fermentation, because it is essential for NAD regeneration and to maintain the metabolic balance. Under stress conditions such as bacterial contamination or osmotic stress, an increase in the production of this sugar-alcohol is also observed (Ren et al., 2012).

## Conclusion

The use of moringa leaf and seed extracts as sedimentation adjuvants in cane juice treatment for ethanol production does not increase flake sedimentation speed. However the sludge compaction is higher when compared with the untreated control. The use of flocculating agents in cane juice treatment is essential to maintain

yeast cell and bud viability in the beginning of the fermentation, and budding rate in the end of the process, when low quality sugarcane is used (RB867515).

The use of different flocculating adjuvants in cane juice treatment does not affect wine quality and ethanol yield.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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