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Effect of Biomass Reduction on the Fermentation of Cider

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ABSTRACT

The aim of this work was to determine the influence of biomass reduction in the cider processing and the quality of the fermented product made on laboratory scale, but in the same conditions usually found in factory units. The must, made with apples of the Gala variety, depectinized and transferred to 500 mL-fermenters, was inoculated with $2.0x10^6$ cfu/mL of natural or commercial yeast, and at each 12 hours biomass was removed by centrifugation in one flask of the experimental set, and the must was left to ferment. All seven flasks of the fermented beverage were analyzed for 20-26 days after the inoculation, and the results showed that the best moment for biomass removal was 1.5-2.0 days of fermentation, leading to a product with significant residual sugars content, a low alcoholic degree, and with a fruity flavor. In addition, it was possible to practically eliminate all nitrogen, which was important to control the natural gasification. It was quite clear that biomass removal could be a very efficient treatment in order to obtain a sweeter and more pleasant alcoholic beverage, a better cider.

Key words: Cider, fermentation, technology

INTRODUCTION

Apple juice fermentation is the technological basis for the fruit wine, which is the raw-material for Brazilian cidermaking. This fermented beverage receives, usually in another processing unit, sucrose and CO_2, is stored at room temperature after a thermal treatment. Processing of cider in Brazil, thus, comprehends apple juice fermentation to dryness and the final making up steps in a traditional way (Nogueira and Wosiacki, 2005). Only one factory in this country is planned to make cider from the apple to the final product in the same plant.

The production of cider in Brazil began when European immigrants tried to implant the pomiculture in the Southeast of the country. The first place chosen, in the state of São Paulo, did not show favorable climatic conditions for apple production, and so the production costs were high and the productivity low. The industries installed there for cider processing, faced the problem to work with a high cost and inadequate raw material (Wosiacki et al., 1997). At the beginning of the 1970's, the apple cultivation was introduced in the Southern States, where the climate was more favorable, with a rapid development of the orchards, reaching a production of 1,000,000 ton/year of edible fruits in less than 30 years

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(ABPM, 2004). The classification procedure for such fruits causes the emergence of a low commercial value by-product that may represent from 10 to 20% of the total apple production, or even up to 30% (Kennedy et al., 1999). For processing, usually 2/3 of the rejected fruits may be used, the so-called industrial apples. One of the best possibilities to add economical value to these fruits is using them as raw-material for cidermaking (Paganini et al., 2004). This is already done by some factories in the vicinity of the apple fields that process the apple juice and sell the fermented apple must to the factories in São Paulo which then benefit it in order to make up the cider.

Cider is usually defined as a gasified and low alcoholic beverage made from apple juice extracted through milling and pressing procedures, followed by two microbiological processes: the alcoholic fermentation and the malolatic conversion (Laplace et al., 2001). The French method includes the oxidative fermentation due to the fact that apiculate yeast, with a low alcoholic fermentation activity, produces fruity and floral smells, thus improving the quality of the final product (Le Quéré and Drilleau, 1998). The alcoholic fermentation can be carried out with commercial inoculum or natural flora from the fruit epidermis. According to Michel et al. (1990), the alcoholic fermentation of the French cider, considered one of the best in the world, has special features: it is slow, from one to two months, partial, with residual sugars and in which the alcohol degree does not exceed 5%, and mixed, because it is carried out by natural microflora (Nogueira and Wosiacki, 2005) but without any make up operations, such as the addition of sugar or sulphite.

These characteristics are hardly observed in Brazilian processing in which high temperatures speed up the fermentation rate, the extremely high content of sulphite (above 350 mg/L) affects all kinds of yeasts and it is always necessary to add commercial inoculum. Because sugar exhaustion is the sole criterion to stop the process and sucrose is always added, above of 50 g/L, dry cider will never exist (Nogueira et al., 2003).

The main microorganisms found in the fermentation of cider comprehend more than 500 strains of yeasts, isolated from the must and from the equipments, both in farm and in industrial factories in Britain and Normandy, France. The strains *Saccharomyces cerevisiae* var. *uvarum* is always the main alcoholic fermentation yeast found (Le Quéré and Drilleau, 1993, 1998; Michel et al., 1988). The main microorganisms in natural fermented cider, according to Michel et al., (1988), are: Yeasts (*Brettanomyces sp., Hanseniaspora valbyensis, Metschnikowia pulcherrima, Saccharomyces cerevisiae* var. *uvarum),* Lactic acid bacteria (*Lactobaclillus brevis, Leuconostoc mesenteróides, Leuconostoc oenos)* and Acetic acid bacteria *(Acetobacter aceti, Gluconobacter oxydans).*

In the French traditional method, the slow fermentation has been considered necessary to maintain the quality, because if the process is too fast, desirable fruity smell notes are overlapped by an undesirable strong yeast flavor that remains in the end-product, although this may be decrease a little in the maturation phase (Lequéré, 1991). According to Drilleau (1991), some compounds, such as diacetyl and acetoin, produced in high amount due to high temperatures and to excess of yeast population, may largely contribute to the low quality of the final product with an unpleasant smell.

Usual environmental conditions in Brazil lead to a rapid fermentation (7-15 days) with the exhaustion of sugars (Nogueira et al., 2003). Therefore, it is possible to find a high amount of unpleasant flavors due to the fast fermentation as well as to a lack of the desirable fruity smell notes caused by the absence of oxidative yeasts (Drilleau and Lequéré, 1998) which were eliminated by the action of the SO_2, usually added at the beginning and during the process.

According to Nogueira and Wosiacki (2005), in the sensorial analysis of juices and fermented alcoholic beverages of 14 apple varieties, values received concerning the attribute **aroma** were between "***I liked it slightly***" and "***I liked it moderately***" for samples of juice and "***I disliked it moderately***" and "***Indifferent***", for samples of wine (dry), which suggests the lack of a pleasant flavor in the fermented samples.

The present work was done with the aim to determine the effect of a biomass reduction in the fermentation process itself and in the quality of an apple wine in a laboratory scale, using similar conditions as in the industrial units.

MATERIAL AND METHODS

Raw material

Commercial and industrial apple samples were obtained at the local market and in a fruit processing unit, respectively, both from the most important commercial variety, namely Gala. The raw material was previously maintained in a controlled atmosphere and showed the usual standard of quality.

Must preparation

The fruits were milled (Processing Metvisa, Type MPA) and pressed during 5 minutes at 3 ton/cm^2 (Hydraulic Press Eureka, Hoppe Ind. Ltda., Brazil), then pectic substances were removed by decanting after an enzyme treatment with Pectinex 3XL during 2 h at 45ºC (3 mL/hL). Must was freeze stored in plastic vessels when not used immediately after processing.

Effect of sulphiting

Different lots of commercial and industrial apples from the Gala variety were processed to obtain a must which was divided into two vessels. In the first lot, 300 mg/L of sulphite (SO_2) was added and after 12 h at 10ºC, an aliquot was removed in order to count the microorganisms in a selective synthetic medium. In the second lot, the control, no treatment was performed and an aliquot was also removed for analysis. The sterilized mediums were PDA for the fermentative yeast (Becton Dickinson Microbiology Systems) and L-lysine for the oxidative yeast (Wickerham, 1951). The colonies of the oxidative yeasts and fermentative yeasts were counted after incubation for 24 h at 25ºC and 24 h at 35ºC, respectively.

Biomass removal

Depectinized must (450 mL) were poured into seven small-scale fermenters (500 mL) and each one was inoculated with $2.0x10^6$ cfu/mL and incubated at 25ºC. The first flask (F-1) was the control, or time zero. At 12 h, the population of yeast of the second flask (F-2) was reduced by centrifuging at 2000 rpm (Centrifuge Fanem Ltda, Model 214) from 20 minutes, after which the centrifuged must was re-inoculated, if necessary, and the fermentation started again. The same procedure was done with the other flasks, one by one, each 12 h, up to 72 h (F-7). The fermentation in all the treatments was stopped after 20 days, at the same time. The parameters analyzed were maximum velocity, calculated from the loss of weight of the fermenter by CO_2 release (dCO_2/dt), determined by automatic measurements of the weight loss of the fermenters every 60 minutes (Bely et al., 1990) and reducing sugars, total acidity, total phenols, ethanol, total nitrogen and N α-amine determined according to official methods (IAL, 1975; RSK, 1987; Tanner and Brunner, 1985).

Fermentation of apple wine with biomass removal

The apple must was depectinized with 3 mL/hL of pectinase (Pectinex 3XL, Novozymes do Brasil) during 3 h at 25°C and transferred to previously sterilized (120°C/20 min.) 250 mL fermenters. The inoculum consisted of the natural micro flora of the fruit or of the commercial yeast *S. cerevisiae* in the active dry form (Uvaferm CK - Danstar Ferment GAC, Denmark), which was rehydrated and used with an initial population of $2.0x10^6$ cfu/mL. The kinetics of fermentation were monitored by weight loss of the fermenters due to the release of CO_2. The yeasts were counted in a Neubauer device (Xb-k-25, SMIC, China) during the fermentation (Lee et al., 1981). The biomass was removed from both fermenters at the end of the exponential phase of growth, around 48 h of incubation, maintaining a yeast population of $2.0x10^6$ cfu/mL in order to start a new fermentation.

Quality profile of the final fermented beverage

In all samples, residual sugar and nitrogen, as well as produced alcohols were determined with official methods (IAL, 1985).

RESULTS AND DISCUSSION

Quality profile of raw material

Table 1 shows some physicochemical features of the apple must obtained from samples of the Gala variety, which are in agreement with the figures already available concerning commercial and industrial fruits (GTM, 2005). The content of sugars (11.96 ± 0.15 g/100mL) classified this must as elite (more than 11.5 g/100mL) for cider production (Beaulieu, 2000). The total acidity, calculated as malic acid, of 0.125 ± 0.01 g/100mL characterized this must as low acid (less than 0.45g/100mL), which was usual in Brazilian raw-material, apples without any commercial value as

table fruit (Beech, 1972; Paganini et al., 2004). The total nitrogen content (90.00 ± 8.15 mg/L) could be considered as an usual value for fermentation, because values below 50 or above 130 characterized the musts as of low or high level, respectively (Nogueira, 2003). The value of phenol compounds, 434 mg/L, classified the must as "bitter-sweet " but it was still very low to interfere with the quality of the wine especially if compared with the values reported about European industrial apples that could reach up to 7000 mg/L (Guyot et al., 2003; Sanoner et al., 1999). However, it was quite above average value (300 mg/L) of Brazilian musts (Vieira et al., 2004) and that suggested by Drilleau (1991), of 200 mg/L as the lowest level of phenol compounds in a bitter must.

Table 1- Same quality feature of the must obtained from the apples samples of the Gala variety.

Physicochemical attributes	Average amount
Total reducing sugars, (g/100mL)	11.96 ± 0.15
Total acidity, (g/100mL)	0.125 ± 0.01
pH	3.92 ± 0.01
Total phenols, (mg/L)	462.20 ± 9.47
Total nitrogen, (mg/L)	90.00 ± 8.15
Alfa-amine nitrogen, (mg/L)	70.00 ± 20.05

Influence of sulphiting on the apple must

In the fresh must obtained in a laboratory scale the initial yeast population was $3.0x10^4$ cfu/mL, but in industrial units such figures could reach up to 10^6 or 10^8 cfu/mL, due to contaminated fruits. Such a number can be explained because the processing of cider occurs in the summer with an average temperature of 25°C and the raw-material has low acidity and low tannin contents. It is usual to add sulphite as soon as the juice is extracted in order to eliminate undesirable microorganisms, but this procedure affects also non-*Saccharomyces* and some bacteria involved with the formation of pleasant flavors (Lea and Drilleau, 2003).

Table 2 shows the initial population of fermentative yeast in the must made with commercial apples from the Gala variety as $6.50x10^4$ cfu/mL, which is similar to the former figures, but when the must is obtained from the so called industrial fruits, in fact those that were removed during the classification process due to some defects including contamination, the yeast population was quite high, reaching $5.50x10^6$ cfu/mL. The added sulphite (300 mg/L) caused an increase in the populations of fermentative yeast, up to $1.20x10^5$ and $8.95x10^6$ cfu/mL in the must obtained from commercial and industrial fruits, respectively. The pH of the must was 3.92. In this assay the counting was done after 12 h at 10ºC, and the growth in the presence of sulphite confirmed the resistance of the yeast *Saccharomyces* sp. The initial populations of oxidative yeast in commercial and industrial must were of $1.35x10^7$ and $2.15x10^8$ cfu/mL, respectively, which characterized a higher population of oxidative than of fermentative yeast. The added sulphite reduced the population of these yeasts to $3.90x10^6$ and $7.80x10^7$ cfu/mL, respectively. The results were similar to those found by Dueñas et al. (2002) in the cidermaking in Astúrias, Spain. Such effect, indeed, can be more significant at lower pH levels in the must, which can be achieved with blending with more acidic must or even with the addition of malic or citric acid because the free form of sulphite is more active (Lea and Drilleau, 2003).

Table 2 - The effect of sulphite addition (300 ppm) after 12 hour at 10ºC on the amount of microorganisms in must prepared with commercial and industrial fruits.

Raw material	Components of the natural flora, cfu/mL			
	Fermentative		Oxidative	
	- SO_2	+ SO_2	- SO_2	+ SO_2
Commercial	$6.50x10^4$	$1.20 x10^5$	$5.50 x10^6$	$8.95 x10^6$
Industrial	$1.35 x10^7$	$3.90 x10^6$	$2.15 x10^8$	$7.80 x10^7$

Some of these sulphite sensitive oxidative yeasts produce pleasant flavors and a reduction in their initial population directly affects the synthesis of these compounds, causing a decrease in quality. As sulphite favors the growth of the fermentative yeasts, they rapidly dominate the medium, making the population of oxidative yeasts even lower than before (Herrero et al., 2003), with even higher losses in quality.

Best moment for biomass removal

The biomass reduction, usual step in the processing of French cider, consists of a partial removal of the yeast population by flotation, filtration or centrifugation (Lequéré, 1991). Fig. 1 shows the right moment of this removal during the log phase up to the beginning of the stationary phase. The yeast population was withdrawn until the residual amount was the usual to start the next growth in each fermentation flask (F-1 to F-7), covering a period of 72 h.

The best time for biomass reduction was within 1.5 - 2.0 days after inoculation (Table 3). It was possible to find, after a 20-day fermentation, a content of residual sugars 6.56 and 4.13 g/100mL and a low alcohol degree of 2.55 and 3.00 ºGL in the assays where the biomass was withdrawn after 1.5 and 2.0 days, respectively. In fact, the fermentation speed remained low, circa of 0.44 and 0.35 g/L.d^{-1}, with an easy operation control. In the referential assay, without biomass reduction, at the end of the fermentation, a total sugar content of only 0.21 g/100mL and an alcoholic degree of 6.35ºGL (Tab. 3) were found, which was a typical feature of the industrial process of total sugar fermentation, down to dryness.

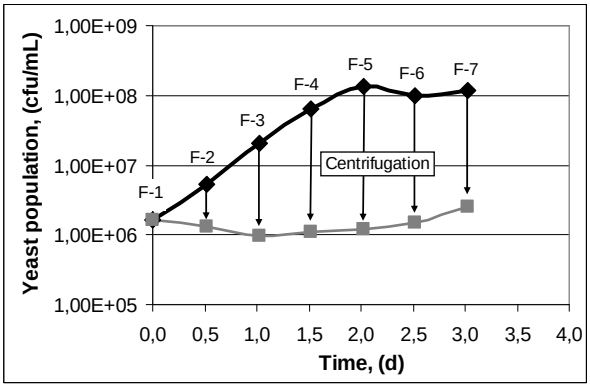


Figure 1 - Identification of the moment of biomass removal and the initial and final microbial population at each time

When the reduction of the yeast population was performed before 1.5 days or after 2.0 days of fermentation, this same satisfactory effect was not achieved. In the first case, the yeast did not grow enough to consume all the nutrients, especially nitrogen that remained in a level of 96.21 mg/L (Tab.3), which was considered high (Nogueira, 2003), leading to a microbiologically unstable product. Also, at the beginning of fermentation, important differences in the residual sugar and final alcoholic degree are not usually observed, as in this study, where the values of the sugar and alcoholic degrees were similar to those in the control. After two days of fermentation, almost all the nutrients, including sugar, had already been consumed, and only a low level of sugars and a high alcoholic degree remained, both next to the figures found in the control essay, and it was too late for the withdrawing process (Table 3).

Influence of biomass removal on the fermentation process

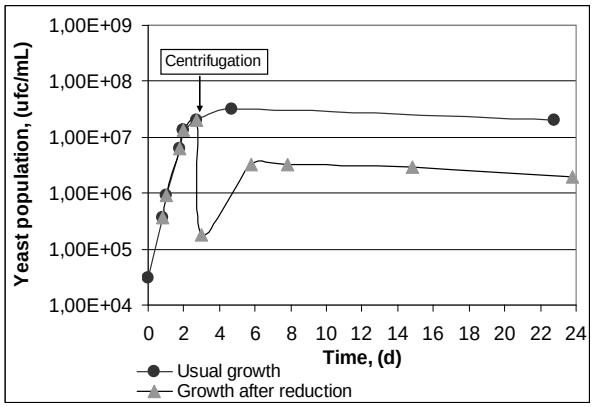
Fig. 2 shows the growth of microorganism in the natural fermentation (2A), evolution of the total sugars (2B) and evolution of the total nitrogen (2C), as well as the respective modifications due to

the biomass removal after 2 days of fermentation. Natural flora reached the maximum population of $3.24x10^7$ cfu/mL after 4.7 days of growth (Fig. 2A) and the stationary phase was followed during the next 23 days until the exhaustion on the fermentable sugars (Fig. 2B), when the nitrogen level was quite high, around 55 mg/L. This fermentation time was considered long in the processing of Brazilian cider and in practice such values could be quite different, because the raw-material might have a large microbial population due to sanitary conditions. The end of the log phase was defined as the best moment for the reduction of biomass and the new yeast growth reached the maximum population of $3.20x10^6$ cfu/mL after 3.8 days of incubation (Fig. 2A), remaining stable, with sugar at 4.06 g/100 mL during 25 days of fermentation (Fig. 2B) and a nitrogen level of less then 30 mg/L (Fig. 2C).

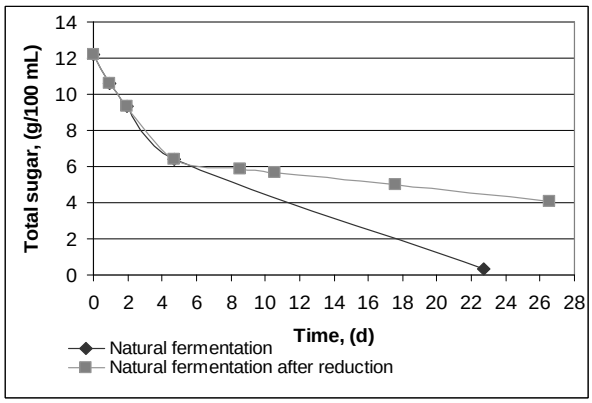
Table 3 - The effect of the moment of biomass reduction on the quality profile of the apple wine

Days of fermentation	**Total sugar g/100mL**		**Total N, mg/L**		**V_{max}, g/L.d^{-1}**	**Ethanol, ^{o}GL**	
	Initial*	Final**	Initial*	Final**		Initial*	Final**
Control	12.05	0.21	165.26	47.19	5.96	0.0	6.35
0.5	11.52	0.28	153.54	73.14	4.07	0.40	6.70
1.0	10.80	0.24	96.21	45.21	3.26	0.50	6.45
1.5	**8.71**	**6.56**	**47.74**	**51.81**	**0.44**	**1.65**	**2.55**
2.0	**6.58**	**4.13**	**49.18**	**53.92**	**0.35**	**2.65**	**3.00**
2.5	4.39	1.57	49.20	47.17	0.24	3.75	4.90
3.0	2.88	3.03	50.74	49.37	0.14	4.90	5.30

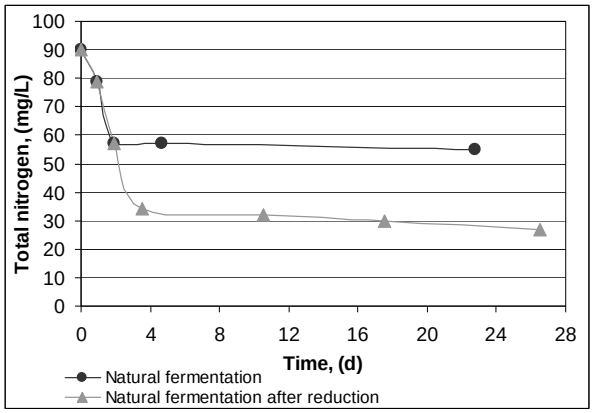
* Initial: at the moment of the biomass reduction; **Final: after 20 days of fermentation.



2A



2B

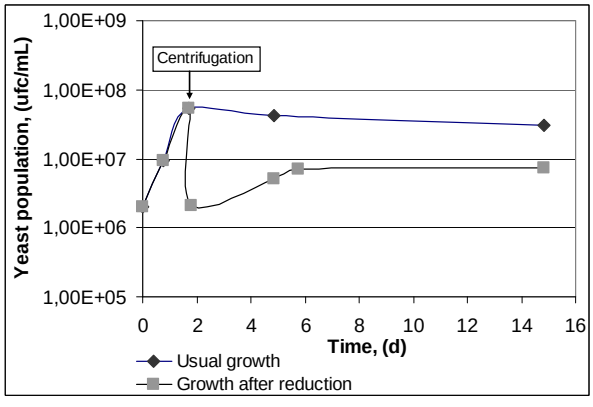


2C

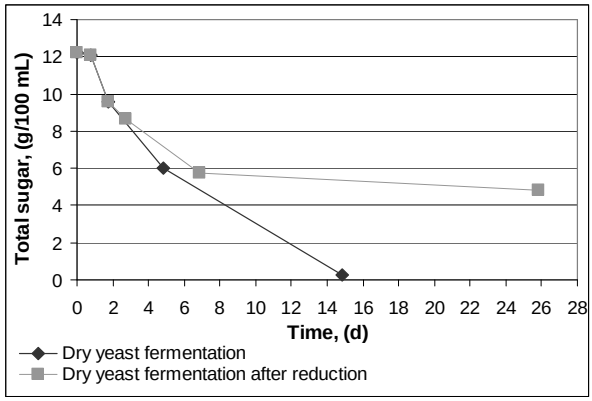
Figure 2 - The influence of the reduction of biomass in fermentation parameters: (2A) growth of natural flora; (2B) consumption of total sugars; (2C) consumption of total nitrogen

The maximum population in the must inoculated with dry yeast (Fig.3) was $5.40x10^7$ cfu/mL after 1.73 days of fermentation (Fig. 3A); the residual sugar content was 0.24 g/100mL after 15 days (Fig. 3B) and the level of nitrogen was higher than 60 mg/L (Fig. 3C). With the biomass reduction at the end of the growth phase, the maximum population was $7.0x10^6$ cfu/mL, after of 3.75 days (Fig. 3A), residual sugar was stable around 4.85 g/100mL after 26 days of fermentation (Fig. 3B) and the nitrogen level dropped down to 20 mg/L (Fig. 3C).

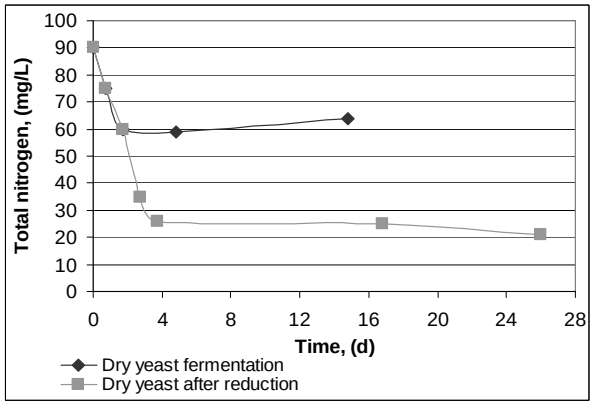
The processing with biomass removal leads to a maximum population lower than the one found when this operation is omitted, due to the consumption of all nutrients (vitamins and nitrogen compounds) by the yeasts in the first growth (Drilleau, 1990). In cider processing, the nitrogen is the main limiting nutrient of the alcoholic fermentation, since it is essential for protein synthesis (Goñi and Azpilicueta, 1999), but in fast fermentation, it is not totally consumed.



3A



3B



3C

Figure 3 - The influence of the reduction of biomass in fermentation parameters: (3A) growth of yeast; (3B) consumption of total sugars; (3C) consumption of total nitrogen

This can be seen in the Figs. 2C and 3C, where 30 and 35 mg/L of nitrogen were consumed, respectively, in the natural and in the inoculated essays. Residual nitrogen could explain the microbiological instability of ciders that were gasified in the bottle or did not receive a thermal treatment, with alteration in the quality of the product and eventual bottle blow up (Nogueira, 2003). With the biomass reduction, in the same Figs. 2C and 3C, such a situation was not observed; since nitrogen was totally consumed, remaining only the not assimilable one, which in apple wine could vary from 20 to 40 mg/L.

Impact of biomass removal on the quality of the final product

At the next growing in a must now with less nutrients, the speed became slower than before (Table 4). At the industry, the processing time for the exhaustion of the sugars is of 7 to 15 days and in this experiment the time was 23 and 15 days, respectively, for natural or inoculated fermentation.

The assays with microflora removal contained 4.0 g/100mL of residual sugar after 27 days, that mean a slow process (0.30-0.40 $g/L.d^{-1}$) easy to control. Such a sugar level, especially fructose, is important for the quality of the product due to the high preference of Brazilian consumers for sweet beverages. Thus, industrial cider is corrected with sucrose which has a higher glicemic index and affects the final flavor

Table 4 - Characteristics of the products obtained by natural and inoculated fermentation and with and without biomass reduction.

Innoculum	Without biomass removal					With biomass removal				
	Time days	Residual sugar, g/100mL	Ethanol, ^{o}GL	Total N, mg/L	Rate, g/L.d^{-1}	Time days	Residual sugar, g/100 mL	Ethanol, ^{o}GL	Total N, mg/L	Rate, g/L.d^{-1}
Natural (none)	23	0.50	7.00	55.00	2.60	27	4.06	2.30	26.96	0.30
Commercial	15	0.24	7.30	64.33	3.50	27	4.85	4.20	21.60	0.40

When the biomass removal step is included in the fermentation, it is possible to observe fruity aroma notes in the final product, a good quality marker that is not present in usual fermentation. As Brazilian processing is carried out basically with *Saccharomyces sp.* inoculated in the active dry form, the aroma contains ferment notes that do not contribute to the quality of the product.

The flavor is positively affected, first by the residual sugar in the end product (circa 4.0 g/100mL) and also by the low alcoholic degree, factors that influence the final quality of the beverage. The biomass reduction also shows other advantages, such as a better control of the process, since the process is slower and the product more microbiologically stable, which would make the use of the champnoise process of gasification possible without any danger of bottle blowing up.

In addition, this operation is not expensive and usually the cider processing industries have equipments for the centrifugation or/and filtration necessary for biomass removal. The fermentation time increases to around 25 days, but the quality and price of the product also rise.

CONCLUSION

The biomass reductions used in Brazilian cider processing provided a beverage of superior quality. The characteristics of this operation were:

[1] to run a slower fermentation that enable an easier control of all operations,

[2] to exhaust the nitrogen compounds, which supported the natural gasification and,

[3] to improve the flavor, with the presence of residual sugars of the own fruit and the pleasant volatiles liberated by oxidative yeasts.

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RESUMO

O trabalho objetivou caracterizar a influência da eliminação de biomassa no processamento da sidra e na qualidade do produto fermentado em bancada de laboratório utilizando condições observadas na indústria. O mosto da cultivar Gala foi despectinizado e transferido para fermentadores de 500 mL e inoculado com 2,0x10^{6} ufc/mL de leveduras naturais ou comerciais. A cada 12 horas em um dos fermentadores a biomassa foi reduzida por centrifugação, em seguida o mosto era deixado fermentar. Os sete fermentados de maçãs foram analisados após 20-26 dias de fermentação, mantendo açúcar residual na bebida, um baixo grau alcoólico e aroma frutado. Além disso, foi possível eliminar todo o nitrogênio assimilável, importante no controle da gaseificação natural. Desta forma, a redução de biomassa pode ser um tratamento eficiente para obter uma sidra suave e de baixo grau alcoólico com uma melhor qualidade.

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