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Influence on the Productivity of Ethanol by two Strains of Yeasts in must Fermentation of Sweet Sorghum

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***Abstract*—*Currently, the world's energetic matrix is in a modification, resulting from the worsening global warming. In this scenario, Brazil presents the generation of electric energy through Hydro consolidated, as well as the ethanol in gasoline replacement in vehicles. This biofuel is produced from the ethanolic fermentation of sugar cane juice or residual molasses of sugar manufacturing. With the high marketing of "flex-fuel" cars in the country, it is estimated that the demand will increase considerably, being necessary to the expansion of sugar cane plantations, or the use of other raw materials that can complement the production. In this context, the sweet sorghum outstands, which have been studied by presenting short vegetative cycle, high-yield in ethanol per area, co-generation from bagasse burning, the possibility of mechanization, besides being cultivated in sugar cane plantations renewal areas. Considering that sweet sorghum is a relatively new crop, there is a lack of information in the literature concerning the ethanol production process. In this sense, one can cite even the behavior of different strains of yeasts in the ethanolic fermentation of sweet sorghum juice and the characteristics of the resulting wine. In this way, the objective of this work was to assess the influence of yeast strains PE-2 and BG-1 in the must fermentation process of sweet sorghum, it was observed that the raw material offers levels of sugars suitable for industrial processing, and the PE-2 adapt more easily to the must sweet sorghum than the BG-1, producing high alcoholic content.*

Keywords*—*Chemical analysis, fermentative efficiency, alcohol content, bio-energy.

I. INTRODUCTION

With high pollution and with the problem of global warming, humanity has been seeking ways to minimize the impacts on the environment. One possible alternative is the generation of renewable energy such as ethanol because it releases less harmful gases into the atmosphere, besides being energy from inexhaustible natural resources.

Brazil has won the worldwide market for the production of ethanol, being today second place in the ranking of the major producers of this biofuel [1, 8, 14].

The sugar cane industry plays an important role in the domestic market since it is the main source of raw material for biofuel ethanol; not only for production, but also the sugar product that most countries and food industries consume, and therefore depend on marketing [22].

Currently, the production of ethanol from sugar cane is approximately 23 billion and estimated a 2020 production of around 64 billion [32]. For this it is necessary to expand the area to be cultivated, creating more jobs, but on the other hand, it causes many impacts not only to the environment and the economy but also social impacts [13, 23, 33].

Targeting an increase in the production of ethanol, the sweet sorghum can be deployed as a rotation culture at the time of the sugar plantation reform, as it is a plant with a shorter life cycle, providing a greater yield of the land already used for cultivation and minimizing the impacts to the environment [5, 30].

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Sweet sorghum is a promising crop, with high potential for use in ethanol production, complementing the culture of sugar cane and has as its main feature the use of water equivalent to 1/3 of the quantity used in sugarcane [26].

The ethanol productivity is directly linked to the type of yeast used in the fermentation process. These yeasts must be genetically selected, taking into account especially the speed with which the fermentation is performed and the transformation of sugar into ethanol [28].

One of the ethanol production processes (extraction of juice, juice clarification, fermentation, and distillation) fermentation is one of the most important, it is the phase in which the sugar is converted into ethanol by yeast activity, with the release of carbon dioxide and energy in the form of heat [6]. This conversion process is carried out by means of 12 chemical reactions, where each one is stimulated and accelerated by different enzymes, which are very influenced by the atmosphere, depending on factors such as temperature and pH, for example, the enzyme activity will be greater or lesser [15].

Industrial processing of sweet sorghum resembles with the production of ethanol from sugarcane as raw material, using the same sugar cane structure, requiring only a few adjustments [31]. The yeast studied, Saccharomyces Cerevisiae, grows easily in temperatures ranging from 30 to 34° C and in environments with an acidic pH around 4.5-5.0 [16].

In the proposal of sorghum usage as raw material, supplementary to the sugar cane, the ideal requirements of yeasts to ferment this material are still not well defined, under this view, should consider the viability of the yeast cells and their stay in the fermenter through the budding and the viability of the buds formed. In this regard the objective of this work was to assess the influence of yeast strains PE-2 and BG-1 in the must fermentation process of sweet sorghum.

II. MATERIALS AND METHODS

CHARACTERIZATION OF THE EXPERIMENTAL AREA AND CROP CONDUCTION

The The experiment was developed at the SagradoCoração University -USC Experimental Farm, located in the region of Agudos, in the state of São Paulo, Brazil (22º28'S, 48º34'W, at a mean altitude of 530m a.sl.). The climate of the region is subtropical (Cwa-Koeppen). The soil was classified as a typic Hapludox. Its chemical and physical characteristics determined in the 0-20, 20-40 and 40-60 cm layers. Were made the plowing and harrowing at the area, and later planting and fertilization, following the recommendation of 80 kg ha^{-1} N, 40 kg ha^{-1} of P_2O_5 40 kg ha^{-1} of K2O and 20 kg .ha^{-1} S.

The sweet sorghum genotype used was Malibu®, the seeds were hand-planted, 2 to 4 seeds per hole, in an area of 504m², the depth of seeding ranged from 3 to 5 cm. The weed control was accomplished with manual weeding and herbicide application in the sown area.The pesticides used were Atrazine herbicide; Fipronil insecticide to control cutting ants (*Atta* .sp) and pyrethroid for military caterpillar control (*Spodopterafrugiperda*).At 26-days after sowing of sweet sorghum was held topdressing with urea. At 115-days after sowing were harvested 300 sweet sorghum stalks, removing leaves and pointers.

PREPARE JUICE, CLARIFICATION AND OBTAINING THE MUST

The juice was extracted by milling, being characterized as to ºBrix, pH, total acidity, reducing sugars, total reducing sugars [9], Pol and Purity [10].

The extracted juice was subjected to clarification using the simple liming process with pH correction to 7.0 ± 0.2 using calcium hydroxide ($Ca(OH)_2$). The pH value was predetermined by preliminary tests and methodologies to obtain maximum enzyme activity. It was then heated to boiling and transferred to 6000 ml vats containing 2 mg L^{-1} of polyelectrolyte Magnafloc® to accelerate the sedimentation of impurities. Later, the juice was cooled and kept at 90 °C for applying the alpha-amylase enzyme Termamyl 2x (Novozyme® 50188) in dosage 0.020 L.Mg^{-1} of the processed sorghum. It remained in the decanter for 60 minutes for hydrolysis of starch. The supernatant was siphoned, for separating sedimented impurities, resulting in clarified juice. To obtain the musts was performed to standardize the ºBrix to 16° ± 0.5, pH 4.5 ± 0.3 with sulfuric acid (10N) and 32ºC.

FERMENTATION PROCESS TESTING TWO YEAST STRAINS

They have used in the experiment two yeast strains Saccharomyces cerevisiae (PE-2 and BG-1), obtained from industrial units of Jaboticabal –SP region. Its main features are the high resistance to pH shocks, long stops during the fermentation and recycle process. Also have low foaming, high capacity deployment and prevalence and high fermentation yield [17]. The fermentation was carried out in stainless steel vats, in a batch process without yeast recovery, in the proportion of 10% of the yeast in must 6L. The feeding occurred in two stages, the

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first stage with 3L and after 2 hours was added over 3L must.

The fermentation process was monitored by the reduction in ºBrix, with the aid of densitometer, every 4 hours, is considered finished when the concentration of soluble solids was less than or equal to 1, or when values kept stable during 1 hour.

Forty minutes after the last feeding and the end of the fermentation process were performed cell viability analysis, budding and viability sprouts.At the end of fermentation (8 to 10 hours), the wines were recovered by centrifugation at 1650g, 25°C for 5 minutes (HIMAC® CR21G centrifugal). They were evaluated according to ºBrix, pH, Total Acidity [9], Residual Sugars Total Reducers and glycerol [7].

The wines were distilled into micro distiller, the distilled was analyzed in digital hydrometer Antoon-Paar to determine the alcohol content. The fermentation efficiency was calculated according to [10].

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was completely randomized with two treatments and three repetitions. The treatments consisted of two yeast strains, used during the fermentation process (BG -1 and PED- 2). The results were submitted to analysis of variance by F test and the averages compared by Tukey test (5 %).

III. RESULTS AND DISCUSSION FEATURES FROM EXTRACTED SWEET SORGHUM JUICE

In the extracted juice treatments and clarified juice (Table 1), there was a decrease in the concentration of starch of 2156 to 296 mg. L^{-1} after enzyme application and as a result, the increase of total soluble solids (°Brix) from 15.2 to 15.8 comparing extracted juice and clarified respectively. These results are in agreement with those obtained by Gomes (2014) [12] in sweet sorghum juice CVSW80007 genotype on order 2183 mg.L^{-1}. According to Nan et al. (1994) [21], the concentration of starch in the juice can range from 300 to 9900 ppm, with the more common average close to the 2000 ppm.

The results for the pH were significantly different, having presented greater values for the clarified juice. The result can be expected because in the process of clarifying the juice, this is treated with 300 ppm of calcium hydroxide and its pH is raised to 7.0 so that the process of coagulation and precipitation of impurities is optimized. On the other hand, the extracted juice pH can represent the condition of maturation of stalk, as well as its quality (deterioration).

Table 1. Analysis of original and clarified juice of sweet sorghum.

Juices	Extracted	Clarified
ºBrix	15,2A	15,8A
pH	4,9B	6,5A
Total Acidity (g.L^{-1} H2SO4)	2,6A	0,96B
Reducing Sugars (%)	1,74A	1,4A
Total Reducing Sugars (%)	12,2A	12,8A
Pol (%)	9,9A	10,8A
Starch (mg.L^{-1})	2156A	296B
Purity (%)	66A	68A

Measures followed by distinct letters differ significantly by Tukey test to p<0 .05.

Physiological behaviour of yeasts

The results obtained for cell viability at the beginning and end of the fermentation process are presented in Table 2. It was observed average values of 93.22% for BG-1 and 87.86% for PE-2 when the beginning of fermentation was assessed. At the end of the process, there were no significant differences between the strains studied. These values were similar to those obtained by Masson et al. (2015) [18], that evaluating fermentation juice of sweet sorghum, cell viability near 90% at the beginning and end of the process when using FT858 yeast.

Table 2. Cell viability of yeasts.

Yeasts	Cell Viability Start	Cell Viability End
	%	
BG-1	93,22A	92,98A
PE-2	87,86B	94,30A
Test F	3,87**	0,19ns
DMS	7,56	8,37
CV	3,68	3,94

BG-1 and PE-2 commercial yeasts. Measures followed by distinct letters differ significantly by Tukey test to p<0.05. ** significant at the level of 1%. NS: not significant. DMS: Differentiates Significant Minimum. CV: coefficient of variation.

For the budding yeast index (Table 3) there are similar values between the two strains, however at the end of the process there was an expected increase in the level, which according to NAGODAWITHANA et al. (1974), cited by MUTTON (1998) [20], with the intensification of fermentation, temperature increase occurs, leading to an increase in metabolic activity of yeasts, and consequently increasing the budding. Higher values for the PE-2 at the

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end of the process were recorded. According to AMORIM et al. (1996) [2], it was reported that the percentage of budding varies from 5 to 15%, with values above this being indicative of increasing temperature or the low maintenance of yeast in fermentation vats.

Table 3. Budding yeast during the fermentation process.

Yeasts	Budding Start	Budding End
	%	
BG-1	14,43A	20,68B
PE-2	18,61A	33,05A
Test F	1,36ns	11,98*
DMS	9,93	9,92
CV	26,48	16,29

BG-1 and PE-2 commercial yeasts. Measures followed by distinct letters differ significantly by Tukey test to p<0 .05. ** significant at the level of 1%. NS: not significant. DMS: Differentiates Significant Minimum. CV: coefficient of variation.

To the beginning of fermentation (Table 4), the yeast BG-1 presented greater quantities of living sprouts about PE-2. However, at the end of the process, the yeast PE-2 presented greater values concerning BG-1. In this sense, one can infer that the PE-2 yeast adapts more easily to the sweet sorghum than BG-1. This fact confirms the results of the analysis of the budding index. Furthermore, the values were similar to those obtained by Masson et al. (2015) [18] who observed more than 90% shoots viability throughout the fermentation process.

Note that the percentage of viable cells and buds during fermentation is of extreme importance to the maintenance of yeast population, being essential it is monitoring, since, in addition to unwanted metabolites contained in the raw material, toxic compounds to yeasts that are produced during fermentation can accumulate in yeast, promoting viability loss and reducing industrial efficiency [25].

Table 4. Viability of buds

Yeasts	Viability of buds Start	Viability of buds End
	%	
BG-1	93,45A	79,25B
PE-2	85,16B	96,48A
Test F	5,08ns	57,55**
DMS	10,21	6,31
CV	5,04	3,17

BG-1 and PE-2 commercial yeasts. Measures followed by distinct letters differ significantly by Tukey test to p<0 .05. ** significant at the level of 1%. NS: not significant. DMS: Differentiates Significant Minimum. CV: coefficient of variation.

CHARACTERISTICS OF WINE AND FERMENTATIVE EFFICIENCY

In Table 5are presented the results obtained for °Brix, Residual Sugars Total Reducers, pH and total acidity of the wine.A significant difference in the concentration of Residual Sugars Total Reducers present in the wine when compared the two strains of yeasts was shown. Larger values of Residual Sugars Total Reducers indicate inefficiency of the conversion of sugars into ethanol. This result confirms with the determined for ºBrix, which was also greater for the resulting wine fermentation using BG-1. However, the values were higher than those determined by Masson et al. (2015) [18], which verified the Residual Sugars Total Reducers 0.09% order.

The values of pH and total acidity differ from those obtained by Silva et al. (2014) [29] and by Ferreira et al. (2015) [11] that found lower pH (4.2 and 3.9) and total acidity (2 $g.L^{-1}$), researching the influence of emerging and enzymatic treatment respectively. Can assign such differences to the action of the yeast strains used in this study. Note also the largest amount of acids produced by the BG-1 about PE-2. For the production of bio-molecules to happen, there is a conversion of sugars in the must by yeast. This fact is due to the reduction in the amount of ethanol produced [4].

Table 5. Wine Analysis

Wine	ºBrix	Residual Sugars Total Reducers ($g.L^{-1}$)	pH	Total Acidity ($g.L^{-1}$ H_2SO_4)
BG-1	3,3A	0,81A	4,4B	3,0A
PE-2	3,0B	0,78B	4,6A	2,7B
Teste F	16,0**	9,8**	202,3**	28,2**
DMS	0,2	0,1	0,0	0,2
CV	2,6	1,9	0,4	2,3

Measures followed by distinct letters differ significantly by Tukey test to p<0.05. *CV: coefficient of variation

The wine glycerol values (Table 6) showed differences for the yeast strains studied. Glycerol is a second compound that is formed in the same path of ethanol and is inversely proportional to its production. Therefore, the idea of fermentation is the lowest possible production of glycerol [2].Differences in glycerol levels according to BEROVIC et al. (2006) [3], can be explained by the strains studied by temperature, substrate concentration, and the osmotic stress.

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Fermentation carried out resulted in a wine with an alcohol content between the two yeasts, BG-1, and PE-2 (Table 6), showing the consumption of sugars by the yeast in this sweet sorghum juice. RIBEIRO FILHO et al. (2008) [27] observed values of 5.9% of the ethanol from sweet sorghum processing, while Masson et al. (2015) [18] found mean values of 6.3%.

RATNAVATHI et al. (2010) [24] achieved efficiency values in the range of 86.5 to 94.7% off sweet sorghum, using yeast Saccharomyces cerevisiae CFTR 01. Masson et al. (2015) [18] determined values of 81%. The results obtained in this study were similar to those reported in the literature and many factors influence the fermentative efficiency, as the quality of raw material, temperature, pH, yeast strains, among others.

Table 6. Wine Analysis

	Glycerol	Alcohol %	Fermentation Efficiency
BG-1	0,66B	8,2A	92,7A
PE-2	0,69A	8,1A	93,3A
Test F	28,0**	0,7ns	5,9
DMS	0,01	0,5	0,9ns
CV	1,2	2,7	2,8

BG-1 and PE-2 commercial yeasts. Measures followed by distinct letters differ significantly by Tukey test to p< 0.05. F-test: Fisher **DMS method: minimum variation Differs. *** CV: coefficient of variation.

IV. CONCLUSION

Sweet sorghum (Malibu ®) offers sugar levels suitable for industrial processing aiming at the production of bioethanol.

Starch concentration reduced after the clarification of the juice, resulting from the action of alpha-amylase which was applied.

The yeasts PE-2 and BG-1 produce alcoholic high levels when you use sweet sorghum as raw material. Although the PE-2 yeast adapts more easily to the concentrated sweet sorghum than BG-1. PE-2 was considered more adaptable because the budding end of the yeast was greater in 12.37%, as well as the viability of these buds was greater 17.23% about BG-1, i.e. almost total.

REFERENCES

[1] AGOSTINHO, F.; ORTEGA, E. Integrated food, energy and environmental services production as an alternative for small rural properties in Brazil. Energy, United States: Jan. 2012. p. 103-114

[2] AMORIM, H.V.; BASSO, L.C.; ALVES, D.G. Processos de produção de álcool – controle e monitoramento. Piracicaba: FERMENTEC/FEALQ/ESALQ-USP, 1996, 93p.

[3] BEROVIC, M.; PIVEC, A. et al. Influence of heat shock on glycerol production in alcohol fermentation. Journal Bioscience and Bioengineering. Osako; v.103, m.2, p.135-139, 2006.

[4] CAMOLEZ, M. A; MUTTON, Influência de Microrganismos Contaminantes sobre o Processo Fermentativo. Jaboticabal. FCAV/UNESP – Compus de Jaboticabal. Vol. 23 nº5. 2005.

[5] CAVAZZA, L.; VENTURI, G.; ANADUCCI, M. T. Technical possibilities for ethanol crops in Italy. RivistaAgronom, [s.l.], v. 17, p. 238-60.

[6] CEBALLOS-SCHIAVONE, C. H. M. Tratamento térmico do caldo de cana-de-açúcar visando a redução de contaminates bacterianos – Lactobacillus - na produção de etanol e eficiência de tratamento do fermento por etanol. Piracicaba: Universidade de São Paulo, 2009. p. 178.

[7] COPERSUCAR. Manual de Controle Químico da Fabricação de Açúcar. Piracicaba, 2001. 1 CD-ROM.

[8] COSTA, A. C. A. da; PEREIRA JUNIOR, N.; ARANDA, D. A. G. The situation of biofuels in Brazil: new generation technologies. Renewable and Sustainable Energy Reviews, [s.l.], v. 14, n. 9, dez. 2010, p. 3041-3049.

[9] CTC. Centro de Tecnologia Canavieira. Manual de métodos de análises para açúcar. 2005. 1 CD ROM.

[10] [FERNANDES, A. C. Cálculos na agroindústria da cana-de-açúcar. Piracicaba-SP; 2000, 193p.

[11] FERREIRA, O. E. Produção de etanol a partir de sorgo sacarino com tratamento enzimático. Tese (doutorado em microbiologia agropecuária). 2015. 79f. Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2015

[12] GOMES, S. C. M. P. Produção de etanol utilizando mix de sorgo sacrino e cana-de-açúcar em processo de maturação. Dissertação (mestrado em microbiologia agropecuária). 2014. 51f. Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2015

[13] HALL, J; MATOS, S; SEVERINO, L; BELTRÃO, N. Brazilian biofuels and social exclusion: established and concentrated ethanol versus emerging and dispersed biodiesel. Journal of Cleaner Production, United States v. 17, suppl. 1, p. S77- S85, Nov. 2009.

[14] LA ROVERE, E. L.; PEREIRA, A. S.; SIMÕES, A. F. Biofuels and Sustainable Energy Development in Brazil. World Development, [s.l.], v. 39, n 6, p. 1026-1036, jun. 2011.

[15] LIMA, U. A; BASSO, L. C; AMORIN, H.V. Produção de etanol In: Biotecnologia. São Paulo: E. Blucher, v.3, p.1-43, 2001.

[16] LIMA, U.A. et al (Coord.). Biotecnologia Industrial: Processos Fermentativos e Enzimáticos. São Paulo: Edgard Blücher, 2001. 593 p.

[17] LNF. LNF Latino Americana. Leveduras selecionadas. 2013. Disponível em:

https://dx.doi.org/10.22161/ijaers.68.8 *ISSN: 2349-6495(P) | 2456-1908(O)*

http://www.lnf.com.br/leveduras.html. Acesso em: 27 ago. 2015.

[18] MASSON, S.I. Produção de bioetanol a partir da fermentação de caldo de sorgo sacarino e cana-de-açúcar. Jaboticabal: Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinária. Ciência Rural, v.45, n.9, set, 2015.

[19] MCBEE, G.G., MILLER, F.R. 1993. Stem carbohydrate and lignin concentrations in sorghum hybrids at seven growth stages. CropSci. 33, 530-534.

[20] MUTTON, M. J. R. Avaliação da fermentação etanólica do caldo de cana-de-açúcar (Saccharumspp) tratadas com maturadores químicos.1998, 178p., Tese (Livre Docência– FCAV, UNESP). Jaboticabal, 1998.

[21] NAN, L.; BEST, G.; CARVALHO NETO, C.C. Ethanol production from sweet sorghum. In: Integrated energy systems in China – The cold Northeastern region experience. FAO, Roma, 1994. Disponível em: <http://fao.org/docrep/T4470E00.htm>. Acesso em: 14 Jun. 2019.

[22] NEVES, M. F.; CONEJERO, M. A. Estratégias para a Cana no Brasil. 1 ed. São Paulo: 2010. p. 16,19,20,91.

[23] PHALAN, B. The social and environmental impacts of biofuels in Asia: an overview. Applied Energy, [s.l.], v. 86, n. 1, p. S21-S29, nov.2009.

[24] RATNAVATHI, C.V. et al. Study on genotypic variation for ethanol production from sweet sorghum juice. BiomassandBioenergy. Kidlington; v.34, n.7, p.947-952, 2010.

[25] RAVANELI, G, C. Qualidade da matéria-prima, microbiota fermentativa e produção de etanol sob ataque de Mahanarvafimbriolata em cana-de-açúcar. 105p., 2010. Tese (Doutorado) Faculdade de Ciências Agrárias e Veterinárias. FCAV/UNESP. Jaboticabal, 2010.

[26] REDDY, B.V.S. et al. Sweet Sorghum- a potential alternate raw material for bio-ethanol and bio-energy. SorghummilletsNewslett, Patancheru, India; v.46v.10, p.79-86, 2005.

[27] RIBEIRO FILHO, N.M. et al. Aproveitamento do caldo do sorgo sacarino para produção de aguardente. Revista Brasileira de Produtos Agroindustriais. Campina Grande; v.10, n.1, p.9-16, 2008

[28] SANTOS, F; BORÉM, A; CALDAS, C. Cana-de-açúcar: bioenergia, açúcar e álcool – tecnologia e perspectivas. Viçosa, Minas Gerais: 2010. In: VASCONCELOS, J. N. Fermentação etanólica. São Paulo, SP, Editora Edgard Bluncher, 2010. v. 1, c. 15, p. 401-434.

[29] SILVA, V. F.N. Avaliação de parâmetros experimentais do fracionamento do bagaço de cana-de-açúcar na obtenção de etanol celulósico e lignina. 2014. 145 f. Tese (Doutorado em Ciências) – Escola de Lorena da Universidade de São Paulo. 2014.

[30] SIPOS, B.; RÉCZEY, J.; SOMORAI, Z. ; KÁDÁR, Z.; DIENES, D.; RÉCZEY, K. Sweet sorghum as feedstock for ethanol production: Enzymatic hydrolysis of steam pretreated bagasse. Applied Biochemistry and Biotechnology, EUA: v. 153. 2009. p. 151-162.

[31] SRINIVASA RAO, P. et al. Sweet Sorghum for bioful and strategies for its improvement. Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, 2009. 80p. (Boletim Técnico, 77).

[32] SORDA, G.; BANSE, M.; KEMFERT, C. An overview of biofuel policies across the world .Energy Policy, United States: v. 38, issue 11, p. 6977-6988, Nov. 2010.

[33] VAN DER HORST, D.; VERMEYLEN, S. Spatial scale and social impacts of biofuel production.Biomass and Bioenergy, [s.l.], v. 35, n. 6, p. 2435-2443, jun. 2011.

[34] TEIXEIRA, C. G.; JARDINE, J. G.; BEISMAN, D. A. Utilização do sorgo sacarino como matéria prima complementar à cana-de-açúcar para obtenção de etanol em microdestilaria. Ciência e Tecnologia de Alimentos, Campinas: v.17, n.3, p.248-251, dez 1997.