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Research Article

Application of Multivariate Analysis Techniques for Selection of Yeast with Potential for Beer Wort Fermentation

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Abstract

Beer is the most consumed alcoholic beverage in the world, produced from water, malt and hops, fermented and carbonated by yeast *Saccharomyces cerevisiae*. Yeasts are so important that they define the type of beer to be produced (Ale or Lager). There are several characteristics that define the potential of a yeast for brewing, which is evaluated by the analysis of the fermentation process. The statistical techniques of multivariate analysis can aid in the tabulation and exploratory analysis of the obtained data, allowing the recognition of patterns. This research aimed to use multivariate analysis methodologies to select yeasts with potential for beer wort fermentation. Ten strains of yeast isolated from the Atlantic Forest and Caatinga biomes of Bahia - Brazil were evaluated in a fermentation conducted in pure malt barley at 12° P, and analyzed for: apparent extract, ethanol production, apparent degree of fermentation, consumption of maltose and maltotriose and production of glycerol. Principal Component Analysis, Hierarchical Clusters Analysis, and Kohonen's Self-Organizing Maps were used for the selection of yeasts. Commercial yeasts S-23 (Lager) and S-04 (Ale), manufacturer *Fermentis,* were used as control parameters. The strains SC52, SC57 and SC82 were selected as potentials for beer production, presenting higher fermentative yields than commercial yeasts. The results analyzed by means of a comparison of the means of the analyses carried out along the fermentation process were identical to those obtained through the multivariate analysis techniques used, evidencing that the methodologies can be used in yeast selection work for the production of beers.

Keywords: Multivariate Analysis; Saccharomyces Cerevisiae; Beer

Introduction

Many are the species of yeasts used in biotechnological processes, the main one being *Saccharomyces cerevisiae* due to its unique fermentative physiology [1].

The yeasts are cultivated to obtain biomass (for yeast industries), derived from cellular components (as in the pharmaceutical and food supplement industries) and the production of compounds (such as ethanol) [2]. For beers, besides producing ethanol and CO_2, they are responsible for conferring flavor, aroma and texture. It is the biological organism that turns the brewer's wort into the final product. They are so important that they define the type of beer that will be produced, classifying them in Ale or Lager, depending on the type of strain used [3-6].

Yeasts considered to be good brewers have the following characteristics: rapid fermentation speed, without excessive cell growth; efficient use of maltose and malt triose; ability to tolerate the stress

imposed by high alcohol concentrations and osmotic pressures of the must [7]. These characteristics are observed from the analysis and consequently evaluation of the data generated throughout the fermentation process. The treatment and the perception of what these data represent are important to evaluate the productive capacity of the yeast under study.

Currently, there are several techniques available for manipulation and processing of data, enabling the highlighting of some information or that intelligently recognize existing and potentially relevant patterns. Among these are the Principal Component Analysis (PCA); Hierarchical Clusters Analysis (HCA) and Kohonen Self-Organizing Maps (SOM), a technique of Artificial Neural Networks for exploratory analysis and pattern recognition [8]. The PCA can be defined as a mathematical method that employs orthogonal transformation to convert a set of variables possibly correlated to a set of linearly uncorrelated variables called main components [9].

The HCA interconnects the samples by their associations producing a dendrogram where the similar samples, according to the chosen variables, are grouped together, having as the basic assumption of interpretation that the smaller the distance between the points, the greater the similarity between the samples [10].

Artificial Neural Networks (ANNs) are patterns recognition techniques that have been widely used in recent years due to the development of problem-solving algorithms that enable mapping, modeling and classification of data [11,12]. The RNAs have been applied to several quality control processes in foods [13-15] and beverages [16-22], including beers [23-26]. Given this scenario and considering that beer is the most consumed alcoholic beverage in the world, as well as observing the growing movement of artisan beer production in Brazil, this work aims to use multivariate techniques as an exploratory analysis strategy to select yeasts for brewing craft beer.

For the selection of the yeasts, comparisons were made between the fermentation data obtained during the process with control yeasts (commercial yeasts), thus evaluating similarities between them. The Kohonen Self-Organizing Maps (SOM) technique was applied, which is an unsupervised Artificial Neural Network method with a competitive learning strategy, with a neighborhood interaction function, in which the clusters (classes) are formed Exploring the similarities or dissimilarities between input patterns based on their inter-correlations. Examples of applications of microbiology include subspecies discrimination using pyrolysis mass spectrometry and the Kohonen networks [27].

Materials and Methods

Yeasts

The yeasts used in the research (Table 1) come from previous research projects, already identified biochemically and molecularly as *Saccharomyces cerevisiae* [28], and intraspecifically differentiated with respect to ethanol tolerance, osmotolerance, thermo-tolerance, H2S production, flocculation capacity and batch fermentation [29]. They are preserved and stored in the Laboratories of Microbiology Research Laboratory (LAPEM) of the State University of Feira de Santana (UEFS) and Laboratory of Applied Microbiology of Agroindustry (LABMA) of the State University of Santa Cruz (UESC), located in the State of Bahia -Brazil, in the cities of Feira de Santana and Ilhéus, respectively. As a control, commercial yeasts were used, manufacturer *Fermentis*, S-23 (lager) and S-04 (ale), purchased from specialized stores in the sector. The stock culture was maintained in sloped test tubes containing Sabouraud agar and stored under refrigeration at 4°C.

Local	Biome	Code
Ibirataia/BA	Atlantic Forest	SC01
Ibirataia/BA	Atlantic Forest	SC37
Ibirataia/BA	Atlantic Forest	SC52
Jaguaripe/BA	Atlantic Forest	SC57
Jaguaripe/BA	Atlantic Forest	SC62
Jaguaripe/BA	Atlantic Forest	SC82
Condeúba/BA	Caatinga	SC174
Condeúba/BA	Caatinga	SC175
Condeúba/BA	Caatinga	SC184
Rio de Contas/BA	Atlantic Forest	SC220
Bélgica	---	S-23*
Bélgica	---	S-04*

Table 1: Yeast strains used in the experiment and their respective places of origin.

Adapted from Silva, 2009.
* Commercial yeasts (S-23, lager, S-04, ale), used as controls, purchased from specialized stores in the sector.

Brewery

The brewer's wort was produced with the following raw materials: water, of the mineral type, obtained in the local commerce of the municipality of Feira de Santana, Bahia-Brazil; malt pilsen type, originating in Belgium, supplied in 25 kg bags; and hops, being an aromatic (in pellets type 90, with approximately 7% of alpha acids, origin U.S.A.) and the other of bitterness (in extract CO_2, with approximately 30% of alpha acids) manufacturer Hopsteiner.

Cultivation and propagation of yeasts

From the stock culture, the strains were picked and inoculated in petri dishes containing Sabouraud agar and kept in an oven at 30°C for 24 h. Then the scrapes were transferred by scraping the agar using a platinum loop and under aseptic conditions in a Biological Safety Cabinet (BSC) to a 250 mL Erlenmeyer flask containing brewer's wort (125 mL) at 12°P (± 0.5°P) to start the propagation step. The flask was incubated at 30°C on a rotary shaker at 150 rpm for 24 h. The fermentation step was started after checking the concentration of the inoculum in the flask (1-2x108 cel mL^{-1}). The maximum volume of 10% of the propagation required to achieve a cell concentration of 1-2 x 10^7 cel mL^{-1} at the start of the fermentation was used. The concentration of the cells in suspension was determined by Neubauer chamber counting methodology (1/400 mm^2 x 1/10 mm) and expressed in cel mL^{-1}. The determination of cell viability (viable and non-viable cells) was performed by the International Method of staining with methylene blue [30].

Fermentation

The fermentation was carried out on a 500 mL Erlenmeyer flask scale, using a useful volume of 250 mL, of which 225 mL of pure malt and 25 mL of wort from the propagation were used. In order to promote the reinvigoration and rapid growth of the yeast, in the initial 12 hours of the fermentation stage the erlenmeyer was kept closed with a sterile capuchin, allowing the yeast respiratory tract, energetically more efficient. The sterile hood was then replaced with an airlock valve to close the system, guaranteeing the anaerobic conditions, as well as to facilitate the collection of samples during the fermentation. This fermentation route results in the production of beer by converting the sugar present in the wort into ethanol and carbon dioxide.

Analytical monitoring of the experiment

During the fermentation process, 5 mL of periodic samples were withdrawn at 12h intervals until the end of the fermentation. The final fermentation time was determined when the attenuation point (non-fermentable sugar concentration of the endpoint assay) was reached. Samples were degassed by vigorous shaking of the falcon tubes for 1 min. Then centrifuged at 5400 g for 10 min and the supernatant obtained was used to quantify: apparent extract (°P); concentration of ethanol (% v v^{-1}); density (g mL^{-1}); and apparent degree of fermentation (%), determined in beer analyzer equipment, Beer Analyzer (Anton Parr, Áustria).

The concentrations of glycerol and sugars maltose and maltotriose were determined at the initial and final points of the fermentation, in order to determine the percentage of production / consumption of these. The determinations were performed by High-Performance Liquid Chromatography (HPLC), in Agilent brand equipment, equipped with an oven, automatic sample injector, refractive index detector Agilent 1100/1200 brand, BIORAD FSX-87H column. The mobile phase used was a mixture of acetonitrile: water in different proportions, with a flow rate of 100 µL min^{-1}, at a temperature of 35°C. Before determination by HPLC, the samples were degassed and centrifuged at 5400 g for 10 minutes, the supernatants filtered through a 0.45 µm membrane and diluted in deionized water, in the ratio of 1:10 and then injected into the chromatograph. The concentrations of maltose and maltotriose were calculated from calibration curves obtained from standard solutions.

Selection of yeasts

For the selection of yeasts, the Principal Component Analysis (PCA), Hierarchical Clusters Analysis (HCA) and Artificial Neural Networks (ANN) methodologies were applied, and the Kohonen Self-Organizing Maps technique was applied. The following factors were used: maltose consumption, maltotriose consumption, glycerol production, Apparent Fermentation Degree (GFA) and Ethanol Production (Et).

Analysis and processing of data

The data were analyzed and treated in the statistical programs MATLAB R2013a and STATÍSTICA 8.0. The significance level of 5% was adopted, and the Tukey test was applied to compare the means of the data obtained.

Results and Discussion

Table 2 shows the apparent extract, ethanol production and apparent degree of fermentation and Table 3 shows the initial contents of maltose, maltotriose and glycerol obtained at the end of the fermentations at temperatures of 15°C and 22°C.

Yeast	Apparent extract (g L^{-1})		Ethanol (g L^{-1})		Apparent Degree of Fermentation (%)	
	15°C	22°C	15°C	22°C	15°C	22°C
Control**	43,65 ± 0,35^{c}	35,42 ± 0,01^{c}	41,30 ± 0,20^{g}	44,74 ± 0,18^{f}	74,92 ± 0,10^{i}	72,50 ± 0,11^{e}
SC01	55,73 ± 0,60^{f}	55,59 ± 0,13^{f}	35,27 ± 0,00^{d}	35,82 ± 0,35^{d}	54,23 ± 0,65^{c}	55,92 ± 0,23^{a}
SC37	71,75 ± 0,28^{i}	62,44 ± 0,05^{g}	27,30 ± 0,32^{a}	32,43 ± 0,03^{a}	54,26 ± 0,31^{c}	58,25 ± 0,20^{c}
SC52	31,03 ± 0,00^{a}	30,40 ± 0,01^{a}	47,10 ± 0,01^{i}	47,91 ± 0,01^{h}	75,00 ± 0,00^{i}	75,00 ± 0,02^{f}
SC57	34,10 ± 0,10^{b}	31,58 ± 0,01^{b}	45,05 ± 0,04^{h}	47,22 ± 0,01^{g}	72,50 ± 0,00^{g}	75,00 ± 0,10^{f}
SC62	54,66 ± 0,20^{e}	56,39 ± 0,01^{f}	35,90 ± 0,00^{e}	35,35 ± 0,52^{c,d}	57,71 ± 0,01^{e}	56,52 ± 0,25^{b}
SC82	34,10 ± 0,10^{b}	31,86 ± 0,02^{b}	45,05 ± 0,23^{h}	47,22 ± 0,05^{g}	74,08 ± 0,01^{h}	74,75 ± 0,35^{f}
SC174	50,55 ± 0,05^{d}	51,08 ± 0,49^{d}	36,77 ± 0,00^{f}	36,61 ±0,18^{e}	58,33 ± 0,00^{f}	59,19 ± 0,08^{d}
SC175	69,42 ± 0,10^{h}	50,59 ± 0,65^{d}	28,88 ± 0,00^{b}	35,03 ± 0,42^{b,c}	43,83 ± 0,01^{a}	56,41 ± 0,36^{b}
SC184	56,43 ± 0,53^{f}	54,26 ± 0,21^{e}	35,27 ± 0,00^{d}	36,77 ± 0,11^{e}	53,93 ± 0,00^{b}	58,18 ± 0,19^{c}
SC220	65,28 ± 0,53^{g}	55,76 ± 0,72^{e,f}	29,90 ± 0,32^{c}	34,72 ± 0,21^{b}	56,88 ± 0,00^{d}	56,72 ± 0,10^{b}

Table 2: Average values and standard deviation of apparent extract, ethanol and apparent fermentation levels, obtained under fermentation conditions at 15°C and 22°C in pure malt.

* Values between the partial means marked with the same letter, in the same column, did not differ significantly ($p > 0.05$) from each other, according to Tukey's test. ** Control: Data presented for temperature of 15°C refer to commercial yeast S-23 and for temperature of 22°C to commercial yeast S-04.

Yeast	Maltose (g L^{-1})		Maltotriose (g L^{-1})		Glycerol (g L^{-1})	
	15°C	22°C	15°C	22°C	15°C	22°C
Wort*	54,22		24,02			0,00
Commercial**	3,59	2,81	6,37	4,85	2,50	2,30
SC01	11,76	8,11	14,34	15,05	4,80	4,25
SC37	11,60	2,93	13,78	15,53	4,50	4,15
SC52	4,86	3,30	9,50	7,48	1,17	1,15
SC57	10,76	5,59	9,72	7,48	1,25	1,17
SC62	18,57	10,86	12,03	14,04	3,50	3,00
SC82	10,32	3,84	9,66	9,20	1,20	1,14
SC174	30,59	18,53	11,07	13,44	3,65	3,55
SC175	37,06	38,24	12,63	14,23	4,75	4,25
SC184	21,63	23,57	11,27	14,41	4,00	4,00
SC220	37,64	38,70	12,67	16,65	4,20	3,80

Table 3: Maltose, maltotriose and glycerol contents in fermentations in pure malt (12°P) wort with selected and commercial yeasts, at 15°C (lager) and 22°C (ale) at the end of the fermentation process.

* Initial concentrations of maltose, maltotriose and glycerol at the beginning of the fermentation process. ** For fermentation at a temperature of 15°C, yeast S-23 and for fermentation at 22°C, yeast S-04, both from Fermentis, were used.

As can be observed in Table 2, in fermentation conducted at a temperature of 15°C (lager), the Apparent Degree of Fermentation ranged from 43.83% (SC174) to 75% (SC52), with SC52 yeasts (75), SC57 (72.5%) and SC82 (74.08%), and SC52 did not differ statistically ($p > 0.05$) from commercial lager S-23 yeast (74.92%). The attenuation was obtained after 120 h of fermentation. The residual Apparent Extract ranged from 30.40 g L^{-1} (SC52) to 62.44 g L^{-1} (SC37). The SC52, SC57 and SC82 yeasts presented the lowest values, which represents a higher consumption of the extract present in the wort, even higher than commercial yeast S-23 lager. The SC52 yeast differed statistically from the others, presenting the highest extract consumption. There was no significant difference between yeasts SC57 and SC82. Ethanol production ranged from 27.3 g L^{-1} (SC37) to 47.1 g L^{-1} (SC52). For this parameter, the yeasts SC52, SC57 and SC82 also had the highest production values, higher than the commercial lager S-23 yeast (41.3 g L^{-1}). Yeasts SC57 and SC82 did not differ statistically from each other ($p > 0.05$), showing the same levels of ethanol production, 45.05 g L^{-1}.

In the fermentation condition at 22°C (ale) the yeasts SC52, SC57 and SC82 also stood out because they presented the best performances, even when compared to the commercial yeast S-04, with emphasis on SC52 yeast, which differed statistically ($p > 0.05$) of the other yeasts, for the items apparent extract and ethanol production, presenting the best results. The apparent fermentation

grade ranged from 55.92% to 72.50%, and the yeasts that showed the best performance (SC52, SC57 and SC82) when compared to commercial yeast (S-04) did not differ significantly ($p > 0.05$) with each other. The apparent extract varied from 30.40 g L^{-1} (SC52) to 62.44 g L^{-1} (SC37), when the attenuation was reached (108 h of fermentation), with emphasis on yeasts SC52, SC57 and SC82 Showed the lowest values of apparent extract, resulting in a higher production of Ethanol. The yeast SC52 stood out with the highest ethanol production, 47.91 g L^{-1}. The yeasts SC57 and SC82 did not differ statistically ($p > 0.05$), showing the same levels of ethanol production (47.22 g L^{-1}), higher than the commercial yeast S-04 (44.74 g L^{-1}). These results were superior to those found in work on lager yeast selection, 44.18 g L^{-1} [31].

Table 3 shows that in fermentation conducted at 15°C, yeasts SC52, SC57 and SC82 presented higher maltose consumption, evidenced by the lower concentrations at the end of the fermentation, when compared to the other yeasts studied. These yeasts consumed over 80% of the maltose present in the must. Also highlighted were yeasts SC01 and SC37, which consumed, respectively, 78.31% and 78.60% of maltose. The commercial yeast S-23 consumed 93.38% of the maltose present in the must. The yeasts SC82 (94.60%), SC57 (93.92%), SC62 (92.92%), SC37 (89.69%), SC52 (85.04%) and SC01 (79.97%); The commercial yeast S-04 consumed 94.82% of the maltose present in the brewer's wort.

All yeasts consumed 70% of the maltotriose present in the brewer's wort, regardless of fermentation temperature (except SC220 yeast which at 22°C consumed just below, 69.29%). The yeast SC57 was highlighted, presenting the highest consumption of maltotriose in the two fermentation temperatures.

With respect to the production of glycerol, SC52, SC57 and SC82 yeasts showed the lowest concentrations (1.15 g L^{-1}, 1.17 g L^{-1} and 1.14 g L^{-1} for fermentation at 22°C; 1.17 g L^{-1}, 1.20 g g L^{-1} and 1.25 g L^{-1} for fermentation at 15°C, respectively) when compared to the other yeasts studied, including commercial yeasts. In comparative terms, for example, 3.75 g L^{-1} was the glycerol content found at the optimum point of fermentation for beer produced using banana as an adjunct [32]. Considering that glycerol is an undesirable secondary compound in beer production, the results obtained were satisfactory.

The distribution of the samples on the PCA graph (Figure 1) shows the differences between the studied yeasts, since yeasts with similar behavior occupy nearby regions in the graph.

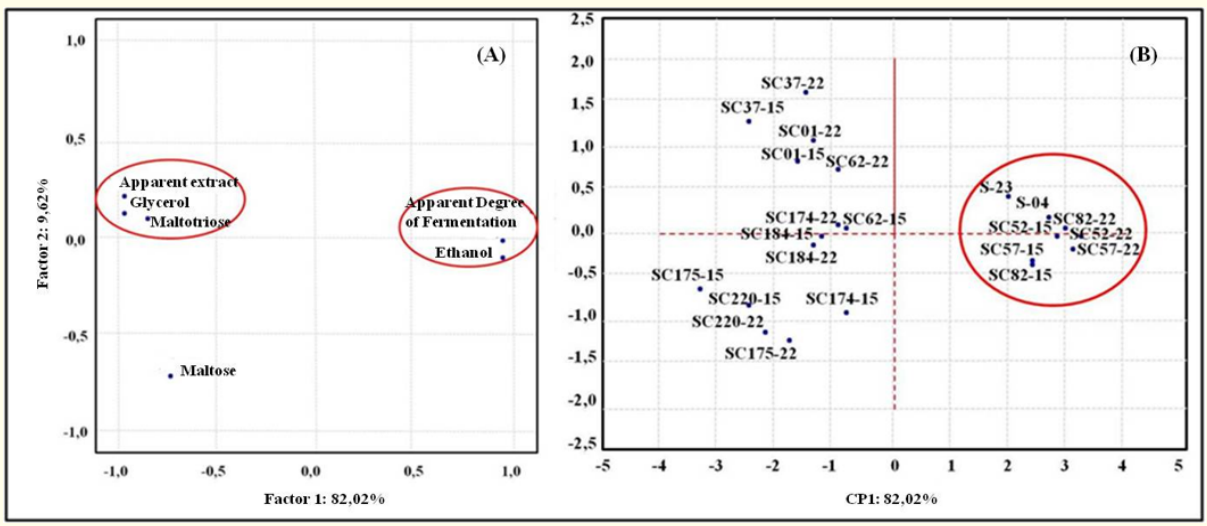

Figure 1: Principal Component Analysis: (A) evaluation factors/parameters and (B) grouped yeasts.

Through the PCA it is possible to explain 91.64% of the variability among yeasts, in which 82.02% of the variation between yeasts is explained by the first major component (PC1), while the second main component (PC2) explained 9.62% of the variability. SC52-15 (SC52, fermented at 15°C), SC52-22 (SC52, fermented at 22°C), SC57-15 (SC57, fermented at 15°C), SC57-22 (SC57, fermented

at 22°C), SC82-15 (SC82, fermented at 15°C), SC82-22 (SC82, fermented at 22°C), corresponding to yeasts which are pooled to control yeasts S-23 (commercial Lager), S-04 (commercial Ale). Therefore, through the PCA chart, we can confirm that these are the yeasts that tend to present the potential for beer production.

In the HCA (Figure 2) we also observed the grouping, as a function of the lower euclidean distances of the SC52, SC 57 and SC82 yeasts at 15 and 22°C, with the yeasts control S-23 (Commercial Lager), S-04 Ale), corroborating with the analysis carried out by the PCA methodology.

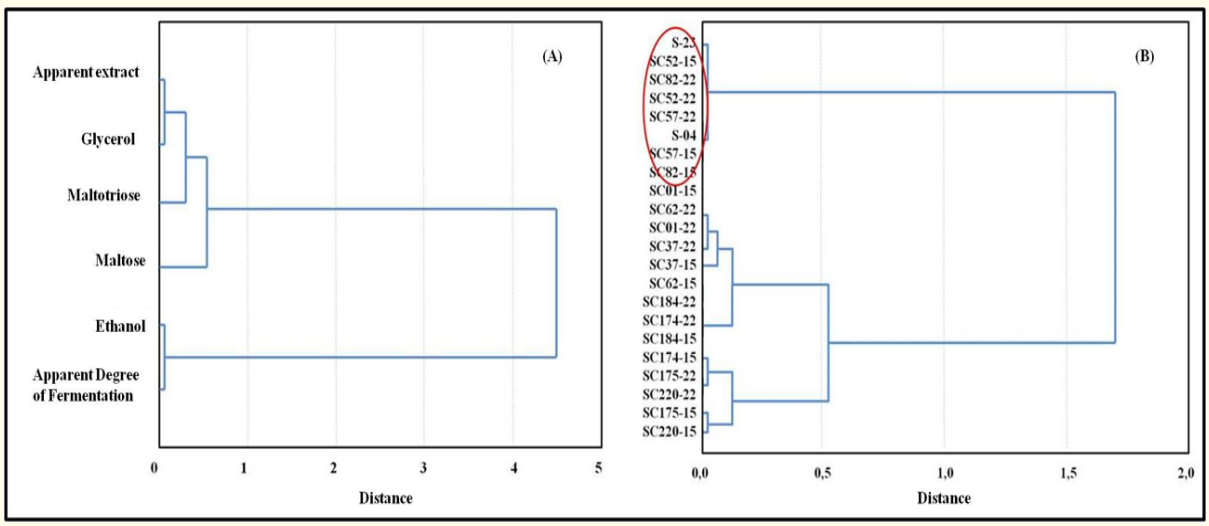

Figure 2: Analysis of Hierarchical Clusters: A) group of evaluation factors/parameters and B) grouped yeasts.

With the objective of reducing the number of dimensions to be analyzed, preserving the original information in order to facilitate the observation and interpretation of the results obtained, after the application of the PCA and HCA, the technique of Artificial Neural Networks - Self-Organizing Maps of Kohonen (SOM).

The resulting network training map shows the existence of groups of neurons with similar activation. These groups can be visualized in matrix U which shows the distances between neighboring map units (Figure 3). Larger distances indicate group delimitation while low values reveal similar activation neurons. The elements of the same group have small distances between them, so they are indicated by dark uniform areas with low values. The visual analysis of the U matrix (Figure 3) suggests three homogeneous areas, which indicate units that are not distant from each other.

In Figure 4 we can clearly observe the three groups formed, in which it is possible to visualize that yeasts SC52, SC57 and SC82 (regardless of the fermentation temperature, 15°C or 22°C) are

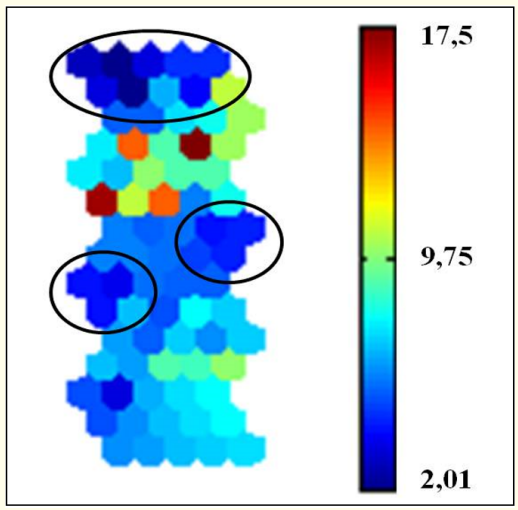

Figure 3: U matrix - 5 x 8 map with 6 variables. *The scale on the side indicates the distance between map units (neurons).

grouped with yeasts S-23 (lager) and S-04 (ale) used as controls for the selection of yeasts, allowing us to infer that due to the similarities presented with the commercial yeasts used as control, these yeasts present potential for brewing good quality, considering the conditions used.

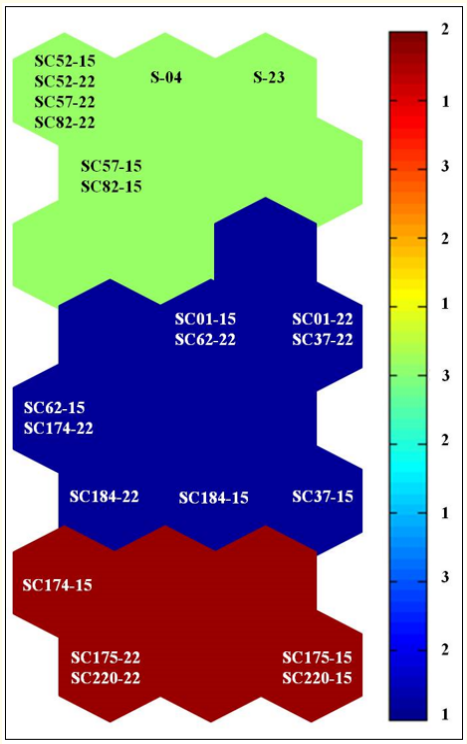

Figure 4

The apparent attenuation of more than 50% is considered satisfactory in beer production processes [33,34]. In this study, the Apparent Grade of Fermentation varied between 43.83% and 75.0% and between 55.92% and 72.50% in fermentations at 15°C and 22°C, respectively, with highlights for SC52 yeasts, SC57 and SC82, which showed the highest attenuations at both fermentation temperatures, including commercial yeasts (Table 2).

The increase in temperature favors the consumption of the apparent extract (apparent concentration of the total sugars), reducing fermentation time [32,35], also observed in this research, since the attenuation at 15°C occurred after 120 h of fermentation, while at 22°C it occurred after 108 h of fermentation.

In relation to maltose and maltotriose, a higher consumption was evidenced with the increase of the fermentation temperature, which may be associated with the fact that it is difficult to find in the nature Lager yeasts, since this one constitutes a hybrid; Therefore, it can be inferred that the yeasts studied are of the Ale type, are more efficient during the fermentation process with regard to the consumption of maltose and maltotriose [36].

Conclusion

From the application of multivariate analysis techniques, it was possible to select three strains of wild yeast for brewing, using commercial yeasts as process control.

The Principal Component Analysis, Hierarchical Clusters Analysis and Artificial Neural Networks using Kohonen's Self-Organizing Maps technique were useful techniques in the identification of correlations and pattern recognition, allowing a quick visualization of the results, providing a better understanding and facilitating the selection process from the formed groups. Therefore, we can conclude that the applied exploratory analysis techniques are indicative of yeast selection works with the potential brewer, using commercial yeasts as a control parameter.

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Conflict of Interest

The authors declares no conflicts of interest in this article.

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Bibliography

1. Johnson., *et al*. "Yeast biotechnology". In: Kurtzman CP, Fell JW, Boekhout T (Ed.). The yeasts: a taxonomic study. 5th edition. New York: Elsevier (2011): 626-627.
2. Tortora., *et al*. "Microbiologia". 6th edition Porto Alegre (Artmed) (2002): 964.
3. Cereda., *et al*. "Biotecnologia alimentos e bebidas produzidos por fermentação". Sao Paulo: Edgar Blucher (1983): 46.
4. Silva., *et al*. "Tecnologia de bebidas". "In: Venturini W, G Filho. São Paulo: Edgar Blucher 15 (2005): 353.
5. Carvalho., *et al*. "Elementos Biotecnológicos Fundamentais no Processo Cervejeiro: 1º Parte - As Leveduras". Revista Analytica, São Paulo 25 (2006): 36-42.
6. Dragone G., *et al*. "Bebidas alcoólicas: Ciência e tecnologia". In Venturini Filho WG. volume 1 São Paulo: Blucher (2010): 31-33.

7. Priest FG and Campbell I. "Brewing Microbiology". 2nd edition. Chapman and Hall, London (1996).

8. Fernandes RCS. "Análise de aglomerados das sequências da protease does HIV-1 de indivíduos infectados utilizando mapa auto organizável e k-médias". Dissertação (mestrado) - UFRJ/ COPPE/Programa de Engenharia Biomédica (2010).

9. Morais JTG. "Análise de Componentes Principais Integrada a Redes Neurais Artificiais para Predição de Matéria Orgânica". Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Engenharia Industrial. Universidade Federal da Bahia. Salvador, Bahia (2011).

10. Moita Neto JM and Moita GC. "Uma Introdução à Análise Exploratória de Dados Multivariados". *Química Nova* 21.4 (1998): 467-469.

11. Marini F. "Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast". *Analytica Chimica Acta* 635 (2009): 121-131.

12. Zupan J., *et al*. "Classification of multicomponent analytical data of olive oils using different neural networks". *Analytica Chimica Acta* 292 (1994): 219-234.

13. Du C., *et al*. "Comparison of three methods for classification of pizza topping using different colour space transformations". *Journal of Food Engineering* 68 (2005): 277-287.

14. Ciosek P., *et al*. "Direct and two-stage data analysis procedures based on PCA, PLS-DA and ANN for ISE-based electronic tongue - Effect of supervised feature extraction". *Talanta* 67 (2005): 590-596.

15. Marini F., *et al*. "Supervised pattern recognition to discriminate the geographical origin of rice bran oils: a first study". *Microchemical Journal* 74 (2003): 239-248.

16. Beltrán NH., *et al*. "Feature extraction and classification of Chilean wines". *Journal of Food Engineering* 75 (2006): 1-10.

17. Raptis CG., *et al*. "Classification of aged wine distillates using fuzzy and neural network systems". *Journal of Food Engineering* 46 (2000): 267-275.

18. Kruzlicova D., *et al*. "Classification of Slovak white wines using artificial neural networks and discriminant techniques". *Food Chemistry* 112 (2009): 1046-1052.

19. Penza M and Cassano G. "Classification of Slovak white wines using artificial neural networks and discriminant techniques". *Analytica Chimica Acta* 509 (2004): 159-177.

20. Ragazzo-Sanchez JA., *et al*. "Classification of Slovak white wines using artificial neural networks and discriminant techniques". *Sensors and Actuators B* 134 (2008): 43-48.

21. Alonso-Salces RM., *et al*. "Chemometric classification of Basque and French ciders based on their total polyphenol contents and CIELab parameters". *Food Chemistry* 91 (2005): 91-98.

22. Jack FR and Steele GM. "Chemometric classification of Basque and French ciders based on their total polyphenol contents and CIELab parameters". *Food Quality and Preference* 13 (2002): 163-172.

23. Ciosek P and Wróblewski W. "The recognition of beer with flow-through sensor array based on miniaturized solid-state electrodes". *Talanta* 69 (2006): 1156-1161.

24. Ciosek P., *et al*. "Electronic tongue for flow-through analysis of beverages". *Sensors and Actuators B* 118 (2006): 454-460.

25. Silva G., *et al*. "Exploratory analysis of the volatile profile of beers by HS-SPME-GC". *Food Chemistry* 111 (2008): 1057-1063.

26. Alcázar A., *et al*. "Multivariate characterisation of beers according to their mineral content". *Talanta* 57 (2002): 45-52.

27. Goodacre R., *et al*. "Sub-species discrimination using pyrolysis mass spectrometry and self-organizing neural networks of ropionibacterium acnes isolated from normal human skin". *Zentralblatt für Bakteriologie* 22 (1994): 124-136.

28. Silva AF. "Caracterização genética de linhagens de Saccharomyces cerevisiae isoladas de fermentações artesanais de cachaças da Bahia". Dissertação de Mestrado (Mestre em Biotecnologia) - Programa de Pós Graduação em Biotecnologia, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana (2009): 117.

29. Pinheiro CSR. "Seleção de leveduras produtoras de etanol como suporte para a produção de álcool combustível e cachaça artesanal por produtores rurais". Tese de Doutorado (Doutor em Biotecnologia) - Programa de Pós Graduação em Biotecnologia, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana (2012): 94.

30. ASBC. "Methods of Analysis of the American Society of Brewing Chemits". Eighth Revised Edition. Minnesota: The Technical Committee and Editorial Committee of the ASBC (1996).

31. Krogerus K., *et al*. "New lager yeast strains generated by interspecific hybridization". *Journal Industrial Microbiology Biotechnology* 42 (2015): 769-778.

32. Carvalho GBM. "Obtenção de cerveja usando banana como adjunto e aromatizante. Tese (Doutorado em Biotecnologia Industrial). Programa de Pós-Graduação em Biotecnologia Industrial. Área de Concentração: Conversão de biomassa) - Escola de Eng. de Lorena da USP. São Paulo (2009).

33. Hendges DH. "Produção de cervejas com teor reduzido de etanol, contendo quinoa malteada como adjunto" (2014): 93.

34. Manzano M., *et al*. "Microbiological Analyses of Dry and Slurry Yeasts for Brewing". *Journal of the Institute of Brewing* 111 (2005): 203-208.

35. Ramirez., *et al*. "Optimal Beer Fermentation". *Journal of the Institute of Brewing* 113 (2007): 325-333.

36. Gibson., *et al*. "Saccharomyces pastorianus: genomic insights inspiring innovation for industry". *Yeast* 32 (2015): 17-27.

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