

Influence of Alcoholic Strength on Sensory Profile of Lager Beers

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Abstract Non-alcoholic beer is a product whose demand by consumers is growing worldwide, however, it is not well accepted by consumers due to its organoleptic characteristics. Sensory evaluation reflects the flavor profile of a product and, more specifically, profile methods are considered to offer the best current solution to the problem of describing beer flavor. This research aimed to evaluate several sensory attributes of commercial regular and non-alcoholic beer (low-alcohol and alcohol-free beers) brands. These attributes were carbonation, body, sweetness, bitterness, astringency, flavor persistence, odour and taste. The results obtained by cluster and Principal Components Analysis are in a good consistency. It has been found that non-alcoholic beers have a higher sweetness than regular beers, which may be due to the brewing process. Besides, the study has allowed to determine the organoleptic characteristics of low-alcohol and regular beers. It has also helped to define the sensory profile of low-alcohol beer most valued by consumers in an attempt to improve the sensory properties of this type of beer.

Keywords: non-alcoholic beer, beer characterization, sensory analysis, flavor

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1. Introduction

Beer is one of the most consumed beverages in the world. Recently studies have shown that moderate beer consumption is associated with an increase in bone density, cardiovascular [1] and immunological benefits; is also associated with anti-inflammatory and antioxidant properties and may also exert higher protection against coronary heart disease than other alcoholic beverages [3].

Previous studies show important differences owing to the reason for beer consumption based on gender. Women drink to relax, have fun, improve their mood, or to escape boredom, while male drink to be brave, cool or be accepted by the group friends [4].

Beer is an alcoholic beverage usually made from malted cereal grain (such as barley), yeast, hops and water. Sometimes adjuncts and additives are also used by the brewing companies in beer manufacturing. There are different types of beer, each of which have specific organoleptic properties encompassing gustative, visual, and aroma perceptions. These properties are affected by the raw material, the fermentation of the wort, and the technological conditions used for production and packaging [5,6]. Beer aroma profile is made by many volatile organic compounds which are responsible for its

particular flavor. Among them, esters and alcohols are the main groups of aroma compounds [7]. These compounds are formed during different stages of the brewing process (mashing, boiling and fermentation), so their profiles depend on the technological procedure and on the particular yeast strain used. At this point, it is important to note that gender influences the preferred alcoholic beverage. It is well known that women prefer sweeter products as flavoured beer, instead of classic beer. The most of female prefer a fruity kind of flavors and smell, while men are known for tolerating any kind of smells better than women [4].

Non-alcoholic beer has experienced a great demand due to issues related to health, diet, safety, or legal restrictions as when a law expressly prohibits the consumption of alcohol in some countries, etc. [8,9]. This type of beer follow different legislative parameters in the different producing countries [10]. In the United States alcohol-free beer (AFB) means that there is no alcohol present, while 0.5% alcohol by volume (ABV) corresponds to the upper limit of so-called non-alcoholic beer or "near-beer". However, in most of the EU countries beers with low alcohol content are divided into alcohol-free beers, less than or equal to 0.5% ABV, and low-alcohol beers with no more than 1.2% ABV [11]. In Spain beers with low alcohol content have less than or equal to 3 % ABV and non-alcoholic beers, no more than 1% ABV. In addition,

commercially, non-alcoholic beers are divided into "0.0%", those containing less than 0.1% alcohol; and "free" with an alcohol content not exceeding 1% [12]. In this work, the term non-alcoholic beer is utilized to refer to beer with very low alcohol content.

The strategies to produce non-alcoholic beers can be divided into two main groups [13,14]:

1. Biological processes.

The main biological approaches are based on limited ethanol formation during beer fermentation [15,16,17]. Cold contact fermentation, arrested fermentation, special yeast strains, yeast immobilization are the principal biological methods employed to elaborate non-alcoholic beer. They are usually performed in traditional brewery equipment using yeast strains with low fermentation abilities [10].

2. Physical processes.

Removing the alcohol in the final product (dealcoholization) using thermal processes (vacuum evaporation, vacuum distillation, centrifugal distillation), extraction processes (solvent extraction, carbon dioxide extraction, adsorption or membrane-mediated processes (nanofiltration, dialysis, reverse osmosis, osmotic distillation or pervaporation) [18,19]. During these processes, the beer undergoes extreme conditions that can cause loss of the original aromas and flavors [7,14,20]. Sensory defects result, in part, from the loss of various compounds such as alcohols, carbonyl compounds, esters and organic acids [13,21,22,23]. However when the process is well optimized the non-alcoholic beers are usually acceptable in organoleptic properties [11, 24].

The main problem in the production of this type of beer is the difficulty of obtaining a product with the same characteristics (taste, aroma, body and foaming properties) as the regular beers. Various factors during the production of non-alcoholic beer influence its characteristics, such as the variety of barley and the malting process, temperature and pH during mashing, sparging, variety of hops added during wort boiling fermentation and storage conditions [10,25]. It is important to emphasize that non-alcoholic beers are not well accepted by consumers due to the loss of compounds responsible for aroma and taste derived from its manufacturing process [7,26,27].

The taste and aroma of beer are the most important factors driving the consumers' choices in the market. A 'harmonious' beer requires a balanced taste of sour, sweet, bitter, acidity and other tastes [28]. Besides esters and alcohols, many other components have an impact on beer sensory profiles, such as organic acids, sugars, hop bitter acids, polyphenols, dimethyl sulfide (DMS) and carbonyl compounds [29]. Several studies have shown that consumers tend to find non-alcoholic beers lacking in the sensory attributes of palate fullness, mouthfeel, and harmony [30,31].

The descriptive profile, technique based on the selection of common terms for a sensory panel of trained tasters, is the sensory methodology commonly used to characterize food products [32].

The American Society of Brewing Chemists, the European Brewery Convention and the Master Brewers Association of the Americas developed a system of

international sensory terminology for the sensory analysis of beers [33]. The terms applied to beer were arranged in a wheel for easy reference and learning, called "The beer sensory wheel".

The flavor wheel gives each distinct flavor a descriptive name and groups them by similar flavors into 14 types, not including subjective terms such as good/bad or balanced/unbalanced.

The main objective of this study focused on sensory description of non-alcoholic lager beers, in order to determine the differential descriptors between regular beers and non-alcoholic beers.

2. Materials and Methods

2.1. Beer Samples

Sixteen commercial bottled lager beers have been evaluated (6 national regular lagers, 2 national low-alcoholic lagers, 4 national alcohol-free, 3 import low-alcoholic lagers and 1 import alcohol-free) (Table 1). The beers were randomly selected from all the brands found in different supermarkets in Valladolid city (Spain). Each bottle was opened just before carrying out the first measurement and used the same day.

The samples were stored in a refrigerator at 4 °C until the moment of analysis.

Table 1. Ethanol concentration, type and origin of beer samples

NUMBER	ETHANOL CONCENTRATION (%)	BEER TYPE	ORIGIN
1	0,0%	Alcohol-free	Nederland
2	0,3%	Alcohol-free	Germany
3	0,0%	Alcohol-free	Spain
4	<1%	Low-alcohol	Germany
5	0,0%	Alcohol-free	Spain
6	0,0%	Alcohol-free	Spain
7	<1%	Low-alcohol	Spain
8	<1%	Low-alcohol	Germany
9	<1%	Low-alcohol	Spain
10	0,0%	Alcohol-free	Spain
11	4,8%	Regular	Spain
12	5,5%	Regular	Spain
13	5,4%	Regular	Spain
14	5,5%	Regular	Spain
15	6,5%	Regular	Spain
16	5,4%	Regular	Spain

2.2. Panel of Judges

A panel of thirteen judges, 8 men and 5 women, consisting of professional enologists from the internal staff of Valladolid University and invited expert beer drinkers were selected on the basis of interest and availability. All persons had previous experience on sensory-analysis panels.

The training of the panel took place in six sessions of 1 h each according to the Standard ISO 8586:2012 [34]. During these sessions, the panel was trained on non-alcoholic beers. To evaluate the performance of the assessors, the initial panel was assessed the different beers with a wide variety of sensory characteristics. Each

sample was evaluated three times by each assessor, using a complete randomized blocks study design. To make a final selection of trained judges, a LS Means test of the taster factor was carried out. The criteria for a possible elimination of a judge that has been considered, is specified for tasters whose score is outside the range of +/- 1 point of the total average 60% of the time. Following this criterion, two tasters have been eliminated. On the other hand, performing the analysis of normality and homogeneity of variances has found an outlier taster. Therefore, the final results are evaluated with the scores of 11 tasters.

2.3. Experimental Design and Procedure

The laboratory of sensory analysis, where sensory profiles were determined, met the general guidance for the design of test rooms provided by ISO 8589 [35]. Assessors had a minimum of three and a maximum of fifteen years of experience in beer sensory analysis.

The judges assessed the 16 commercial beers in duplicate in two sessions. In each session, four to six beers were randomly presented to the panelists. The testing sessions took place in a room at a controlled temperature (25 °C).

The beer samples were coded and served in a random manner. All beers were tasted at 4 °C and evaluated for selected descriptors on a ten-point scale where 1 represented poor and 10, excellent. To eliminate fatigue and so as not to carry over the effects of beer taste, the judges applied water to rinse their mouths and ate a piece of bread between subsequent samples [36].

Two studies were conducted and consisted of: 1) Sensory analysis of non-alcoholic beers (national and import) to determine the sensory differences, 2) sensory comparative analysis between national lager beers (regular and non-alcoholic) in order to know the differential sensory descriptors between each other.

In the first tasting session, a descriptive evaluation was made of 10 non-alcoholic lager beers (6 national beers and 4 import beers). Each judge valued the different beers analyzes. For the evaluation of each sample by the judges, an approximate time of 10 minutes was given. At the same time, in order not to lose skill in the senses, a 15-minute break was made at the end of the evaluation of the fifth sample.

Eight descriptors were selected and used in this study: carbonation (CO₂), body (Bd), sweetness (Sw), bitterness (Bi), astringency (As), aftertaste (Retro), flavor and taste. Abbreviations used in the results section are reported in brackets.

Moreover, the presence or absence of the 13 sensory attributes, hoppy, grainy, malty, green apple, grassy, cooked vegetables, caramel, sweet butter, caprylic acid, sour, herb, yeast and fruit esters were evaluated by the assessors. All these attributes characterize the sensory defects of non-alcoholic beer. They comprised most of the classes and some first-tier terms reported in the beer sensory wheel [33].

In the second tasting session a descriptive evaluation of the 6 regular lager beers was carried out, the procedure that has been carried out as well as the tasting sheet used was identical to the first tasting session.

2.4. Data Analysis

Statistical analysis was performed using Statistica 8 (Stat Soft. Inc., Tulsa, USA). Analysis of Variance (ANOVA), Cluster Analysis (CA) and Principal Component Analysis (PCA) were applied to the sensory data.

Initially, an ANOVA was performed to discriminate among different beers. The generated model for the analysis of the results was a nested model. The null hypothesis was that all beers were equal, therefore significant (rejection of the null hypothesis) in samples where p value was greater than 0.05.

Cluster Analysis (CA) and Principal Components Analysis (PCA) were applied in order to establish if the sensory attributes correlate with each other. The same methods have been used to determine if the different beers can be grouped according to their alcoholic strength. Cluster Analysis is a statistical classification method in which a set of samples with similar characteristics are grouped together in groups called clusters. Each sample is treated as a point in a n-dimensional space (one dimension for each variable). The samples which belong to the same category will appear close in this n-dimensional space, while samples that are different will appear separately.

Principal Component Analysis (PCA) allows transforming a big number of correlated variables into a reduced number of uncorrelated variables called principal components. By this method, a small number of components, determined as a linear combination of the measured variables, are used to replace the original variables measured in the experiment.

3. Results

3.1. Descriptive Sensory Analysis of Non-alcoholic Beers (National and Imported)

The results obtained for all descriptors analyzed are shown in Table 2. Comparing the imported non-alcoholic beers, we can observe that there was no statistically significant difference in carbonation, aftertaste, astringency, body, flavor and taste. However, statistically important differences have been found in bitterness and sweetness descriptors. Beer 2 shows lower sweetness and a higher bitterness than the other analyzed beers.

On the other hand, comparing the Spanish non-alcoholic beers, no statistical difference were found for carbonation, astringency and flavor descriptors. However, statistically important differences have been found in all other descriptors analyzed.

Finally, comparing all non-alcoholic beers analyzed (national and imported), no statistical difference were found for carbonation and astringency descriptors, however, statistically important differences have been found in all other descriptors analyzed.

Regarding the flavors present in non-alcoholic beers a different behavior has been observed for the two types of beers analyzed. Imported non-alcoholic beers have very different characteristics. Beer 1 has an increased cereal and caramel flavor. Beer 2 has an increased hoppy, yeast and sour flavor. Beer 4 has an increased hoppy and fruit esters flavor. However, national non-alcoholic beers present a similar trend (Figure 1).

Table 2. The means comparison of the sensory attributes in 16 beers

Code	SENSORY ATTRIBUTES							
	CO ₂	Retro	As	Bd	Bi	Sw	Flavor	Taste
1	3,7 ^a	4 ^{ab}	2,6 ^{ab}	3,9 ^{ab}	2,7 ^a	4,3 ^a	5 ^a	5,2 ^a
2	4,5 ^a	4,3 ^{ab}	3,2 ^{ab}	3,7 ^{ab}	6,1 ^b	2,6 ^b	4,5 ^{ab}	4,8 ^{ab}
4	5 ^{ab}	5,3 ^b	2,9 ^{ab}	4,8 ^b	3,5 ^a	4,5 ^a	5,3 ^a	6,1 ^a
8	4,3 ^a	4,7 ^{ab}	2,6 ^{ab}	4,4 ^b	3,8 ^a	4,7 ^a	5,2 ^a	4,8 ^{ab}
3	4,25 ^a	3,3 ^a	3,3 ^{ab}	3,6 ^a	3,5 ^a	3,4 ^{ab}	4,2 ^b	4,3 ^b
5	4,8 ^{ab}	4,4 ^{ab}	2,9 ^{ab}	4 ^{ab}	3,9 ^a	3,7 ^{ab}	4,3 ^b	4,1 ^b
6	4,4 ^a	3,1 ^a	2,6 ^{ab}	2,9 ^a	3,5 ^a	3,3 ^{ab}	3,7 ^b	3,8 ^b
7	4,6 ^{ab}	4 ^{ab}	2,3 ^a	3,6 ^a	3,2 ^a	4,8 ^a	3,2 ^b	4,2 ^b
9	4,6 ^{ab}	5,2 ^b	3 ^{ab}	4,8 ^b	5,7 ^b	2,7 ^b	4,6 ^{ab}	5,1 ^a
10	4,5 ^{ab}	4,8 ^{ab}	2,6 ^{ab}	3,4 ^a	3,3 ^a	3,2 ^{ab}	3,9 ^b	4,5 ^b
11	6,4 ^b	3,9 ^{ab}	3,3 ^{ab}	4,2 ^b	4,6 ^b	2,9 ^b	4,45 ^{ab}	4,5 ^b
12	5,4 ^{ab}	4,6 ^{ab}	3,6 ^b	4,6 ^b	5,3 ^b	3 ^b	4,1 ^b	4,7 ^b
13	5,6 ^{ab}	4,7 ^b	3,6 ^b	4,2 ^b	5,2 ^b	2,9 ^b	4,2 ^b	5,1 ^{ab}
14	5,5 ^{ab}	4,2 ^{ab}	3,2 ^{ab}	4,4 ^b	4,8 ^{ab}	3,3 ^b	4,6 ^{ab}	4,4 ^b
15	6,3 ^b	5,1 ^b	3,8 ^b	5,1 ^b	5,5 ^b	2,5 ^b	5,2 ^a	5,4 ^a
16	6,3 ^b	4,6 ^{ab}	3,6 ^b	4,4 ^b	4,6 ^{ab}	3,2 ^b	4,3 ^{ab}	4,8 ^b

These flavor differences between different non-alcoholic beers could be related to raw materials and production process differences. Spanish analyzed beers are mostly produced using adjuncts (wheat or corn) that substitute a large proportion of barley. Sometimes, fermentation is carried out at higher temperatures for a shorter period of time. Very often, a combination of these modifications is used, and this affects the final sensorial profile of the beer. The brewing techniques can lead to higher concentrations of 2-3 butanediol imparting sweet and butterscotch flavors [37].

In terms of dealcoholization processes, most Spanish beers use vacuum distillation, which results in an unbalanced content of volatile compounds with the loss of more than 75% of higher alcohols and 100% of esters [11], in this sense, the lower concentration of higher alcohols and esters along with the removal of ethanol could intensify the sweet flavour [37].

3.2. Descriptive Sensory Analysis of Regular and Non-alcoholic Beers

Comparing regular beers, no statistical differences were found for carbonation, body, sweetness, bitterness, astringency and aftertaste descriptors. However, statistically important differences have been found in flavor and taste descriptors, higher for beer 15.

Comparing all national beer analyzed (regular and non-alcoholic) statistically important differences have been found in all other descriptors analyzed.

Regarding the flavors present national beers, regular national beer generally have an increased malty flavor, which are associated with high content of higher alcohols, which indicates that these compounds have been eliminated during the dealcoholization process. Higher alcohols have boiling points close to ethanol and,

consequently, are largely eliminated in beers produced by thermal processes [38]. The yeast descriptor decreases after the removal of ethanol in non-alcoholic beers (Figure 1), in which the cereal taste is more pronounced than in the regular beer.

On the other hand, all national beers analyzed (regular and non-alcoholic) do not have herb, sour, caprylic acid, sweet butter, caramel, cooked vegetables and green apple flavor.

After an individual analysis of each descriptor, through a comprehensive means test for all non-alcoholic beer samples analyzed, it can be concluded that beer 4 (German beer) was the most appreciated by the tasting panel, being the best valued in five of the eight descriptors analyzed (carbonation, aftertaste, body, flavor and taste).

Taking into account that, in the analysis of non-alcoholic beer, sample 4 has been the best rated beer, we proceeded to assess the differential factors with other beers. This beer is significantly different in intensity of taste and flavor, generally much more intense than other non-alcoholic beers. Also, it differs in most cases in the aftertaste and body, resulting better appreciated. Furthermore, its significant features, that were above the rest, are hop flavors, cereal and fruit esters, being green apples (acetaldehyde) the only default flavor.

3.3. Cluster Analysis

Cluster Analysis was performed in order to find natural groupings of correlating variables. Cluster analysis of the evaluated variables (astringency, bitterness, sweetness, flavor, taste, body, aftertaste and carbonation) shows two distinct clusters (Figure 2a). The first one corresponds to astringency and sweetness that are independent variables, and the second cluster groups the other 6 variables. In this

second cluster it can be seen that bitterness and carbonation are independent, while flavor and taste are associated with each other, just as are the descriptors body and aftertaste.

Performing a cluster analysis to different samples of beers two distinct clusters were observed (Figure 2b). The

first corresponds to samples 1, 4 and 8 (non-alcoholic imported beers), and the second to the rest of samples. It can be observed that samples number 1 and 15 are independent of any other variable. It is also noted that samples 11, 12, 13, 14 and 16 are grouped (alcohol beer samples).

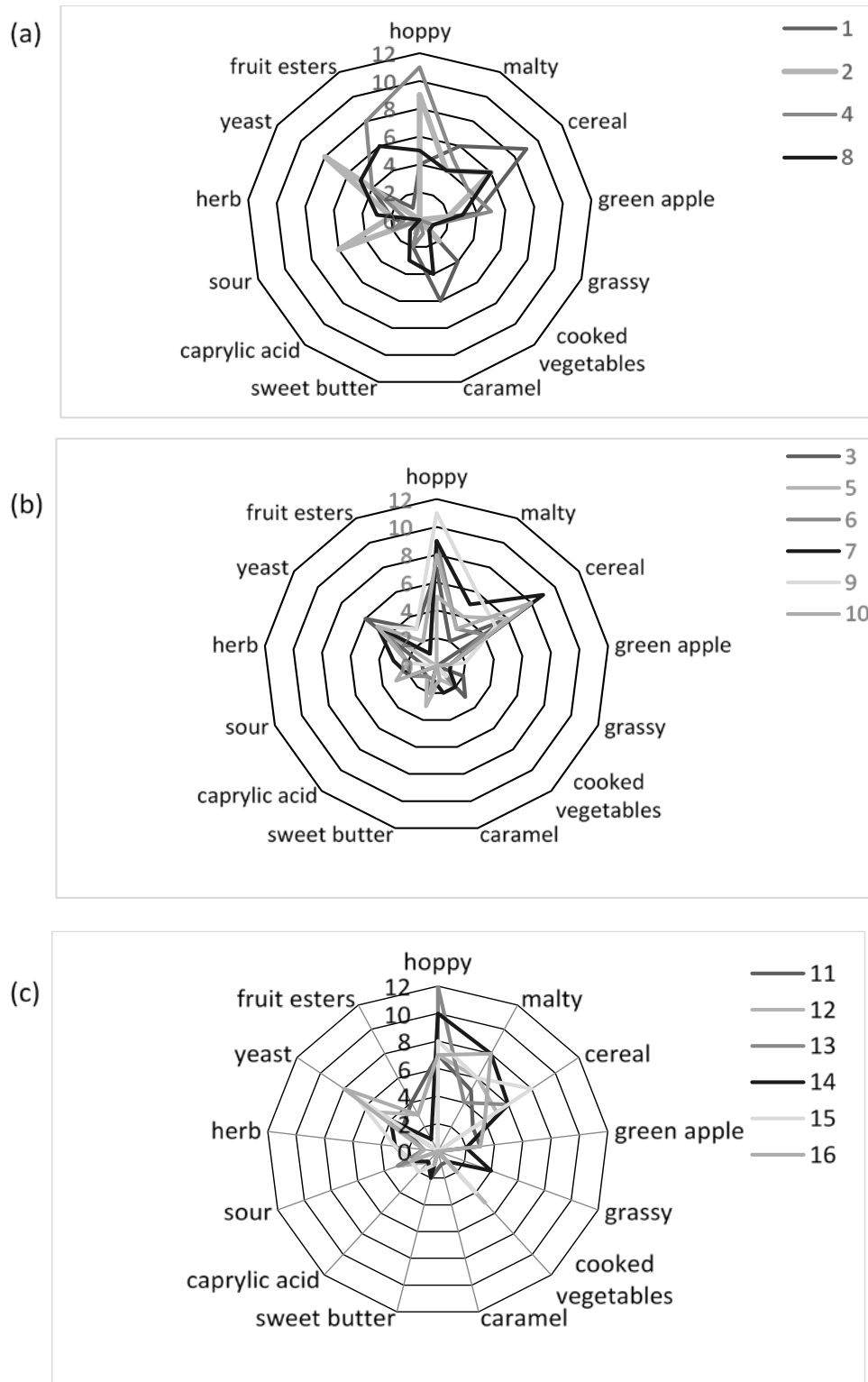


Figure 1. The spider diagram of flavors on import non-alcoholic (a), national non-alcoholic (b) and national regular beers (c)

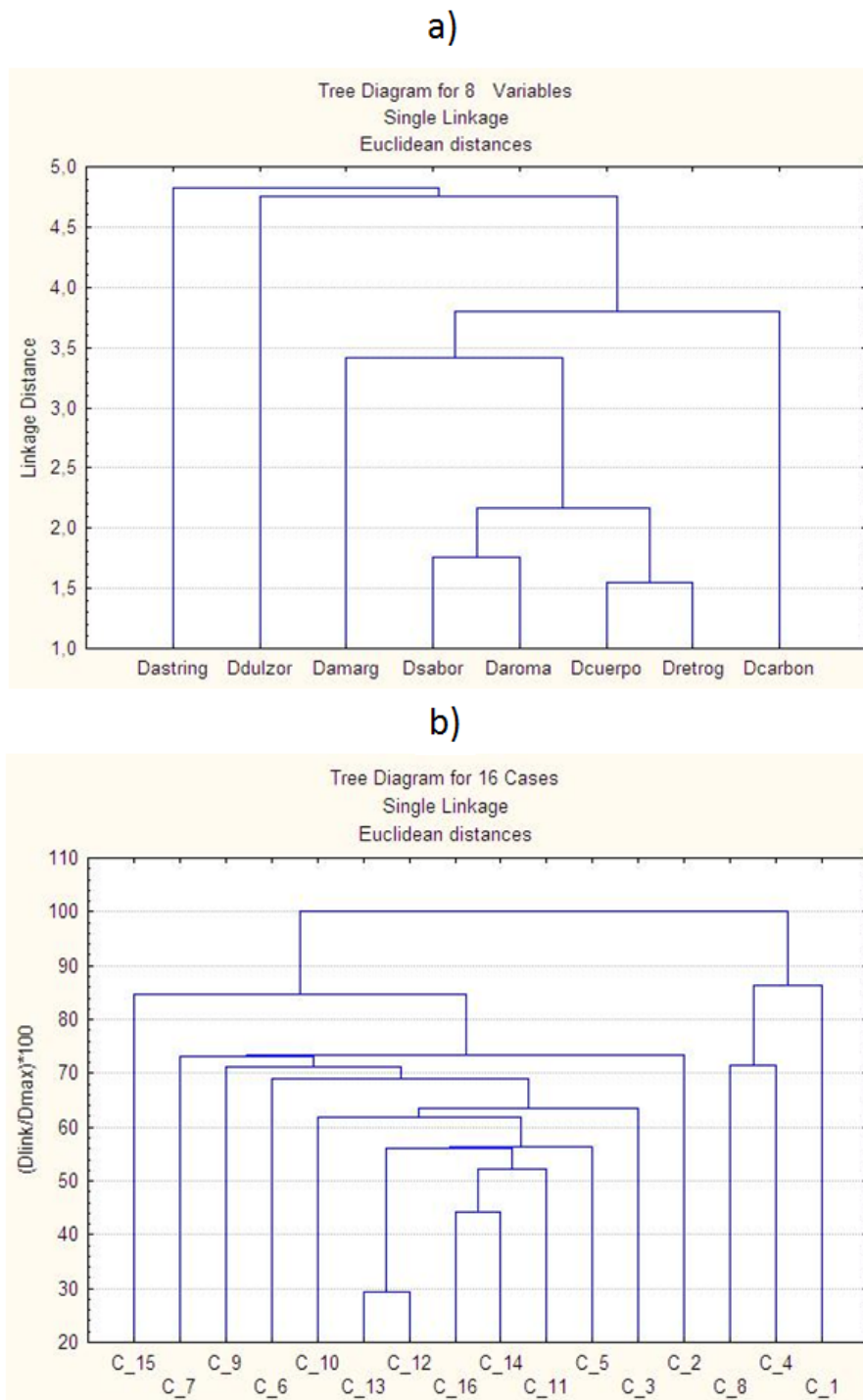


Figure 2. Cluster analysis of sensory attributes (a) and beer samples (b)

3.4. Principal Component Analysis (PCA)

In order to corroborate the correlations obtained in the Cluster Analysis, a Principal Component Analysis (PCA) was performed. Performing a PCA of sensory variables, two factors that explain the 81.32% of the variance associated with variables are obtained. Representing our variables on these two factors, it appears that the sweetness variable is totally independent of the rest studied variables and is associated with a positive value of Factor 1 whereas Factor 2 has a negative value (Figure 3a).

The Factor 1 had negative loadings for carbonation, astringency, bitterness, body, aftertaste, taste and flavor as

well as high positive loadings for sweetness. Objects close together have similar characteristics and therefore astringency and bitterness variables appeared to be highly positive correlated, as well as flavor and taste. The Factor 2 had negative loadings for body, aftertaste, taste, flavor and sweetness as well as high positive loadings for carbonation, astringency, bitterness.

Results are consistent with those obtained in the cluster analysis.

The score plot (Figure 3b) showed the position of the 16 samples of beer in the multivariate space of the first two PCs. Scores were arranged in four areas. The clear separation between the samples pointed out the differences in certain investigated sensory parameters.

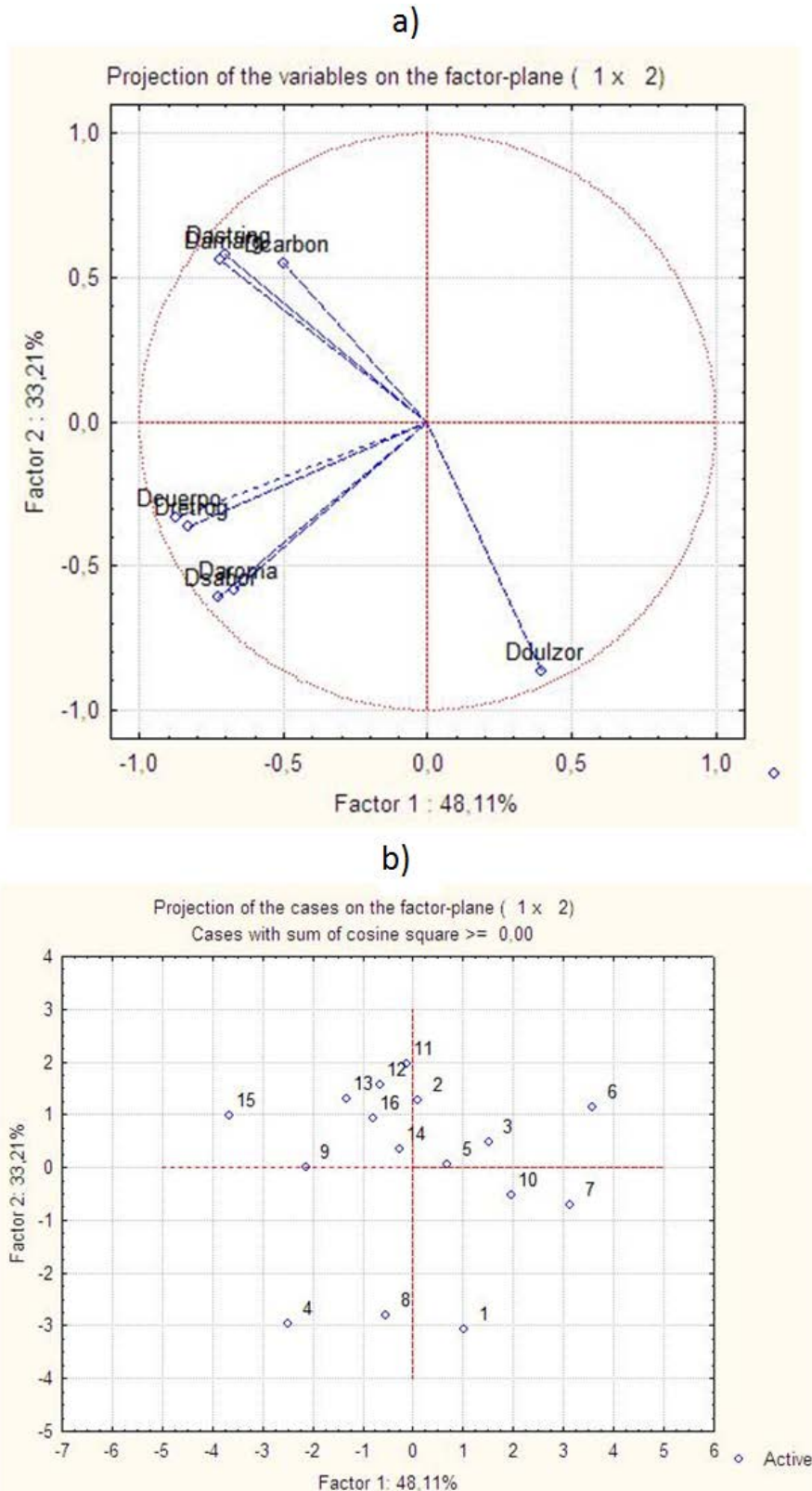


Figure 3. Principal component analysis of sensory attributes (a) and beer samples (b)

Through Factor 2 was possible to discriminate between national beers and the most of non-alcoholic beers analyzed. On the other hand, it was not possible to separate the different types of national beers (regular and non-alcoholic beers).

Beer 1 is negatively correlated with Factor 2 and it is correlated with sweetness descriptor; 2, 3, 5, 6, 7 and 10 samples are plotted in the positive quadrant of Factor 1

and they are weakly correlated with all analyzed descriptors; sample 4 is plotted in the negative quadrant of Factor 1 and Factor 2 and it is strongly correlated with body, aftertaste, flavor and taste descriptors; sample 15 is plotted in the negative quadrant of Factor 1 and positive quadrant of Factor 2 and it is strongly correlated with astringency bitterness and carbonation descriptors; while the samples 11, 12, 13, 14 and 16 (non-alcoholic beers)

have high carbonation and lower body, aftertaste, flavor and taste (Figure 3b).

4. Conclusions

The results show that non-alcoholic beers have a higher sweetness than regular beers. This could be due to the addition of additives such as glycerol or saccharin after the dealcoholization process. Moreover, the addition of dextrans into beer has been reported to improve the flavor profile of non-alcoholic beer through their action on the retention and/or perception of flavor active compounds [11,22]. In the case of limited fermentation, residual sugars from fermentation, could be associated a sweet taste.

Large differences between two types of non-alcoholic beers (national and import) have been found. The first exhibits large values of carbonation, while the last presents higher values of aftertaste, body, flavor and taste.

On the other hand, regarding the sensory variables analyzed it was found that the sweetness is a completely independent variable, while the taste and odour are closely related as with the body and aftertaste.

These results are very valuable not only for brewers and researchers in this field who seek to improve the quality of non-alcoholic beers but also for those interested in social, technological and economic concerns.

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Competing Interests

The authors have no competing interests.

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