Vol.50, n. 5 : pp. 887-892 September 2007
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Identification of Yeasts Isolated from the Pulp in Nature and the Production of Homemade "Umbu" Wine

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ABSTRACT

The goal of this work was to select strains of fermentation yeasts and test them in a small-scale production of " umbu" fruit wine. The fruits were collected in N. Sra da Glória city in Sergipe, Brazil. The *fruit pulp was diluted and aliquots were inoculated on YMA plates. The yeasts strains were submitted to fermentation using "umbu" fruit juice as substrate, exception with to those strains of species related to pathological processes. The yeasts tested produced a beverage characterized between dry to light due to its alcoholic content with very nice taste, translucent appearance, of green to light yellow color.*

Key words: Spondias tuberosa, wine of fruit, non-saccharomyces

INTRODUCTION

The yeasts are microorganisms that belong to the Fungi due to the lack of photosynthesis absence of motion structures, presence of rigid cellular wall and nucleus membrane; however, they differ from filamentous fungi by showing themselves, predominantly under unicellular form. The majority is classified as ascomycetous and show themselves as spherical, oval or cylindrical cells, with cellular division through budding (Pelczar, 1980; Madigan et al., 2004). The distribution of yeasts "in natura" is done by insect vector and wind. The flowers and fruits are important habitats to their development due to the high concentration of simple sugars and low pH (Pelczar, 1980). Many authors have isolated yeasts with fermentative capacity from fruits, citric concentrates and other sugar substrates (Brannon; Pollit, 1935; Trindade et al., 2002).

Due to their high fermentative capacity, the yeasts are essential in the production of alcoholic beverages. Among them, *Saccharomyces cerevisiae* is universally employed in processes for the production of alcoholic beverages, especially wines. The term wine is only used for the fermentation of grapes, however, many other fruits can be used in the same process (Martins, 1998, Madigan et al., 2004, Dorneles et al., 2005), as well as other species of yeasts associated to these fruits (Trindade et al. 1999).

Among the fruits that have the potential for the production of beverages, the fruits of S*pondias tuberosa* present appropriate characteristics (sugar content, pH, and others). Moreover, they are much appreciated both nature and as processed products

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such as sweets, juices and popsicles. In this context, this work focused on the selection yeast strains from the fresh and frozen pulps of "umbu" for the production of homemade "umbu" wine.

MATERIALS AND METHODS

The fruits from the "umbu" tree were collected in Nossa Senhora da Glória/SE, Brazil. Thirty samples were collected aseptically in plastic bags and then processed in a blender (10% sterile saline; 0.5g of fruit samples). The isolation of the yeasts was done by inoculations of decimal dilutions of the samples in YM Agar plates (0.3% extract of yeast, 0.5% peptone, 2% of glucose and 2% of agar), with 100mg/L of chloramphenicol. The plates were incubated at 25°C±3°C for 5-8 days. After that, the selection and purification of the morph types was done. The isolated yeasts were identified according to Yarrow (1998), and Kurtzman and Fell (1998). After selecting and purifying, the isolates were stored on GYMP broth at -80°C. Among the isolated species that showed fast glucose fermentation profile at least one isolate was used in the test for production of homemade "umbu" wine. The test was performed using a protocol adapted from Trindade and colleagues (1999) according to the following steps: a) Preparation of "umbu" must: Brix was corrected to 20°, because the pulp is sugar poor. The must was sterilized by autoclave; b) Production of pre-inocullum: yeasts were grown on Sabouraud Agar for 24h and then 15ml from the must was added until a visible gas production and a °Brix reduction; c) Production of "umbu" wine: pre-inocullum content was put in 500ml of corrected and sterilized must. The broth was homogenized and SST was verified every day until stabilization. The sensorial analysis was done according to Teixeira (1987), aiming the comparison of the wines produced by different species and selecting the best one. For this purpose, a quantitative test was conducted comparing different wines using a structured standardized questionnaire with the following items: appearance, aroma and taste, the evaluation varying within a Hedonic rating (from "I really hated it" = score 1 to "I really loved it" = score 9). The wine tasters were selected randomly, among students and staff from Universidade Federal de Sergipe. The experiment was set in a casual scheme with three repetitions. Analysis of variance (ANOVA) was performed and the average grades were submitted to Tukey's test at 5% of probability using the ASSISTAT program (Silva, 1996; Silva; Azevedo, 2002).

RESULTS AND DISCUSSION

Seventeen species were identified comprising 54 isolates (Table 1). These results corroborated with several authors, Brannon; Pollit (1935), Owama; Saunders, (1980); Furlaneto et al., (1982); Morais et al., (1995), Trindade et al., (1999); Trindade et al., (2002) that isolated yeasts from fruits. The genera *Candida, Rhodotorula, Kluyveromyces, Pichia, Trichosporon, Kloeckera, Zygosaccharomyces* have been isolated from natural food such as honey, fruit juices, milk and others, as well as from industrialized food (Cook, 1958; Jay, 1970; Ivo, 1982; Wiskiewski et al., 1988 in Magalhães; Queiroz, 1991).

Table 1- Frequency of occurrence of identified yeast species.

Species (Ascomycetous)	**number of isolates**	***Species* (Basidiomycetous)**	**number of isolates**
Pichia membranifaciens	2	*Cryptococcus flavus*	1
Candida floricola-like	1	*Cryptococcus humiculus*	1
Candida geochares	1	*Cryptococcus laurentii*	1
Candida parapsilosis	4	*Black yeast*	1
Candida sergipensis	13	*Trichosporon moniliforme-like*	1
Candida sorbosivorans	9		1
Candida spandovensis	11		
Candida tennuis-like	1		
Candida valida	3		
Kloeckera japonica	1		
Kluyveromyces marxianus	1		
Issatchenkia occidentalis	2		
TOTAL			**54**

The prevalence of ascomycetous yeasts was in agreement with the results of Santos et al., (1996). These authors stated that the basidiomycetous yeasts were predominant in flowers and green fruits, and as the fruit ripened, the ascomycetics and black yeasts dominate. The species with higher occurrence (24%) was the *Candida sergipensis*, described by Trindade and colleagues (2004). Considering that this yeast was isolated only from the frozen substrate, it could be inferred that its predominance was related to the selective pressure from the low temperatures that led to low competition.

Recent works have confirmed that Brazil has a great potential in diversity of yeast communities, which included *Wickerhamiella australiensis, W. cacticola, W. occidentalis, C. drosophilae, C. lipophila, Starmerella* gen. Nov. *S.bombicola* sp. Nov., *Kodamaea anthophila, K. nitulidarum, C. restingae, C. batistae, C. hagleri, C. sergipensis, Saturnispora hagleri, C. riodocensis, C. cellae, C. bromeliacearum* and *C. ubatubensis*, isolated from natural substrates such as flowers, vector insects and cactus (Rosa; Lachance, 1998; Rosa et al., 1999a,b; Trindade et al., 2004; Morais et al., 2005; Pimentel et al., 2005, Ruivo et al., 2005).

Out of the 54 yeasts isolated from the fruits of the "umbu" tree, 50 presented high-fermentative ability (gas production ≥ 50% Durhan tube after 24h). As emphasized by Hagler et al. (1995), some substrates in which yeasts are far from being the ideal for these microorganisms and the **"**umbu" pulp could be one of them, due to the low amount of sugar. However, the ability of the yeasts in using a wide range of organic compounds (Phaff, 1991) allowed the occupation of these niches and explained the expressive presence of the yeasts in the "umbu" pulp.

Table 2 presents the results of the homemade "umbu" wine production expressed as amount of alcohol, total soluble solids, total acidity, pH and the fermentation period.

Table 2 - Representation of the alcoholic content - AC (°GL), total acidity - TAA (% acetic acid), total soluble solids - TSS (°Brix), of the "umbu" wine produced by different yeast strains.

Strain	Specie of yeast	TA (°GL)	TSS (°Brix)	TAA (%acetic acid)	Time of activity (days)*
R-101	*Candida sergipensis*	10a	12.6	0.82	12
R-94	*Candida sergipensis*	8c	16.0	0.97	16
R-03	*Candida valida*	10a	14.0	0.99	16
R-100	*Candida sorbosivorans*	9b	15.8	0.94	14
R-133	*Kluyveromyces marxianus*	9b	14.2	0.93	12
R-109	*Candida tennuis – like*	10a	13.8	0.94	16
R-48	*Kloeckera japonica*	8c	12.0	0.92	12
R-279	*Candida geochares*	8c	15.8	0.96	12
R-107	*Candida floricola-like*	10a	15.2	0.82	18

Average followed by the same lower case letter in the column did not differ (Tukey's test; $\alpha = 0.05$)

The "umbu" wines produced by different yeast strains presented significant variations related to the amount of alcohol and consumption of available sugars (Table 2). The strains (*C. spandovensis*), R-03 (*C. valida*), R-109 (*C. tennuis-like*) and R-107 (*C. floricola-like*) produced wines with the highest amount of alcohol (10%GL). According to required time for stabilization of the fermentative process, strain R-101 was most efficient (Table 2).

As for the sensorial characteristics, the wines showed transparency, very nice taste, color between green and light yellow and could be classified as between dry to light**.** Tables 3 and 4 showed the results of sensorial analyses, which contained, respectively, the average scores and the percentage of acceptance and refusal of the "umbu" wines, according to the hedonic tests. Sensorial characteristics of *C. floricula*-like R-107 were superior to *C. sergipensis* R-101.

The percentage of acceptance and refusal (Table 4) for the appearance and the aroma of the beverages scored between 20 and 63.33%. Considering all the aspects, the beverage produced by *C. floricola*-like R-107 had the highest acceptance percentage for the parameters (50, 63.33 and 80% for appearance, aroma and taste, respectively) and lowest refusal for aroma and taste (0%) whereas *C.*

sergipensis R-101 and *Klockera japonica* R-48 showed the lowest % of acceptance for appearance and highest percentage of refusal for the taste.

Table 3 - Average scores of "umbu" wine according to analysis of hedonic scale of nine points answered by 30 not trained tasters.

	Average of scores								
ATTRIBUTES	**R-101**	**R-94**	**R-03**	**R-100**	**R-133**	**R-109**	**R-48**	**R-279**	**R-107**
Appearance	5.4bc	5.6abc	5.1c	5.2c	5.7abc	6.2ab	5.8abc	6.6a	6.3ab
Aroma	6.2ab	5.4b	5.6b	5.5b	6.2ab	5.8b	5.8ab	5.9ab	6.8a
Taste	5.8cd	6.5abc	5.5d	6.1bcd	6.7abc	6.2bcd	5.9bcd	6.8ab	7.5a

Average followed by the same lower case letter in the column did not differ (Tukey's test; $\alpha = 0.05$)

Table 4 - Percentage of acceptance and refusal of "umbu" wine according to analysis of hedonic scale of 9 points answered by 30 not trained tasters.

	% of acceptance scores between 7 and 9								
ATTRIBUTES	R-101	R-94	R-03	R-100	R-133	R-109	R-48	R-279	R-107
Appearance	33.33	40.00	36.67	20.00	36.67	40.00	33.33	53.33	50.00
Aroma	46.67	33.33	30.00	40.00	46.67	36.67	43.33	36.67	63.33
Taste	46.67	60.00	33.33	46.67	56.67	36.67	46.67	60.00	80.00
	% of refusal scores between 1 and 3								
ATTRIBUTES	R-101	R-94	R-03	R-100	R-133	R-109	R-48	R-279	R-107
Appearance	16.67	20.00	26.67	16.67	13.33	6.67	13.33	0.00	10.00
Aroma	6.67	20.00	13.33	16.67	13.33	20.00	6.67	20.00	0.00
Taste	23.33	13.33	20.00	13.33	3.33	6.67	13.33	6.67	0.00

CONCLUSIONS

The fruits from the "umbu" tree (*Spondias tuberosa*) presented a diverse yeast community dominated by ascomycetous species in accordance with the literature on yeast habitats, and included which the recently described *C. sergipensis*, *C. spandovensis* and *C. sorbosivorans* as the prevalent ones. The yeast community showed a predominance of fermentative species (92.6%); all the strains tested were able to produce wines with acceptable physical, chemical and sensorial characteristics, when compared with grape wine.

C. floricola-like R-107 was able to produce "umbu" wine statistically superior to the other strains. Was concluded that yeast associated with "umbu" and other for the fermentative process and that beverages production could be an alternative use for these fruits and an input in the local economy.

RESUMO

As leveduras são essenciais na fabricação de bebidas alcoólicas devido a sua alta capacidade de fermentação. Dessa forma, o objetivo deste trabalho foi selecionar linhagens de leveduras fermentadoras e testá-las na produção de vinho semi-artesanal. Os frutos foram coletados em Nossa Senhora da Glória - SE, Brasil. A polpa foi diluída e alíquotas foram inoculadas em placas contendo YMA. Após essa etapa foi realizadas a seleção, purificação e identificação dos morfotipos. As leveduras foram posteriormente submetidas ao teste de fermentação, excetuando-se aquelas relacionadas a processos patológicos, As leveduras testadas produziram vinhos com teor alcoólico que os caracterizaram entre seco e suave, sabor e aparência agradáveis, translúcidos, e cor intermediária entre o verde e o amarelo claro.

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Received: December 29, 2005;
Revised: May 19, 2006;
Accepted: April 02, 2007.