Original Article

YEAST COMPOSITION OF SUGAR CANE JUICE IN RELATION TO PLANT VARIETIES AND SEASONALITY

COMPOSIÇÃO DE LEVEDURAS DO CALDO EM RELAÇÃO ÀS VARIEDADES DE CANA E SAZONALIDADE

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ABSTRACT: In the production of the artisanal cachaça, a beverage obtained after distillation of the fermented sugar cane juice, natural starter ferment ("fermento caipira") is utilized, in which crushed corn, rice bran and citric fruit juice are added to sugar cane juice. The primary microbial source is the juice itself, and although the cachaça sensorial quality is recognized when this ferment is utilized, difficulties in the quality control due to the high level of contaminants and extensive preparation periods are reported. In this context, this work aimed the evaluation of the yeast composition and physico-chemical characteristics of the juice extracted from 10 sugar cane RB-varieties during the harvest season in an area under organic management, seeking for information to contribute to the varietal management allowing a faster and efficient ferment preparation. A significant decrease in the yeast numbers in the juice was observed when the maximal point of maturity was reached for the majority of the varieties. However, the proportion (%) of *Saccharomyces* increased with the cane maturity, recommending the early and medium maturity varieties (RB835054, RB835486, RB845210 and RB855156) to be utilized at the beginning of the harvest period for the ferment preparation, which could result in diminished preparation time and faster fermentation. The variety RB845210 is indicated because it also presented high reducing sugar and protein concentrations in the juice. The varietal management can facilitate the production and performance of the natural starter ferment, in order to contribute for the organic cachaça production.

KEYWORDS: Varietal management. Natural ferment. *Saccharomyces.*

INTRODUCTION

The agro industrial system of sugar cane has been going through a period of great growth since 2002 that reminds the gold period of Proálcool, from 1974-1983 (SEBRAE/IEL, 2005). Currently the usage of hybrid, genetically improved, less nutritional-demanding, more resistant to diseases and more productive sugar cane varieties, is predominant, highlighting the RB (República do Brasil) varieties produced formerly by PLANALSUCAR and now by the Universidade Federal de São Carlos. These varieties occupy roughly 58% of the total area cultivated with sugarcane in Brazil (RIDESA, 2010). Several characteristics are searched in order to screen a variety like good productivity expressed as stalks per hectare; high sucrose content; resistance to disease and pests; good adaptation profile to different types of soil and climate; a long period of industrial utilization; and so on. However, the microbial composition of the sugar cane juice from different varieties and its influence over the technological processes for fuel alcohol and cachaça production have been scarcely studied.

In the artisanal process for cachaça production the sources of nutrients are natural, without the addition of chemical (artificial) products (RIBEIRO, 2002). Generally, the small producers preferably utilize the natural ferment ("fermento caipira") rather than mixed natural ferment or the baking yeasts utilized by the most updated producers. The pure yeast cultures, isolated from the regional unities, have presented good results but they demand appropriate techniques for cell multiplication, adequate setup and controlled hygienic conditions (LIMA, 1999). The number of wild yeasts in the juice is found to be low, which may be the cause of failure when the inoculum for the natural ferment is prepared. This preparation results from the growth of the native yeasts found in the sugar cane juice by using treatments that stimulates their multiplication. The primary source of microorganisms, especially the contaminant organisms, is the raw material, i.e. the juice itself (YOKOYA, 1991; LIMA, 1999). The most important microorganisms associated with the sugar cane juice are essentially the ones coming from soil and plants, in which the molds, yeasts, lactic and sporulated bacteria predominate (GALLO; CANHOS, 1991; OLIVEIRA et al., 2007).

Quantitatively, sugar cane juice is basically comprised by water (80%) and total dissolved solids (20%). Among the total solids, sucrose (17%),

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glucose (0.4%) and fructose (0.2%); organic non-sugars, as nitrogenous substances, waxes, fats, pectins, organic acids and coloured materials; and inorganic non-sugars, are found. High water activity, pH range of 5.0-5.5, high concentration of organic and inorganic nutrients and maintenance at 25ºC-30ºC are favorable conditions for a great and diverse microbial community (OLIVEIRA et al., 2007).

The study of the microbiological characteristics of the juice from different sugar cane varieties can help producers of artisanal cachaça in the preparation of a more effective ferment, looking for varieties that possess a higher and/or more diverse yeast community, especially with a greater occurrence of *Saccharomyces,* which was the aim of the present work.

MATERIAL AND METHODS

Sugar cane juice was sampled as following: samples of 10 sugar cane stalks of ten different sugar cane varieties (RB72454, RB835054, RB835486, RB845210, RB855156, RB855453, RB855536, RB867515, RB925211 and RB928064) were collected from an area under organic management in the city of Araras (SP), which belongs to the Universidade Federal de São Carlos, Centro de Ciências Agrárias, in May, September, and December 2007. The stalks were grinded without the straw from the first to the fifteenth internode. They were transferred to a shredding machine (for forage plants), and the resulting dough was subject to pressure in a hydraulic press (250 kg/cm^2) for around 2 minutes while collecting the juice extracted (3 liters) in bottles which were frozen immediately. After each procedure, the equipment was washed with distilled water three times, discarding the initial volume of the pressed juice. Samples were collected in three periods of the year 2007 (May, September and December).

Sugar cane juice samples were evaluated for pH (by direct measurement in a pH-meter), titratable acidity (by titration of 25 mL of the sample with NaOH 0.1N until pH 7.0), phenolic compounds (BSES, 1991), Pol, Brix, purity, reducing sugar, total reducing sugar (FERNANDES, 2003), and protein (by the determination of nitrogen content through Kjeldahl´s method following multiplication by 6.25).

For yeast determination, 10 mL of each sugar cane juice sample were centrifuged at 3000 rpm for 5 minutes; the supernatant was discarded, and the cell mass was washed and centrifuged again under the same conditions. The cells were resuspended in sterile saline solution (NaCl 0.85%), serially diluted in the same solution, and aliquots of 100µL were plated onto WLN, WLD (WLN + 50 ppm cycloheximide) and Lysine Agar for counting (CECCATO-ANTONINI, 2010). The plates were incubated at 30ºC for 3 days. To estimate the number of *Saccharomyces* yeasts (CFU/mL) for each sugar cane variety during the harvesting period and the proportion of *Saccharomyces* yeasts (%) during the sampling periods, the following formula was applied: [(CFU/mL in WLN medium - CFU/ml in Lysine Agar medium)] and [(CFU/mL of *Saccharomyces*) / (CFU/mL in WLN medium)] X 100, respectively.

The results of yeast numbers (transformed to log) were analyzed using the SAS software through the analysis of variance and Tukey´s test for comparison among treatments (periods of sampling, culture media and sugar cane varieties).

RESULTS AND DISCUSSION

The maximal Brix value was reached in September for the majority of varieties, decreasing afterwards. The varieties RB72454 and RB867515 have presented a different trend, with growing values of Brix from May to December, probably because they are medium/late to late varieties. The same observation is valid for parameters Pol and purity (Table 1).

Low temperatures and/or water deficit are demanded for the occurrence of maturity, according to Andrade (2006). This process occurs naturally from April-May, with climax in August, but the maturity may be anticipated by the application of ripeners. This allows getting more productive varieties, improving the technological characteristics of sugar cane, with early maturity (GUIMARÃES et al., 2005; VIANA et al., 2007); however the effectiveness depends on the application period, climate conditions and genetic characteristics of the varieties (LEITE et al., 2008).

For a group of five varieties (RB72454, RB835486, RB855156, RB925211, and RB835054), higher protein levels were observed in September, varying from 0.6% to 1%, while for the others, the juice presented the lowest levels in this month (Table 1).

Concerning the reducing sugars and total reducing sugars, the values decreased for the first parameter and increased for the second, from May to September, exception for the variety RB72454, which presented relatively constant values of total reducing sugars along the period (Table 1).

Table 1. Physico-chemical parameters analyzed in the sugar cane juice of ten varieties cultivated under organic management in different time periods in the year 2007.

Variety	Month/2007	Pol[1]	Brix[2]	Purity[1]	RS[3]	TRS[3]	Protein[1]	Acidity[4]	pH	Phenolic Compounds[5]
RB72454	May	13.74	16.50	83.27	1.62	22.16	0.44	0.36	5.40	60.35
	September	17.46	20.00	87.30	0.72	23.33	0.88	0.16	5.65	91.70
	December	19.69	20.90	94.21	0.23	23.92	0.44	0.17	5.59	253.40
RB835054	May	11.19	14.40	79.13	2.09	12.05	0.63	0.36	5.31	45.85
	September	18.67	19.80	94.29	0.63	21.68	0.56	0.21	5.38	207.50
	December	18.70	18.70	88.56	0.36	16.98	0.44	0.16	5.20	195.45
RB835486	May	10.60	14.30	74.34	2.64	10.39	0.63	0.53	5.28	125.45
	September	21.19	22.40	94.60	0.33	20.93	0.69	0.16	5.55	96.50
	December	19.85	21.40	92.76	0.32	22.53	0.38	0.14	5.54	166.50
RB845210	May	9.89	14.40	68.68	2.86	13.57	0.94	0.54	5.45	33.73
	September	20.18	22.00	91.76	0.63	21.17	0.44	0.14	5.50	224.40
	December	19.03	20.50	92.82	0.21	17.69	0.81	0.12	5.32	217.20
RB855156	May	13.36	15.80	84.56	1.42	14.13	0.56	0.57	5.40	55.50
	September	20.45	21.90	93.38	0.97	20.00	0.88	0.19	5.17	91.70
	December	19.28	21.00	91.81	0.39	21.09	0.44	0.10	5.61	267.80
RB855453	May	11.79	14.90	79.13	2.09	12.25	0.50	0.44	5.42	60.35
	September	20.32	21.40	94.95	0.34	24.09	0.44	0.24	5.48	289.55
	December	16.59	18.80	88.24	0.75	14.86	0.63	0.26	4.95	253.40
RB855536	May	10.44	14.50	72.00	2.56	11.19	0.75	0.62	5.42	50.65
	September	19.98	21.90	91.93	0.41	22.29	1.06	0.31	5.48	108.06
	December	17.08	19.70	86.70	1.09	18.14	0.56	0.22	5.25	214.75
RB867515	May	9.56	13.00	73.54	3.15	10.13	0.56	0.40	5.47	19.30
	September	17.41	19.40	89.74	0.75	19.61	0.44	0.28	5.46	202.07
	December	19.85	21.30	93.19	0.19	16.98	0.44	0.10	5.52	183.40
RB925211	May	12.09	15.80	76.52	2.12	15.32	0.56	0.54	5.40	55.50
	September	21.42	23.00	93.13	0.34	22.56	0.81	0.21	5.46	147.20
	December	17.96	21.30	90.71	0.53	19.81	0.44	0.10	5.58	195.50
RB928064	May	10.12	14.40	70.28	2.40	11.02	0.69	0.68	5.35	43.45
	September	18.11	20.20	89.65	0.66	22.78	0.44	0.29	5.36	209.95
	December	19.20	19.20	92.76	0.40	17.83	0.56	0.19	5.30	166.50

RS= reducing sugars; TRS= total reducing sugars; [1] (%); [2] (º); [3] (g/100 mL); [4] (g H_2SO_4/L); [5] (ppm galic acid)

The higher value of juice acidity was observed in May, decreasing substantially further, in spite of a slight variation in pH (5.0-5.5), regardless the variety. However, there were great variations in the concentration of phenolic compounds among varieties and time periods. For some varieties, the concentration increased continuously from May to December (RB855156 and RB72454), reaching values as much as 250 ppm in December. For other varieties, the concentration increased from May to September, with stabilization or decrease further. The variety RB855453 distinguished itself from the others because it presented high concentration of phenolic compounds along all the period (Table 1).

The microbiological analysis of the sugar cane juice of 10 varieties showed a significant difference in the number of yeasts among them. The variety RB835486 presented the lower number while the varieties RB855156 and RB925211 the highest ones (Figure 1).

Concerning the sampling periods, the number of yeasts was significantly lower in September, showing no significant differences in May and December. The WLN culture medium, as a non-selective medium, showed the highest yeast numbers but regarding the selective media (WLD and Lysine), the numbers of yeasts were higher in Lysine Agar (non-*Saccharomyces* yeasts) (Figure 1).

There was a significant interaction ($p \leq 0.05$) between varieties and culture media. Using selective media (WLD and Lysine Agar), which allow the isolation and enumeration of wild yeasts (resistant to cycloheximide and non-*Saccharomyces,* respectively), more significant differences were observed among the varieties compared to the results with WLN (Table 2).

A variation in the number of yeasts in the sugar cane juice was observed according to varieties and time periods. Higher numbers of yeasts were observed in May for three varieties (RB72454, RB855536 e RB925211); in September, for one variety (RB855156); and in December, for also one variety (RB855156), Table 2.

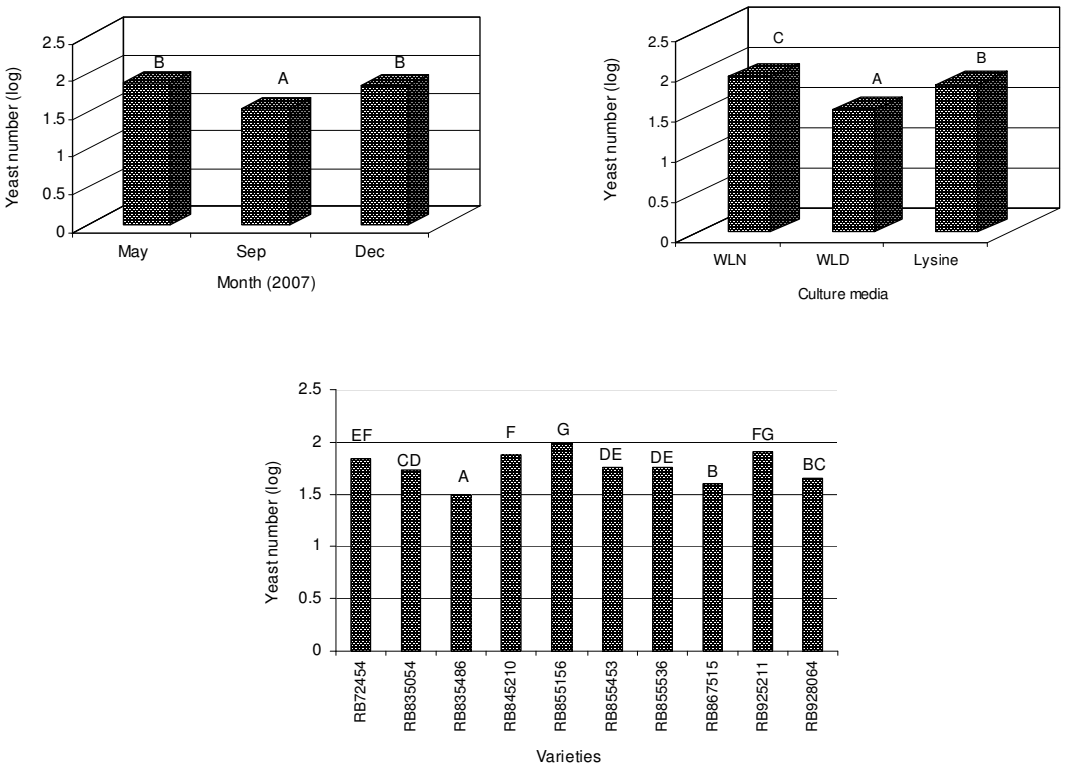

Figure 1. Number of yeasts in the sugar cane juice in relation to the varieties, seasonality and culture media. Different letters over the bars indicate significant differences by Tukey´s test ($p \leq 0.05$).

Table 2. Log of yeast number in the juice from ten sugar cane varieties in three periods of analysis during the 2007 year, using three different culture media for yeast isolation.

	Culture medium			Month (2007)		
Varieties	WLN	WLD	Lysine	May	Sep	Dec
RB72454	1.99 cd	1.58 cd	1.93 de	2.28 e	1.59 cde	1.64 b
RB835054	2.01 cd	1.33 a	1.87 cde	1.93 cd	1.71 e	1.56 b
RB835486	1.67 a	1.28 a	1.50 a	1.37 a	1.41 b	1.67 b
RB845210	2.04 cd	1.60 cd	1.97 e	1.74 b	1.62 de	2.24 f
RB855156	2.10 e	1.85 e	1.98 e	1.80 bc	2.11 f	2.02 d
RB855453	1.91 bc	1.51 bc	1.83 bcde	1.67 b	1.40 b	2.19 ef
RB855536	1.90 bc	1.56 bcd	1.79 bcd	2.26 e	1.14 a	1.86 c
RB867515	1.81 ab	1.27 a	1.69 b	1.96 d	1.53 bcd	1.29 a
RB925211	2.03 cd	1.732 de	1.96 e	2.28 e	1.45 bc	1.98 cd
RB928064	1.81 ab	1.40 ab	1.74 bc	1.48 a	1.43 bc	2.03 de

Different letters in the columns mean significant difference by Tukey´s test ($p \leq 0.05$)

The number of *Saccharomyces* yeasts may be deduced from the results obtained in the media WLN and Lysine Agar. The proportion of *Saccharomyces* in relation to the total number in the varieties seemed to vary along with sugar cane maturity (Figure 2). From May to September, this percentage decreased for the early varieties and increased for the medium/late and late varieties. For the early/medium and medium ones, some varieties behaved as late, and some as early, probably due to the low rainfall conditions observed in the year 2007 (data not shown).

The increased proportion of *Saccharomyces* in the juice seemed to be coincident with the maturity peak of the varieties. Sugar cane juice presents a diversified microbial community that responds differently to the increase in sugar concentration due to maturity process, which probably favours *S. cerevisiae* species, highly resistant to high sugar concentrations (MORAES et al., 1997; PATARO et al., 2000).

The early and early to medium varieties (like RB835054, RB835486, RB845210, RB928064 and RB855156) should be utilized preferably at the start of the harvest for the preparation of natural ferment. In this period, the proportion of *Saccharomyces* yeasts is greater in these varieties, which would result in a shorter preparation time and a faster fermentation rate. During the propagation of the natural ferment, the activity of the microorganisms coming from the sugar cane, air and soil, promotes the medium acidification, resulting in the increase in the alcoholic content along with the disappearance of some yeast species. These changes in pH, alcohol content and increased sugar concentration (due to the constant addition of sugar cane juice) of the broth influence the selection of yeasts that prevail in the cachaça production (MORAES et al., 1997; PATARO et al., 2000). The last authors also showed that when baking yeasts are added as inoculum in the fermentations, they are gradually replaced by the natural microbial community of the sugar cane, i.e. by more adapted yeast strains to industrial conditions.

Significantly lower number of yeasts in September may suggest that a negative correlation between yeast number and maximal point of sugar cane maturity may be present, which is reinforced by the fact that the medium and medium/late maturity varieties like RB72454, RB855536 and RB925211 showed higher yeast numbers in May, when the minimum value of Pol for industrialization is to be reached, around 12-13% (Table 1).

Not only a transient distribution of the yeasts was observed along with sugar concentration gradient. Martini et al. (2010) also verified that the yeasts are concentrated in the upper section (from the eleventh internode to the top) of the sugar cane stalk, indicating a spatial distribution of the yeasts in the cane stalk. The combination of both factors – temporal and spatial – could result in a better management for the preparation of natural ferment.

The variety RB845210 presented also more elevated concentration of reducing sugars and protein (Figure 1), which would be interesting to improve the microbial growth, when juice is successively added for the preparation of the natural ferment. Further tests are demanded, but there is an indication that the varietal management could avoid failures during the preparation of the natural ferment.

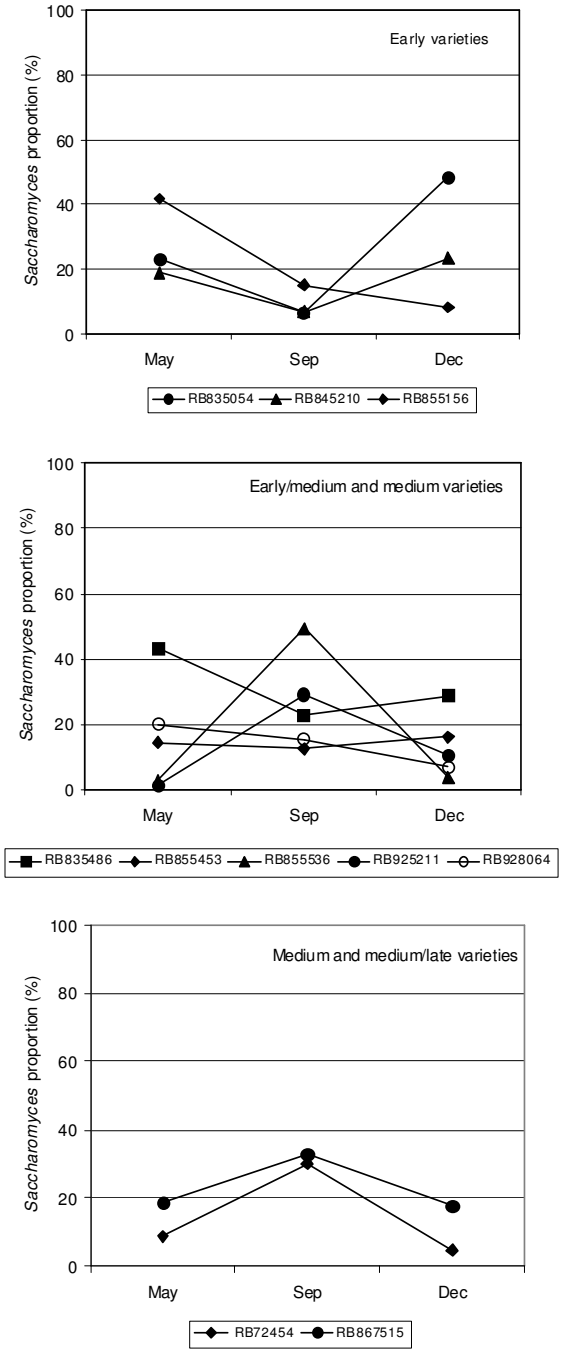

Figure 2. Proportion of *Saccharomyces* (%) in the juice extracted from sugar cane varieties of different maturity cycles during the year 2007.

CONCLUSIONS

A significant decrease in the yeast numbers in the juice was observed when the maximal point of maturity was reached for the majority of the varieties. However, the proportion (%) of *Saccharomyces* increased with the cane maturity, suggesting that these yeasts show a transient distribution along with sugar concentration gradient. Due to the variations in the yeast number and composition along the harvest season, the early and early to medium maturity varieties studied in this work (RB835054, RB835486, RB845210, RB928064 and RB855156) may be indicated for the preparation of the natural ferment, because they presented higher proportion of *Saccharomyces* at the beginning of the harvest.

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RESUMO: Na produção artesanal de cachaça, bebida obtida através da destilação do caldo de cana-de-açúcar fermentado, tradicionalmente utiliza-se o fermento natural ou também chamado de caipira, resultado da mistura de vários ingredientes como milho moído, farelo de arroz e suco de frutas cítricas com caldo de cana. A fonte primária de microrganismos é o próprio caldo da cana, e embora se reconheça a qualidade sensorial da bebida quando este tipo de fermento é utilizado, há alguns inconvenientes como dificuldades no controle de qualidade devido ao alto nível de contaminantes e longos períodos de preparação. Neste contexto, o objetivo deste trabalho foi avaliar a composição de leveduras e as características físico-químicas do caldo em relação às variedades de cana orgânica (10 variedades RB) e à sazonalidade, no intuito de gerar informações para o manejo de variedades que permita o preparo do fermento caipira de forma mais eficiente e rápida. Os resultados indicaram uma diminuição significativa no número de leveduras próximo ao ponto máximo de maturação da cana-de-açúcar para a maioria das variedades. Porém, observou-se que a proporção (%) de *Saccharomyces* aumentou em decorrência da maturação da cana. Sugere-se as variedades precoces e precoces/médias (RB835054, RB835486, RB845210 e RB855156) a serem utilizadas no início da safra para o preparo do fermento caipira, o que poderia proporcionar uma diminuição no tempo de preparo do fermento e uma fermentação mais rápida. A variedade RB845210 é indicada por apresentar também maior concentração de açúcar redutor e proteína no caldo. O manejo varietal pode facilitar a produção e eficiência do fermento caipira, contribuindo assim para a produção de cachaça orgânica.

PALAVRAS-CHAVE: Manejo varietal. Fermento caipira. *Saccharomyces.*

REFERENCES

ANDRADE, L. A. B. Cultura da cana-de-açúcar. In: CARDOSO, M. G. **Produção de aguardente de cana-de-açúcar**. Lavras: UFLA, 2006. p. 25-67.

BSES. Bureau of Sugar Experiment Stations. **The standard laboratory manual for Australian sugar mills**. Brisbane, 1991. v. 2.

CECCATO-ANTONINI, S. R. **Microbiologia da fermentação alcoólica**: a importância do monitoramento microbiológico em destilarias. São Carlos: EDUFSCar, 2010. 105p.

FERNANDES, A. C. **Cálculos na agroindústria da cana-de-açúcar**. Piracicaba: STAB, 2003. 240p.

GALLO, C. R.; CANHOS, V. P. Contaminantes bacterianos na fermentação alcoólica: revisão. **STAB**, Piracicaba, v. 9, n. 1, p. 35-40, 1991.

GUIMARÃES, E. R.; MUTTON, M. A.; PIZAURO JUNIOR, J. M.; MUTTON, M. J. R. Crescimento, acúmulo de sacarose e atividade de invertases em cana-de-açúcar submetida ao tratamento com etil-trinexapac. **Científica**, Jaboticabal, v. 33, n. 1, p. 20-26, 2005.

LEITE, G. H. P.; CRUSCIOL, C. A. C.; SILVA, M. A.; VENTURINI FILHO, W. G. Reguladores vegetais e qualidade tecnológica da cana-de-açúcar em meio de safra. **Ciência e Agrotecnologia**, Lavras, v. 32, n. 6, p.1843-1850, 2008.

LIMA, U. A. **Aguardente:** fabricação em pequenas destilarias. Piracicaba: FEALQ, 1999. 187p.

MARTINI, C.; MARGARIDO, L. A. C.; CECCATO-ANTONINI, S. R. Microbiological and physicochemical evaluations of juice extracted from different parts of sugar cane stalks from three varieties cultivated under organic management. **Ciência e Tecnologia de Alimentos,** Campinas, v. 30, n. 3, p. 808-813, 2010.

MORAES, P. B; ROSA, C. A.; LINARDI, V. R.; PATARO, C.; MAIA, A. B. Characterization and succession of yeast populations associated with spontaneous fermentations during the production of Brazilian sugar-cane aguardente. **World Journal of Microbiology and Biotechnology**, Amsterdam, v. 13, p. 241-243, 1997.

OLIVEIRA, A. C. G.; SPOTO, M. H. F.; CANNIATTI-BRAZACA, S. G.; SOUSA, C. P.; GALLO, C. R. Efeitos do processamento térmico e da radiação gama na conservação de caldo de cana puro e adicionado de suco de frutas. **Ciência e Tecnologia de Alimentos,** Campinas, v. 27, n. 4, p. 863-873, 2007.

PATARO, C.; GUERRA, J. B.; PETRILLO-PEXOTO, M. L.; MENDONÇA-HAGLER, L. C.; LINARDI, V. R; ROSA. C. A. Yeast communities and genetic polymorphism of *Saccharomyces cerevisiae* strains associated with artisanal fermentation in Brazil. **Journal of Applied Microbiology**, Oxford, v. 89, n. 1, p. 24-31, 2000.

RIBEIRO, J. C. G. M. **Fabricação artesanal da cachaça mineira.** Belo Horizonte: O Lutador, 2002. 221p.

RIDESA. Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro. Liberação nacional de novas variedades "RB" de cana-de-açúcar. Ridesa: Curitiba, 2010. 64p. Available at: http://pmgca.dbv.cca.ufscar.br/htm/downloads.php. Access: 21 February 2011.

SEBRAE/IEL. **O novo ciclo da cana**: estudo sobre a competitividade do sistema agroindustrial da cana-de-açúcar e prospecção de novos empreendimentos. Brasília: IEL/NC; SEBRAE, 2005. 344p.

VIANA, R. S.; MUTTON, M. A.; BARBOSA, V.; DURIGAN, A. M. P. R. Maturadores químicos na cana-de-açúcar (*Saccharum* spp) aplicados em final de safra. **Revista de Biologia e Ciências da Terra,** João Pessoa, v. 7, n. 2, p. 100-107, 2007.

YOKOYA, F. Problemas com contaminantes na fermentação alcoólica. **STAB,** Piracicaba, v. 9, n. 6, p. 38-39, 1991.