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Research Letter

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Effect of the clarification pH of sorghum juice on the composition of essential nutrients for fermentation

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One sentence summary: Effect of the clarification pH of sweet sorghum juice on ethanol fermentation.

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ABSTRACT

The growing demand to replace fossil fuels with renewable alternatives has generated an urgent and imminent global need to find new non-fossil sources. Sweet sorghum is widely recognized as a highly promising biomass energy crop with the particular potential to complement sugarcane for ethanol production. Our aim in this study was to evaluate the influence of pH during the clarification process on the composition of essential nutrients in the sorghum juice and observe how this affects the efficiency of the ethanol fermentation process. We found that a higher pH directly affected residual concentrations of key nutrients (P, Ca, Zn and Mn) and consequently the efficiency of ethanol fermentation. In conclusion, we recommend a clarification procedure at pH 6–6.5 in order not to significantly affect nutritional parameters important for the yeast fermentation process.

Keywords: sweet sorghum; clarification process; bioethanol; bioenergy; bioprocess

INTRODUCTION

Petroleum is the main provider of global energy, representing 35.7% of global energy consumption (International Energy Agency 2015). Fossil fuels such as oil and natural gas are primarily used for transportation, and due to the constant expansion of this sector there is an urgent need to find economically and environmentally viable sources of renewable fuels in order to overcome this petroleum dependency (Energy Information Administration 2011). In addition, the combustion of petroleum represents a significant part of carbon dioxide emissions to the atmosphere, raising concerns about global climate changes (Cárdenas, Diez and Quaia 2007). One of the most promising candidates to replace fossil energy as transportation fuel is bioethanol. Apart from a very low net emission of CO_2 to the atmosphere, the combustion of bioethanol generally results in minor emission of non-combusted hydrocarbons, carbon monoxide (CO), nitrogen oxides and exhaust volatile organic compounds, making it an environmentally friendly fuel (Cárdenas, Diez and Quaia 2007). Bioethanol is produced by sugar fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn and sugarcane, but other sugar feedstocks like sweet sorghum have recently attracted growing interest. Sweet sorghum is widely recognized as a promising sugar feedstock crop due to its efficient C-4 photosynthetic pathway, easy and rapid cultivation from seeds, low fertilizer and water requirements, and is considered an agricultural crop resistant to drought due to its ability to stay in a dormant state during dry periods (Woods 2001; Teetor *et al.* 2011). Other authors describe sweet sorghum as an ideal complement to the cultivation of sugarcane because it allows the

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use of the same equipment and facilities as sugarcane (Durães 2011; Romero *et al.* 2012). This crop is economically interesting as it provides sugars for ethanol production and lignocellulosic materials for electricity generation (May *et al.* 2012; Romero *et al.* 2012).

Ethanol production from sorghum juice fermentation can be categorized in three major steps: juice extraction, ethanol fermentation and ethanol separation. As the last two steps depend on the quality and concentration of the raw material, many studies have been conducted in order to evaluate the impact of juice concentration on ethanol yield in the fermentation step (Datta *et al.* 2012; Sasaki *et al.* 2014).

An important industrialization step of sweet sorghum for ethanol production is the clarification of the mixed juice used for fermentation. The mixed juice is a complex mixture of sugars, minerals, organic non-sugars and foreign matter components extracted from the plant by pressure and imbibitions of plant stems (Simpson 1996). The goal of clarification is to stabilize juice with respect to microbial deterioration and remove suspended and turbid particles (Andrzejewski *et al.* 2013).

The clarification method most commonly used is a simple defecation, where heat and the addition of lime are used to obtain a clear juice suitable for further processing (Eggleston, Monge and Pepperma 2002). Special features of the sorghum juice (fiber content, starch, reducing sugars and a different impurity profile) in relation to sugarcane juice, require a different clarification process in order to obtain a juice suitable for alcoholic fermentation and/or concentration.

There are very few studies published that link the clarification process of sweet sorghum juice and its influence on the efficiency of fermentation process. Our aim in this study was to evaluate the influence of pH during the clarification method on the composition of essential nutrients in the sorghum juice and to assess how this affects the efficiency of the ethanol fermentation process.

MATERIAL AND METHODS

Sweet sorghum

Hybrid seeds of sweet sorghum variety Argensil Bio 165 provided by Argenetics Semillas were used in all studies. All sorghum stalks were defibered (open cell ≈ 95%) and pressed (240 kg/cm^2) using a hydraulic press for 1 min, obtaining primary juice and bagasse. To generate mixed juice, 20% water was added to the bagasse and the mixture was pressed again. Both juices were mixed and filtered through a mesh of 0.5 mm porosity.

Clarification of mixed juice

The pH of juice was adjusted to pH 6.0, 6.5 and 7.0, $Ca(OH)_2$ was added before heating to boiling point; 3 mg/L of flocculant (Magnafloc LT27, Ciba SC) was added and allowed to settle for 1h in a water bath at a temperature close to boiling point (98°C). A juice heated at pH 5.2 was used as a control. The juice treatment studies were run in triplicate.

Soluble solids (°brix)

The °brix was measured using an Index Instruments (Kem Kyoto, Japan) RA-620 temperature-controlled refractometer accurate to ± 0.01°brix, and results were expressed as an average of three independent measurements.

Assimilable and ammonia nitrogen

The assimilable and ammonia nitrogen were determined by the methods described by da Silva (2003a,b). Distillation for the determination of nitrogen ammonia was performed in a Kjeldal-type distiller (Buchi B-324 Distillation Unit). Results were expressed in mg/L of nitrogen.

Total phosphorus and total starch

Total phosphorus and starch in juices were determined using colorimetric techniques described in ICUMSA (ICUMSA 2013a,b); results were expressed in mg/L of elemental phosphorus and mg/kg of total starch.

Determination of metals

For the analysis of metals (K, Ca, Mg, Mn, Fe, Zn and Cu), samples were mineralized in a muffle at 650°C and subsequently solubilized with HCl (1%). For analysis of Ca and Mg, 0.5% of lanthanum chloride and 0.5% potassium chloride were added to solubilize samples. For determination of K, only 0.5% lanthanum chloride was used. Measurements were performed by atomic absorption spectrometry with air/acetylene flame in an AAnalyst-100 atomic absorption spectrometer (Perkin Elmer, Ohio, USA).

Fermentation conditions

For this study we used commercial baking yeast. *Saccharomyces cerevisiae* was re-suspended in sterile water. The pH was adjusted to 2.5 with sulfuric acid and the suspension stirred for 1 h, mimicking the treatment in distilleries. Thereafter, 80 mL of mixed or clarified juices (17–18°brix) were inoculated with 20 mL of 7–8% of treated yeasts (10^8 CFU/mL) in an Erlenmeyer flask (125 mL) with a foam stopper. Inoculated juices were incubated statically at 30°C for 12 h. Total fermentable sugars (TFS), final alcohol content and cell viability in fermented mash were measured. All fermentation studies were run in triplicate. Fermentation efficiency was calculated as the ethanol yield divided by the maximum theoretical yield multiplied by 100.

Total fermentable sugars and ethanol

The TFS concentration was determined in fermented mash and in the mixed and clarified juices using a High-performance liquid chromatography with a quaternary pump and injector (Waters Alliance model 2695), a refractive index detector (Waters 410) and a Waters Sugar Pack column (mobile phase H_2O + 0.05 g/L EDTA; column temperature 85°C, detector temperature of 45°C and flow 0.5 mL/min).

The TFS was calculated as:

$$\text{TFS\%} = \text{sucrose\%} * 1.05 + \text{glucose\%} + \text{fructose\%}.$$

To determine the percentage of ethanol, 50 mL of the sample were distilled into a Büchi B-324 distillation unit and subsequently the alcoholic tenor (°GL) was determined with a digital hydrometer, Rudolph DDM2911 (da Silva *et al.* 2003c).

Viability and percentage of yeasts

The viability of yeasts was determined by counting live and dead cells in a Neubauer chamber, using the technique of methylene blue (Copersucar 1987). The percentage of yeasts (v/v) was

Table 1. Effect of clarification of sorghum juice at different pHs on the concentration of assimilable (YAN) and ammonia nitrogen, soluble solids (°brix), total fermentable sugars (TFS), phosphorus and starch.

	Treatments				
		Clarified Juices			
Parameters	Mixed Juice	pH 5.2	pH 6.0	pH 6.5	pH 7.0
YAN (mg/L)	218 ± 6[a]	233 ± 31[a]	231 ± 32[a]	233 ± 21[a]	233 ± 12[a]
Ammonia (mg/L)	109 ± 3[a]	108 ± 4.6[a]	114.0 ± 1.0[a]	125 ± 14[a]	128 ± 12[a]
°Brix (g/100 g)	16.5 ± 1.7[a]	16.5 ± 2.0[a]	16.6 ± 1.9[a]	16.7 ± 1.7[a]	16.8 ± 1.6[a]
TFS (g/100 mL)	12.5 ± 1.3[a]	12.1 ± 1.6[a]	12.5 ± 0.9[a]	12.4 ± 1.1[a]	13.0 ± 1.0[a]
Phosphorous (mg/L)	179 ± 20[a]	164 ± 23[a]	128 ± 26[b]	84 ± 6[c]	43 ± 2[d]
Total starch (mg/kg °brix)	4061 ± 328[a]	3345 ± 282[b]	3313 ± 156[b]	3348 ± 246[b]	3322 ± 312[b]

[a–d]Mean values in rows followed by the same letter do not differ statistically from each other (Tukey, $P = 0.05$).

Table 2. Effect of clarification of sorghum juice at different pH on the concentrations of metals important for alcoholic fermentation.

	Treatments				
		Clarified Juices			
Parameters	Mixed Juice	pH 5.2	pH 6.0	pH 6.5	pH 7.0
Ca (mg/kg)	453.60 ± 75.69[a]	376.80 ± 98.32[a]	597.40 ± 139.42[a,b]	626.60 ± 176.70[a,b]	811.60 ± 219.09[b]
Mg (mg/kg)	196.80 ± 37.81[a]	215.20 ± 48.10[a]	195.20 ± 55.37[a]	212.60 ± 44.27[a]	231.20 ± 31.07[a]
K (g/kg)	4.58 ± 1.13[a]	4.94 ±1.55[a]	4.94 ± 1.34[a]	5.18 ± 1.45[a]	5.78 ± 1.21[a]
Na (mg/kg)	36.60 ± 18.08[a]	41.80 ± 14.79[a]	37.60 ± 20.21[a]	43.40 ± 21.74[a]	40.50 ± 22.84[a]
Cu (mg/kg)	0.82 ± 0.26[a]	0.68 ± 0.36[a]	0.56 ± 0.11[a]	0.68 ± 0.22[a]	0.70 ± 0.24[a]
Zn (mg/kg)	3.56 ± 0.80[a]	3.84 ± 1.31[a]	2.33 ± 0.17[a,b]	2.48 ± 0.94[a,b]	1.78 ± 0.82[b]
Mn (mg/kg)	3.45 ± 0.30[a]	3.46 ± 0.68[a]	2.20 ± 0.51[b]	1.86 ± 0.69[c]	0.98 ± 0.22[c]
Fe (mg/kg)	6.78 ± 1.06[a]	4.20 ± 0.89[b]	2.86 ± 0.68[b]	3.22 ± 1.09[b,c]	2.76 ± 0.91[c]

[a–c]Mean values in rows followed by the same letter do not differ statistically from each other (Tukey, $P = 0.05$).

determined after centrifugation of 10 mL of a homogeneous sample (10 min at 3000 rpm), analyzing the relationship between the volume decanted of yeast and the total volume (da Silva 2003d).

Data analysis

All analytical determinations were performed in a laboratory certified under ISO 9001: 2008 compliance with standards established to ensure the quality of the analytical data obtained.

Statistical analysis of data was performed by analysis of variance with a totally random design using the software INFOSTAT (Infostat 1998). Outliers were removed by applying the Grubbs test with a confidence level of 95%. The comparison of means was performed by Tukey test at a confidence level of 95%.

RESULTS AND DISCUSSION

Total fermentable sugars

No significant differences ($P < 0.05$) were detected in TFS concentrations after the process of clarifying sorghum juice by heating at different pH (Table 1). This is an important aspect since the TFS content in the juice is critical for the fermentation process. Our result is in agreement with a similar study in sweet sorghum, in which Andrzejewski *et al.* (2013) reported that the loss of fermentable sugars (sucrose + glucose + fructose) are insignificant after clarification with temperature, and that only a slight decrease was observed when using pH treatment. During the industrial clarification of sugarcane juice, lime is added to neutralize acidity and avoid acid degradation of sucrose, glucose and fructose during clarification and subsequent thermal evaporation of the clarified juice. The acid degradation (inversion) of sucrose into glucose and fructose is not a concern for sweet sorghum processing to an industrial fermentation feedstock because the goal is to preserve total fermentable sugars. However, the acid degradation of glucose and fructose into organic acids is of concern (Eggleston and Amorim 2007).

Assimilable nitrogen and ammonia

In a similar way to TFS concentration, no significant differences ($P < 0.05$) were found in nitrogen content, indicating that the soluble nitrogen is not affected under the conditions assayed (Table 1). In accordance with these results, it has been reported that no difference in nitrogen content was observed in sugarcane juice clarified by liming (Simpson 1996). Furthermore, the nitrogen levels measured in all treatments were higher than the minimum required for optimum alcoholic fermentation as is shown in Table 2, detailed by Godoy *et al.* (2008). Nitrogen is an important nutritional source due to its role on the yield and kinetic of the fermentation process (Blanco, Quicazán and Cuenca 2012). Most of the nitrogen is metabolized by yeast during the synthesis of proteins for sugar transport, increasing its requirement when the concentration of sugars increases. For this reason, nitrogen deficiency directly affects the rate of sugar consumption by yeast, fermentation rates and yields (Lagunas *et al.* 1982; Jiranek, Langridge and Henschke 1995).

The content of yeast assimilable nitrogen (YAN) regulates the growth and metabolism of fermentation yeast. In the sugar fermentation process the optimal value of YAN is considered to be 60–70 mg/kg (Godoy *et al.* 2008). Yeast cells are subjected to stress during alcoholic fermentation by non-optimum YAN availability. Low YAN is associated with lagging and incomplete fermentation, and sulfide evolution, whereas an excess is associated with unbalanced production of some aromatic compounds (Bell and Henschke 2005). In order to predict YAN-related fermentation problems it is necessary to measure the initial concentration of YAN in the must. It is recommended to supplement deficient musts at the start of fermentation to ensure an adequate population of yeast (Bell and Henschke 2005).

Total phosphorus

Total phosphorus concentration decreased substantially ($P > 0.05$) when the sorghum juice was clarified (Table 1) with increasing pH. When juice clarification was performed at pH 7.0, total phosphorus content in the juice was found to be lower than that recommended for alcoholic fermentation (Godoy *et al.* 2008). This result is consistent with those reported by other authors in sugarcane juice where the formation of precipitates of calcium and magnesium phosphates during the clarification process of sugarcane juice when using heat and $Ca(OH)_2$ have been observed (Simpson 1996; Thai, Bakir and Doherty 2012).

The mechanism of primary flocculation involves precipitation of calcium phosphate *in situ*, where Ca^{2+} ions adsorbed on juice particle surfaces act as nuclei for this precipitation and impurities become incorporated into the coagulum with the calcium phosphate acting as bridges between the impurity particles (Simpson 1996). The success of the primary flocculation is dependent upon the extent of the calcium phosphate precipitation and, therefore, on the concentration of the calcium and phosphate ions. It is generally accepted that phosphate levels of approximately 300 mg/L are required for good clarification (Simpson 1996; Perez Capote *et al.* 2000).

In addition, phosphorus is important for the metabolism of yeast since it is a compositional part of nucleic acids, phospholipids and other active compounds of oxidative degradation and energy exchange processes (ATP, ADP, AMP, NADP) (Steindl 2011). In mixed sugarcane juice it is known that phosphates are one of the major components present as organic and inorganic forms (Zossi *et al.* 2010).

The reactions between lime and phosphates present in sugarcane juice are complex due to the presence of various organic and inorganic constituents in the juice. The impurity content (phosphate, silica, potassium and calcium) in juice is not only dependent on cane variety, maturity and climate, but also the growing conditions and harvesting methods (Naidoo and Lionnet 2000).

Total starch

A feature that clearly distinguishes sorghum juice from sugarcane juice is the higher starch content (Cárdenas, Diez and Quaia 2007). Table 1 shows a significant decrease in the concentration of starch when comparing mixed juice to clarified juices with no significant difference between the individually treated and clarified juices. This may be due to precipitation of the less soluble starch with the insoluble calcium phosphate salts (Prati and Moretti 2010; Thai, Bakir and Doherty 2012) formed by calcium and phosphates naturally present. These same results were reported by Andrzejewski *et al.* (2013), who analyzed the decreased levels of different substances (protein, calcium, phosphate and starch) in clarified juices.

Calcium

Among the undesirable cations present in the juice, calcium is the most important because of its inhibitory effect on the invertase enzyme activity in yeasts and damage to the distillation equipment (Godoy *et al.* 2008; Chotineeranat *et al.* 2010). It is therefore recommended that the Ca^{2+}concentration is as low as possible (Steindl 2011).

In the juices clarified by increasing pH liming it was observed that the residual concentration of calcium also increased with higher pH. When clarified juices were neutralized at pH 7.0, significant differences were found compared to clarifying at pH 5.2 ($P < 0.05$), with a Ca^{2+} increase of approximately 100% (Table 2). A very similar effect was also reported by Andrzejewski *et al* (2013).

Amongst the many reactions and interactions which take place during the clarification of sugarcane juice, the chemistry of calcium phosphates forming during liming is the most important aspect for juice-solid separation. Sugarcane juice contains soluble inorganic phosphates which react with available Ca^{2+} ions from the addition of lime/lime saccharate to form a calcium phosphate precipitate, which take active part in the floc formation. Similar reactions to those reported for sugarcane should occur in sorghum juice clarification (Prati and Moretti 2010).

Magnesium

Magnesium is involved in the ethanol stress tolerance by yeasts (Dombek and Ingram 1986), assuming an optimum concentration of 200 mg/kg during fermentation (Godoy *et al.* 2008). Table 2 shows that the pH and heat did not affect the magnesium levels in clarified juices. Simpson (1996) reported a decrease in the magnesium concentration when lime was added to sugarcane juice. High levels of magnesium may influence clarification as a pH greater than 7.0 increases magnesium precipitation, which could result in an increased calcium content of the solution, possibly affecting the settling rate of the calcium phosphate.

Potassium

As with magnesium, potassium concentrations showed no significant differences ($P > 0.05$) in all clarifying treatments as compared to the mixed juice. It is interesting to notice that the presence of this cation was 10–1000-fold higher compared to the other metal ions analyzed in this work (Table 2). This high concentration could be attributed to soils rich in potassium, or to the amount of potassium containing fertilizer applied to the sorghum field (Romero, Digonzelli and Scandaliaris 2009). Simpson (1996) and Andrzejewski *et al.* (2013), reported similar concentrations with clarified sugar cane and sorghum juice, respectively.

Sodium

Total sodium levels did not vary after the clarification process at different pH (Table 2). In general, the sodium levels could be responsible for the increasing formation of looser flocs during the clarification process. The changes in floc structure, along with the increasing Na^+ concentration, could be associated with the dispersive properties of Na^+ ions. The general increase in size may be due to electrostatic repulsion between calcium

phosphate particles and/or electrostatic repulsion between the microflocs because of the dispersive action of Na^+ ions (Thai, Moghaddam and Doherty 2015).

Iron

The mixed juice had an initial iron content of 6.78 mg/kg and after clarification with pH ≥ 6.0 the Fe concentration decreased by 40–60% ($P < 0.05$) (Table 2). It has been reported in the literature that total elimination of iron occurs when sugarcane juice has been clarified at pH 8.0 (Simpson 1996). Quantification of this cation is important as it is implicated in ribosome biogenesis, protein translation and in the replication and repair of nucleic acids in all eukaryote organisms (Kispal *et al.* 2005).

Zinc

The clarification of sorghum juice leads to a significant decrease of zinc at pH ≥ 6.0 when compared to the mixed juice ($P > 0.05$). When the juices were clarified at pH 7.0 there was a decrease of Zn concentration below recommended levels (2 mg/kg) (Table 2). Simpson (1996) reported the complete elimination of zinc of sugarcane juice after this treatment at pH 7.0. Since zinc is involved in the tolerance of yeast to ethanol stress by reprogramming the cellular metabolism network, it is important to monitor the presence of this cation during the clarification process (Zhao *et al.* 2009).

Copper

At low concentrations, copper is an important micronutrient for yeast. Since the high concentration of this cation has an inhibitory effect on sugar transporters, with a consequent negative effect on the fermentation process, it is recommended to maintain a low but steady copper concentration (Pearce and Sherman 1999). As it shown in Table 2, no significant difference was found in copper concentrations among the different treatments. Furthermore, in all treatments assayed the concentrations were lower than the concentration considered inhibitory (6 mg/kg) (Mrvčić *et al.* 2007).

Manganese

Manganese is implicated in the removal of toxic radicals in yeast cells, where an optimum concentration of 2 mg/kg has been estimated (Godoy *et al.* 2008). The manganese concentration decreased significantly with a pH clarification >6.0, but was not affected when the mixed juice was heated at pH 5.2. An 80% metal concentration reduction was observed at pH 7.0 (Table 2). A similar effect was reported in sugarcane where Simpson (1996) reported the complete removal of the cation when juices were limed at a pH > 7.0.

Fermentative efficiency and cell viability

The fermentation efficiency obtained when using mixed juice (without heat treatment) as substrate was found to be low (75.62 ± 8.38%), probably due to a high natural microbial content in the mixed juice (bacteria, wild yeasts, etc.) which produce deleterious components and competition for nutrients (Valsechi 2005). Due to this high microbial load, heat treatment is an important pretreatment step in order to reduce the negative effect of other microorganisms on the fermentation process. In contrast Andrzejewski *et al* (2013) reported no significant differences between the ethanol yields obtained from the raw and clarified syrup under sterile conditions.

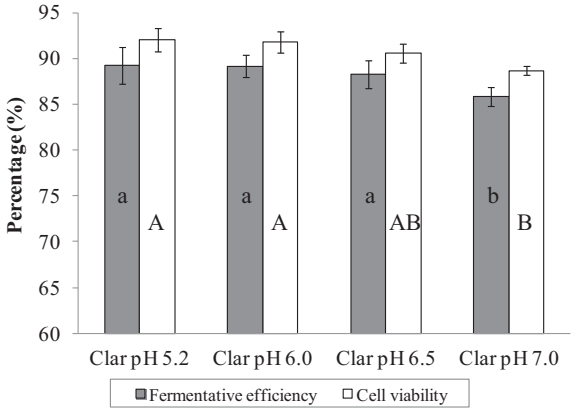

Figure 1. Clarification pH of sorghum juice: effect on the fermentative efficiency and viability of the yeast. Fermentation conditions: 7–8% of treated *Saccharomyces cerevisiae* (sulfuric acid, pH 2.5); must 17–18°brix; incubation 30°C for 12 h. All fermentation studies were run in triplicate. Fermentation efficiency was calculated as the ethanol yield divided by the maximum theoretical yield multiplied by 100.

Due to the low fermentation efficiency found for the untreated mixed juice, this substrate was omitted from the study in order to avoid a wide dispersion of the results. As can be seen from Fig. 1, the only significant decrease in fermentation efficiency was observed when juice clarification was performed at pH 7.0.

Yeast viability is another important parameter to be considered when analyzing a fermentation process. This characteristic depends mainly on the nutritional state, stress conditions and presence of competing microorganisms (Valsechi 2005; Basso *et al.* 2008; Godoy *et al.* 2008). After fermentation, it was observed that the cell viability in untreated mixed juice was relatively low (84.61 ± 2.77%), which is probably due to the presence of microbial contaminants and their metabolites (Valsechi 2005). It is interesting to note that when fermentation was carried out with clarified juices, high cell viability >90% was found for all treatments except the highest pH, where a significant decrease of cell viability was found (Fig. 1). This result indicates a negative effect of clarification under high pH on yeast viability. As shown in Table 2, when the pH increases, calcium concentration increases, whereas manganese, zinc, iron and phosphorus concentrations are reduced. This observation at least partly explains the negative effect of clarification at high pH on cell viability.

To develop optimum conditions for sweet sorghum juice clarification, many different parameters must be considered in order to obtain a proper balance and amount of the individual nutrients essential for yeast metabolism (Birch and Walker 2000; Beltran *et al.* 2005), directed to minimize cell growth and production of fermentation inhibitors in order to maximize ethanol productivity (Mrvčić *et al.* 2007; Steindl 2011). Our results show that a clarification process >pH 6.5 has negative effects on yeast fermentation since the amounts of phosphorus and other micronutrients (Fe, Zn and Mn) are severely reduced while calcium concentration is increased.

Clarification at pH close to 6.0 prevents alkaline degradation of reducing sugars to organic acids, which are harmful compounds for fermentation (Valsechi 2005). Based on the nutritional characteristics of sugarcane juice, authors have recommended a liming at pH 6.2 or 6.3 to work with clarified juice,

with a final pH of 5.6–5.8, but such a low pH may cause corrosion problems in the plant when the juice is concentrated (Steindl 2011).

CONCLUSIONS

The efficiency of the fermentation depends directly on the physiological state and metabolism of the yeast, mediated by nutritional factors and environmental conditions. In the yeast there is an important correlation between fermentation efficiency and stress resistance representing the ability of a yeast strain to adapt to a changing environment and unfavourable growth conditions (chemical, physical and process parameters). Chemical parameters perceived during must fermentation include the concentration of certain nutrients (fermentable sugars, assimilable nitrogen, vitamins, minerals, lipids, oxygen, etc.), and the presence of inhibitory substances (ethanol, acids, sulfite, bacterial substances, etc.). Physical signals include temperature, pH, agitation and osmotic pressure. The most important process parameters are fermentation time, acid treatment of yeasts, alimentation type, the must/yeast relationship, etc.

To optimize the clarification process, it is essential to know the amounts of the individual components in the mixed and clarified juices. In this study we report that magnesium, potassium, sodium and copper levels remain unchanged by the clarification process and are not affected by different pH treatments. In contrast, the levels of other cations such as manganese, zinc and iron studied in this work are almost completely removed (50–80%) at pH clarification >6.0. The clarification process eliminates about 10–15% of starch, and although the amount eliminated is not large, the partial removal of this substance is an important result of the clarification process. We therefore consider an optimal pH near 6.0 for sorghum juice clarification because it does not affect the nutritional quality of the clarified juice giving rise to high fermentation rates as well as high yeast viability.

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Conflict of interest. None declared.

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