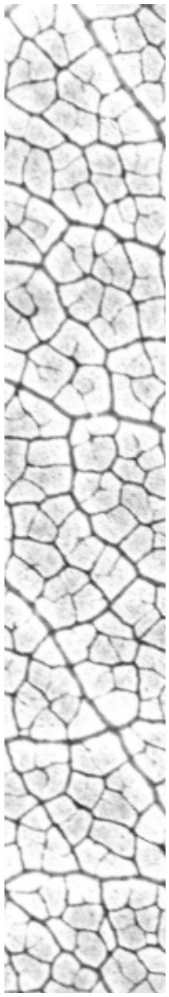

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Study of the anaerobia fermentation of native and commercial yeast (*Saccharomyces cerevisiae*) and its use in brewing

I. F. Bayas-Morejón [1*], A. Tigre-León [1], R. Ramón-Curay [1], J. Guamán [1], J. Segura [1]

[1] Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Universidad Estatal de Bolívar, Campus Agropecuario Laguacoto II, Cp: 020150, Guaranda, ECUADOR
*Corresponding author: isba@doctor.upv.es

Abstract
In this work, it was proposed to obtain beer using native yeasts. Thus, 10 samples of wheat flour and 10 samples of tangerine crust were analyzed by culture in plate with selective medium, after this analysis only two strains with typical characteristics to *Saccharomyces* were isolated from the tangerine crust (TC), these isolated were purified and used in the fermentation process. The physicochemical and organoleptic properties of the obtained beer were analyzed and compared with a control beer (commercial yeast). In alcoholic grade (GL), the results obtained were 4,78 in TC 7 beer and 4,77 in TC10, while in commercial beer it was 5,27%. The parameters of density and CO_2 were 0,90g / cm^3 in TC7 and TC10, these results were like those obtained in the control. Finally, the beer had a high acceptance by the tasters.

Keywords: beer, anaerobic fermentation, *S. cerevisiae*

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INTRODUCTION

The first utilized microorganisms as source of proteins were the yeasts, mainly the *Saccharomyces cerevisiae* that even nowadays is the main source of protein unicellular (SCP). The yeast *S. cerevisiae is* probably the broadly most utilized microorganism per the man through the time; although one didn't have, in a principle, it makes aware plenum of the participation of the microorganism in the elaboration of diverse allowances like the bread or the alcoholic drinks (Botstein and Fink 2013).

The beer is defined as "a resulting drink of fermenting by means of selected yeasts, the must coming from malt of having only fed or blended with other transformable amylases products in sugars for enzymatic digestion, cooking and perfumed with flowers of hop" (Botstein and Fink 2013).

Several types of beer exist with different physiochemical estates, color, flavor, and appearance. The most popular is the beer Lager Pilsener or European, which is manufactured starting from five basic ingredients: malt, enclosed, hop, ferments and water.

In the world, many classes of beer exist and each one possesses an aroma, flavor, color and body; many times, they take the name of the towns of which are you would originate.

Although all are manufactured with the same ingredients, malted barley, hop, yeast and water, what establishes the difference between an and another is the variations of those raw materials and the type of experienced fermentation (Martínez Garay 2016).

According to Kunze (1996), the fermentation is a key stage in the productive process, in this the must or broth of beer becomes alcohol thanks to the intervention of special yeasts. Depending on the class of used yeast, the beers are classified internationally in two basic categories: beers of high fermentation or Ale (20°C), and beers of low fermentation or Lager (about 11-13°C), (Lacerveceria.com). In the obtaining of beer, it is used two yeast types *Saccharoomyces carlsbergensis* for the beer type lager and *Saccharoomyces cereviasiae* for those of the type "ale".

In both cases, during the fermentation for the yeast CO_2 is produced, part of which is retained in the beer (García et al. 2004). The yeasts are the most important microorganisms from the industrial, because many of the species can convert the sugars in ethylic alcohol and carbon dioxide. Since the cereal grain contains starch, it should be sacred (hydrolysis of the starch until the glucose), the malt is added because it contains the

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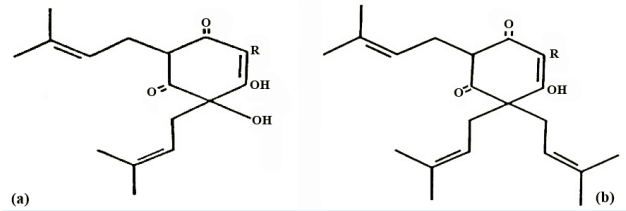

Fig. 1. Structures of the acids alpha (a); Structures of the acids beta (b)

enzyme amylase that hydrolyze the starch (García et al. 2004). The most extended use is framed in the bread industry and in the production of beers and wines. The inactivate yeast for temperature is used as source of nutriments in the animal and human feeding, so much in form of entire yeast as starting from its derivates. This yeast is one of the species considered as microorganism GRAS, for what has been approved for its use as alimentary preservative. It has been asserted that the yeast cream *S. cerevisiae* concentrated, reaches values of dry matter 18-20% and a gross protein content of 32-36% it has more than enough it bases dry (35). On the other hand, some authors sustain that the composition average of true protein is of 40,20%, while other authors, registered inferior values of 39% (Suárez et al. 2016). Now, the beer has been characterized to be a product of high acceptance inside the domestic and international market. In the Ecuador 300 million liters at year is consumed, what is equal to a total of 25 liters per capita (AmBev 2007). The characteristic bitter flavor of the beer not comes from the glandular secretion of the feminine flowers fecundated of the hop, which contains two compounds classified as resins; the humulones or sour alpha lupulin and the lupulins or sour beta lupulin (**Fig. 1**) (Schmidt-Hebbel 1966).

With all these records, in this work intended to visualize the characteristics that the products have of the fermentation with the commercial yeast and the isolated yeast starting from mandarin bark (*Citrus reticulata*). In such a way, that the following objectives were to know the procedure of elaboration of beer by means of the use of two yeasts: commercial yeast and isolated autochthonous yeast of the mandarin and this way to evaluate the acceptability by means of organoleptic analysis of the obtained beers.

MATERIALS AND METHODS

Sampling

A total of 20 samples of natural origin was evaluated, same that were obtained of the central market of the City of Guaranda (Ecuador) between April and July of 2017. The samples were: 10 of mandarin bark (*Citrus reticulata*) and 10 of integral wheat flour (*Triticum aestivum*) that were processed in the experimental laboratory of the "Facultad de Ciencias Agropecuarias of the State of Bolivar University".

Cultivation

The samples so much of wheat like the mandarin barks were analyzed aseptically of agreement with the norm UNI EN ISO 6887-3/2003. For the mandarin barks, with a scalpel its proceeded to peel and to cut in very small pieces, for both sample types, 10 g of the each one of them was homogenized with 90 mL of solution water buffered peptone (1:10 w/v), it was taken to incubate at 28 and 37°C during 24h.

After this time, 100 µL from homogenized was transferred to plates with malt extract agar (Merk, 105398, EE-UU) it was allowed to incubate, at 28ºC during 24 hours, the characteristic colonies to be *Saccharomyces cerevisiae* were selected and confirmed by microscopy, the colonies were re-cultured this in order to obtaining a mass considerable quantity. The obtained yeast was purified and used in brewing.

Brewing

For the brewing process the following procedure was considered:

Reception of the raw material: 2,5kg of barley (Ecuadorian variety *Cañicapa*) *was* weighed, then was carried out the selection and laundry of the barley with 1mL of chlorine for each 5 liters of water (3 times), obtaining a total humidity of 34.95%.

Malt preparation. The barley was subjected to maceration so that the seed absorbs water in an appropriate proportion, generally until 45%. The maceration was carried out during 5 days to a temperature of between 20 and 25ºC, with the gradual addition of water every day, this condition helped to the seed germination that goes accompanied by the synthesis of indispensable enzymes for the scarification process. When the cotyledon reaches a certain development, the germination is stop by the drying in a dehydrator of trays and toasted in a griddle until reaching a temperature of 80ºC (Pilsen beer). Once dry and toasts take off the raicillas for friction and next we proceed to the mill in a disk mill (Cepco, 2053, Quito, Ecuador) (Bokulich and Banforth 2013, Sanaguano et al. 2017).

Must Obtaining. Water is added to the malt, until obtaining a must with 20% of solid substances, ground malt was added 1.12kg, "machica" (barley toast flour) 0.20kg, 10L of water the mixture underwent cooking at 75ºC during one hour. This elaboration phase, denominated cooking, it includes a progressive heating for stages to get the good temperature of the different enzymes, in accordance with the requirements that settle down.

This way a must is obtained with high content of fermentable sugars that is necessary to decant to separate the out cereal residuals, after this time the hop it was added and was underwent boil during 2,5 hours. In this process, it starts the inactivation of enzymes, the must is sterilized, substances of the hop are extracted,

the precipitation of proteins is caused and a relative caramelization of sugar takes place. The ºBrix goes rising adding sugar until arriving at 22ºBrix (Bokulich and Banforth 2013).

Concluded the cooking with the hop and regulated the ºBrix, its filters and cools down until arriving at 24ºC.

Fermentation. In this process, the mixture was filtered to separate most of the solids in suspension and was separate the must and it inoculates the yeasts (*Saccharomyces*): commercial and autochthonous isolated (fermentation in parallel).

Cut of the fermentation. After the maturation, the obtained beer was filtered, then sugar was added until arriving to 0.5% of CO_2 and low pressure was packed. Since it is not a sterile product and the concentration of ethanol and the substances given by the hop they are not enough guarantee of biological stability, it was necessary to pasteurize the beer inside the containers (Ingraham 1998, Morona 2016).

Physical-chemical Analysis

GI of the beer. For the determination of the grade of alcohol, the methodology was used described by Schmidt-Hebbel (Schmidt-Hebbel 1966).

pH in beer. According to Association of Official Analytical Chemists (AOAC 1995), the analysis was based on the determination of the concentration of ions hydrogen with a meter of adjusted pH at 4.0 and 7.0 with buffer solution, for that which a pH meter was used (Hanna pH-200, EE-UU).

Density. For the determination of the density, the methodology was used described by Schmidt-Hebbel (Schmidt-Hebbel, 1966), using a densimeter (Vos Broken 90/25 Haffmans, EE-UU).

Determination of foam in beer. The determination of stability of the foam is carried out using the formator of foam (Haffmans, digital CO2, Gehaltemeter, type: DGM, serial: Nº 877030, EE-UU.) at 20ºC. The stability of the foam of the beer was measured, for that which the method of Nibem was applied. This principle is based on the mensuration of the time (in seconds), in the one which, the necklace of foam descends 10, 20 and 30 mm (Rodríguez 2003). The total acidity was also made expressed as agreement lactic acid with the Alimentary Safe-deposit Sanitary Agency of Argentina (ASSAL 2010).

Sensorial Analysis

For this process the chosen tasters those that have drunk beer of grateful marks, were so that he/she has as relating the flavor, odor and color of their favorite beer.

RESULTS AND DISCUSSION

Cultivate and Isolation

Of the 20 analyzed samples (10 of wheat flour and 10 of mandarin bark), after cultivation in alone badge of 2 samples of mandarin bark (CM7 and CM10) it was possible to isolate yeast with characteristic like the gender *Saccharomyces* as much in badge as in microscopy when comparing them with a stump control (commercial yeast).

Table 1. Value averages of the obtained physical-chemical parameters

Parameter	autochthonous yeast		commercial
	CM7	CM10	yeast
Alcoholic grade (% v/v)	4.78	4.77	5.24
pH	4.28	4.30	4.90
Density (g/cm^3)	0.90	0.90	0.98
CO_2 (g/cm^3)	0.90	0.90	0.91
Total acidity (expressed as lactic acid)	0.34	0.32	0.42

After the brewing using, the two isolated and multiplied. The obtained beer showed an appearance something cloudy, characteristic of a handmade beer. All beer should present a characteristic aspect, being this, limpid or cloudy, with or without presence of sediments, characteristic of the beer (Suárez et al. 2016).

Physical-chemical Analysis of the Obtained Beers

The alcoholic grade of the beer with autochthonous yeast was of 4.78 ºGL (CM7) and 4.77 ºGL (CM10), while of the beer with commercial yeast was of 5.24 ºGL. According to (INEN, 2003), the beers should be in a range from 2.0 to 5.0% of alcohol in volume, however, for the Technical Norma of the Latin American Association of Makers of Beer it should be in the range from 2.5 to 9% (ALAFACE 1999). The pH of the beers with autochthonous yeast was of 4.28 (CM7) and 4.30 (CM10), while of the beer with commercial yeast was 4.90, in accordance with the Technical Norma of Latin American Brewers (ALAFACE 1999), it indicates that the pH should be among the securities from 3.0 to 4.8. According to (INE 2003) the sample should have a pH among 3.5 at 5.0. In a work carried out by Luján and Vásquez (2010), they obtained a pH value of 3.90.

The density of the beer with autochthonous yeast was of 0.90 g/cm3 while of the beer with commercial yeast was of 0.98 g/cm3, value that is justified for the presence of CO2 and ethanol in its composition (Rodríguez 2003).

The concentration of CO2 of the beers with autochthonous yeast was of 0.90% p/v, while of the beer with commercial yeast was of 0.91% p/v. The norm INE, (2003), mentions that, the concentration of CO2 should be in the range of 2.2-3.5% (p/v), on the other hand, Luján and Vásquez (2010), obtained a concentration of CO_2 of 0.91%. The low concentrations of CO_2 are characteristic of handmade beers that use sugar like producing of CO_2 (Vogel 2003).

The acidity expressed as lactic acid of the beer with autochthonous yeast was of 0.34% (CM7) and 0.32% (CM10), while of the beer with commercial yeast was of 0.42%. The INE (2003), it indicates that the acidity value should be to not more than 0.3%, our securities overcome that indicated, however, Luján and Vásquez

Table 2. Sensorial analysis for the beer fermented with commercial and autochthonous yeast

	autochthonous yeast			comercial yeast		
	Mean values for the two types of beer (CM7 and CM10) after three replicas			Mean values after three replicas		
Acceptability	Odor	flavor	Color	Odor	flavor	Color
Very bad	0	0	0	1	0	0
Bad	1	1	2	1	1	2
Regular	3	0	1	1	2	1
Good	5	3	4	3	3	3
Very good	1	6	3	4	4	4
Total	10	10	10	10	10	10

(2010), they obtained a value of total acidity of 0.87%. The lactic acid formation is caused by the lactic bacteria that are in the young beer, deviating the cycle of formation of the alcohol in the moment of the pyruvate formation. For the formation of alcohol, the first transformation that suffers the glucose is to pyruvate, then to acetaldehyde and finally to ethanol (Bokulich and Banforth 2013).

Sensorial Analysis

In this analysis 10 semi-trained tasters they were the in-charge ones of carrying out the evaluation, after this analysis, in odor of the beer with autochthonous yeast, 5 of the tasters gave a qualification of good the product, 3 of regulating and alone 1 qualified likewise as very good 1 as bad, while, in the beer with yeast commercial 4 tasters gave a qualification of very good and 3 as a good odor.

On the other hand, in the characteristic *flavor*, 6 of the 10 tasters qualified as very good to the beer with autochthonous yeast, 3 as good and alone one as bad, while, with the commercial yeast 4 qualified as very good, 3 as good, 2 as regulating and 1 as bad.

The color of the beers so much with autochthonous yeast as commercial was quite similar, in such a way that 7 tasters qualified to both beers like of among very good and good.

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