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Sensory Characterization of Commercial Lager Beers and Their Correlations with Iso-α-Acid Concentrations

Carlos A. Blanco[1,*], Dieudonné Nimubona[1], Encarnación Fernández-Fernández[1], Inmaculada Álvarez[2]

[1]Dpto. Ingeniería Agrícola y Forestal (Área de Tecnología de los Alimentos) Universidad de Valladolid. E.T.S. Ingenierías Agrarias. Avda. Madrid 44. 34004 Palencia, Spain
[2]Dpto. Tecnología de Alimentos. Universidad Politécnica de Valencia, C/ Camino de Vera s/n, 46023 Valencia, Spain
*Corresponding author: cblanco@iaf.uva.es

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Abstract A total of 40 commercial lager beers (6 dark, 28 pale and 6 alcohol free) were analysed by High-Performance Liquid Chromatography (HPLC) and evaluated by a descriptive sensorial panel. Discriminant Analysis was applied to 15 sensory descriptors to obtain a classification by types of beer. Cluster Analysis identified 4 clusters grouping the 15 sensory descriptors. The Correlation Matrix confirmed a correlation between them and the subsequent Principal Component Analysis. A stepwise discriminant analysis was used to eliminate the less significant descriptors, leading to 100% of the samples being correctly classified with a reduced number of variables (colour intensity, smell of caramel, smell of toasted, persistence and viscosity). The analysis of the correlations between iso-α-acid concentrations and sensory descriptors shows a good relationship with bitter taste. Thus, HPLC data can be used for predicting lager beer bitterness through the mathematical correlation developed in this paper.

Keywords: *lagerbeer, iso-α-acids, sensory descriptors, bitterness, correlation*

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1. Introduction

Beer is a very complex mixture of constituents varying widely in nature and concentration levels. It is brewed by fermentation from raw materials including water, yeast, malt, and hops and contains a broad range of different chemical components that may react and interact at all stages of the brewing process [1,2]. Brewing and aging of beer are complex processes during which several parameters have to be controlled to ensure a reproducible quality of the finished product. These include chemical parameters measured instrumentally, as well as taste and aroma properties evaluated by sensory panels [3,4]. Practical problems associated with the sensory assessment of beer and other foodstuffs are well known. Training and maintaining of professional sensory panels is necessary for ensuring reproducibility of the results but this is expensive. In addtion the rapid saturation of the assessors, as only a limited number of samples may be assessed during the same tasting session. It is therefore not surprising that significant efforts are being directed to the development of chemical methods for analysis of attributes that can be correlated with sensory attributes appreciated by consumers. Several sensory parameters (alcohol, estery, grainy, malty) have been predicted using chemical analysis data which comprised concentrations of volatile compounds as well as original and final density, colour, etc. [5,6,7,8].

In order to characterize beers, it is necessary to determine their analytic or organoleptic profile. An appropriate statistical treatment of the analytical data obtained will establish which compounds show similar behaviour in a certain type of beer. Consequently, these compounds will be the main discrimination factors allowing differentiation between different types of beers [1,9].

To the characterization and differentiation of beverages several statistical techniques have been applied (Discriminant Analysis, Canonical Analysis, Principal Components Analysis (PCA), HJ-biplot, Multiple Regression, Artificial Neural Networks, etc.), in terms of the different parameters such as colour, aroma, flavour, strength, ingredients, production method, recipe, origin, etc. [10-14]. Among all these techniques, Discriminant Analysis is frequently used to differentiate alcoholic beverages [15,16,17,18]. It is applied to populations or groups of samples previously identified as such, enabling the classification of each individual later on, so as to verify that it belongs to the established group. The differentiation of the beers can be carried out if a set of beer components or a certain group of compounds is taken into consideration, such as volatile components [17,18,19,20], sensory profile [4,21,22], quality [23], countries, raw material and brewing process [24,25,26].

There are many studies of beer bitterness [24,27,28,29,30,31]. The intensity of bitterness has been related to the concentration of iso-α-acids [24,27,28,31,32]and to a lesser extent with the alcohol content in beer [33]. In particular, the iso-α-acids are responsible for approximately 70% of the bitterness of beer [34,35,36,37]. It is generally recognised that the main bittering principles of beer are the hop-derived iso-α-acids [38]. Beer bitterness could be estimated by several methods [41]. An approximate measurement is commonly obtained in breweries by spectrophotometry at 275 nm [40]. When the total amount of bittering agents is measured by HPLC, more accurate results can be obtained. Recently, using an electronic tongue based on a polypyrrole sensor array, we have determined bitterness intensity in a single measurement [41]. Theresults agree quite well with the total amount of iso-α-acids obtained by HPLC in 21 samples of beer.

The aim of this work is to characterize the organoleptic profile of different types of commercial lager beers, to obtain sensory-instrumental correlations in order to predict sensory profiles. The analysis of iso-α-acids content in order to establish a final correlation between both analytical and sensory data would add interesting conclusions.

2. Experimental

2.1. Material

Commercial lager beer samples

A total number of 40 (6 dark, 28 pale and 6 alcohol free) botlled commercial lager beer samples were obtained from local supermarkets (Valladolid, Spain). The samples were stored in a refrigerator at 4°C until they were analysed. These same samples were used in our previous study [27].

2.2. Chemical Analysis: HPLC Measurements

A commercially available International Calibration Standard (ICS) for HPLC analysis of iso-α-acids (IAA) was used (Labor Veritas, Zurich, Switzerland). The ICS-I1 DCHA-iso-α-acids (dicyclohexylamine salt of iso-α-acids) standard was 64.5% (w/w). The methanol used was LiChroSolv gradient grade from Merck (Darmstadt, Germany). Orthophosphoric acid (85%) and ethylenediaminetetraacetic acid disodium salt, 0.1 mol L−1 (Na_2EDTA) were from Panreac S.A (Barcelona, Spain). Deionized water HPLC grade was used (Milli Q; Millipore, Bedford, MA, USA). A Waters HPLC system equipped with a Mod. 600 solvent delivery pump, a 996 Photodiode Array (PDA) detector, and a Waters HPLC U6K injector were used (Waters Corp., Milford, MA, USA). The column was a octadecyl reversed-phase column (Supelco Discovery C18, 250 mm ×4.6 mm ×5 µm; Supelco, Bellefonte, PA, USA). The chromatographic method used was based on Biendl method [42,43], with a small modification consisting in the addition of 0.1 mol L-1 Na_2EDTA (Panreac S.A, Barcelona, Spain) to the mobile phase B to improve the resolution. The UV detector was set to a wavelength of 270 nm, which corresponds to an absorbance maximum for iso-α-acids. The injection volume was 10 µL, and the flow rate was 1 mL per min at 35°C. Mobile phase A consisted of 100% methanol (Panreac S.A, Barcelona, Spain), while mobile phase B contained 75% methanol, 24% H2O, 0.9% phosphoric acid (85%) and 0.1% Na_2EDTA (0.1 mol L-1). A gradient elution was used, consisting of 0-17 min at 100% B, 17-25 min at 35% A and 65% B, 25-30 min at 100% B. The peak area was recorded by using Empower Pro software (Waters). The HPLC chromatograms areas data were processed with PeakFit v4.11.05 software for Windows (SYSTAT Software, Chicago, IL, USA). Using the calibration line and HPLC chromatograms processed by PeakFit software, the concentration of iso-α-acids contained in each beer sample was calculated.

2.3. Sensory Evaluation

The beer samples sensory analysis was conducted in the sensory analysis room of the Department of Agricultural and Forest Engineering (Food Technology Area, University of Valladolid). The panel was composed by 28 trained assessors (17 men and 11 women with an average age of 29 years) according to the Standard ISO 8586:2012 [44]. 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. The performance of assessors was evaluated checking the discriminatory capacity, the reproducibility of the answers and the agreement among assessors and, after doing a two-way ANOVA with interactions, 11 tasters were eliminated and the group was left with 17 tasters who came to assess the 40 commercial beers in duplicate. Following descriptors were evaluated: external appearance (colour intensity and turbidity), smell (hop, malt, toasted, caramel, odour intensity), mouth feel (carbon, viscosity, astringency) and gustatory sensations (sour, bitter and sweet taste, intensity and persistence). The samples were presented to the tasters in glass cups at 4°C and were evaluated in a single cabin. Tasters rated the beer samples descriptors on a 10 cm unstructured line scale from 0 to 10. All beer samples were stored at 4°C before the analysis.

2.4. Data Analysis

The data analysis was performed with Statgraphics plus V5.1 software (Statpoint Technologies, Varrenton, VA, USA) and BMDP. Analysis of Variance (ANOVA), Discriminant Analysis (DA), Cluster Analysis (CA) and Principal Component Analysis (PCA) were applied to sensory descriptors used in this study. Correlation analysis was applied to sensorial data and iso-α-acids concentration.

Initially, an ANOVA was performed to discriminate between the three types of beer. Afterwards a Discriminant Analysis (DA) of 15 variables selected as sensorial descriptors, was performed to establish if it is possible to differentiate by these sensorial panel the three different types of beer analysed (dark, pale and alcohol free beers). In order to specify the discrimination associate to each discriminant function, the difference in average values and dispersion has been studied for the different groups, in relation to themselves. The normality of the variables has been previously verified. As for the homoscedasticity of the data, which is another basic assumption to apply the technique of discriminant analysis, the results obtained in this study indicate no significant difference between the variances of the discriminant functions in the three types of beers. As a result, the initial hypothesis could be validated. Similarly, it has been confirmed that the Mismatch ratio and the Kurtosis are within the acceptable values.

Cluster Analysis (CA) and Principal Components Analysis (PCA) were applied in order to establish if the sensory parameters studied correlate with each other. Cluster analysis is an unsupervised technique operating without a priori categories or classes in which samples could be grouped. Each sample is treated as a point in an n-dimensional space (one dimension for each variable). The distance between points in the space created by the n variables is determined. The samples belonging to the same category will appear close in this n-dimensional space, while those which are different will be separated. Subsequent natural groupings can be used to select variables for classifying future cases of unknown categories.

By using PCA, is possible to transform a great number of correlated variables into smaller number of uncorrelated variables called principal components. By this way, 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 [4,45,46]. In order to establish the main components the correlation between variables has been previously checked using the correlation matrix, KMO test and Bartlett test of sphericity.

Once verified that there are important correlations between the sensory descriptors under study - in order to reduce the number of variables - the Stepwise Analysis will be applied to the set of variables and beers, with the aim of differentiating correctly the three types of lager beers with the minimum number of variables.

3. Results and Discussion

3.1. Composition of Beers: Sensory Evaluation and Iso-A-Acids Concentration in Lager Beers

Table 1 shows the mean values of parameters evaluated in sensory analysis and the mean concentrations of total iso-α-acids, Table 2 shows the average values, standard deviation and ANOVA of the variables in the three types of lager beers studied. ANOVA shows that it is not possible to distinguish the three types of beers considering the parameters evaluated individually.

Table 1. Mean values of sensorial parameters and concentrations of iso-α-acids

BEER Code	External Aspect		Odour					Mouth sensation			Gustatory and olfactory sensation					[IAA] (mg/L)
	IN1	TUR	HOP	MAL	TOS	CAR	IN2	CARB	VIS	AST	SOUR	BIT	SWE	IN3	PERS	
1	7.9	0.5	2.8	5.9	7.2	6.0	6.0	4.5	4.8	4.6	3.2	5.6	3.6	5.5	5.6	67.0
2	7.9	0.4	2.9	6.8	6.5	6.6	6.1	4.2	5.4	4.6	3.4	5.3	4.6	5.8	5.8	55.5
3	5.9	0.4	3.2	4.9	4.8	4.9	5.1	4.4	4.4	4.0	3.7	4.8	3.6	4.6	4.5	39.0
4	6.4	0.5	2.7	6.3	5.3	5.5	5.6	4.8	5.9	5.1	4.1	5.7	4.4	6.2	5.9	72.6
5	7.7	0.5	2.9	6.3	6.6	6.1	5.8	4.4	5.6	4.9	3.5	6.1	4.0	5.6	5.8	56.5
6	8.2	0.4	2.5	6.2	6.2	5.7	5.3	4.4	5.1	4.5	3.3	5.5	3.9	5.2	5.3	46.8
7	3.9	0.3	4.3	3.4	2.5	2.3	4.0	4.7	3.7	4.0	3.9	4.8	3.0	4.5	4.2	39.6
8	3.3	0.3	5.1	2.7	2.4	2.0	3.8	4.5	4.0	4.0	3.9	5.4	2.4	4.4	4.7	60.6
9	3.7	0.5	4.3	4.3	3.5	3.6	4.4	4.0	3.8	4.0	3.7	4.9	2.9	4.1	4.2	72.2
10	2.8	0.3	4.7	2.7	2.4	2.4	4.1	4.4	3.8	3.7	3.4	4.9	2.7	4.0	3.9	39.6
11	2.8	0.3	5.0	2.8	2.3	2.1	3.8	4.5	3.0	3.2	4.0	4.6	2.6	3.8	3.9	37.7
12	2.9	0.4	4.1	2.5	2.2	1.8	3.6	4.7	3.3	3.3	3.6	4.0	2.7	3.7	3.6	39.9
13	3.5	0.3	4.6	2.4	2.2	1.8	3.6	4.3	3.5	3.6	3.8	4.4	2.7	3.7	3.7	49.9
14	2.8	0.4	3.6	2.8	2.3	2.6	3.1	4.4	3.2	2.9	3.5	4.3	3.3	3.6	3.5	35.1
15	3.8	0.3	5.0	3.0	2.2	2.2	3.9	4.3	4.0	3.7	3.9	4.8	2.6	4.0	4.1	78.1
16	4.7	0.4	3.3	5.7	4.3	4.1	5.4	4.5	4.8	4.2	3.8	5.5	3.3	5.2	5.2	104.7
17	3.2	0.3	5.0	3.0	2.2	2.4	4.1	4.9	4.1	3.8	3.8	4.9	2.8	4.3	4.2	82.3
18	3.7	0.5	4.2	2.8	2.4	2.5	3.7	4.5	3.5	3.8	3.5	5.0	2.6	4.1	4.3	107.4
19	3.8	0.4	4.3	3.5	2.9	2.6	3.5	4.0	3.8	3.3	3.8	4.6	2.9	3.8	3.8	64.9
20	3.6	0.4	4.3	3.2	2.9	2.7	4.0	4.8	3.8	3.7	4.1	4.8	2.9	4.1	3.9	45.8
21	5.9	0.5	3.3	3.2	4.6	2.9	5.8	4.4	4.7	4.5	2.6	4.7	3.8	5.9	6.1	33.2
22	5.8	0.4	3.5	4.8	5.0	4.3	4.5	4.8	5.1	4.9	3.6	5.7	3.1	5.3	5.4	76.5
23	3.2	0.4	4.7	2.7	2.0	2.3	3.4	4.5	3.7	3.9	4.3	5.4	2.7	4.3	4.2	63.5
24	3.4	0.4	4.5	2.6	2.3	1.9	3.8	4.7	4.1	4.1	3.6	5.1	2.7	4.3	4.4	54.1
25	3.8	0.4	4.5	3.4	2.4	2.8	4.1	4.5	3.6	3.8	4.2	4.9	3.2	4.3	4.3	54.2
26	3.7	0.4	4.5	2.8	2.4	2.0	3.8	4.7	3.8	4.3	3.9	5.3	2.2	4.3	4.6	70.5
27	4.0	0.3	4.6	3.2	2.9	2.8	4.1	4.9	4.7	4.5	4.3	5.4	2.8	4.8	5.1	60.5
28	5.4	0.4	4.9	3.7	3.4	3.2	4.5	4.7	5.0	4.3	4.1	5.9	3.1	5.3	5.4	61.7
29	2.7	0.3	4.2	3.7	2.6	2.6	4.2	4.6	3.6	4.1	3.6	5.8	3.0	4.8	4.6	79.5
30	3.8	0.4	4.4	3.0	2.5	2.3	3.9	4.7	3.9	3.6	3.9	5.2	2.4	4.5	4.2	79.3
31	4.7	0.4	4.0	3.9	3.8	3.5	4.2	5.0	4.7	4.3	3.9	5.6	2.9	4.9	4.7	98.8
32	5.4	0.4	3.6	4.4	4.0	3.9	4.7	4.4	4.9	4.6	3.6	5.7	2.8	5.3	5.6	77.3
33	4.5	0.8	4.6	3.4	3.0	2.7	4.0	5.0	4.7	4.3	4.0	5.6	2.4	4.9	4.9	51.7
34	5.8	0.5	2.9	5.7	3.5	5.5	5.7	4.7	4.9	4.5	4.0	5.5	4.0	5.4	5.3	51.4
35	3.9	0.4	4.7	2.9	2.6	2.4	4.9	4.9	2.9	3.3	3.8	4.6	3.0	3.6	3.4	50.3
36	4.3	0.4	3.8	3.9	3.0	3.0	4.4	4.5	2.8	3.3	3.8	4.0	3.0	3.4	3.3	52.1
37	3.9	0.4	4.6	2.5	2.1	2.3	4.9	4.3	2.1	3.3	3.9	4.5	3.4	3.8	3.7	41.6
38	4.0	0.3	5.1	2.7	2.4	2.8	4.5	3.9	2.3	2.6	3.8	4.2	3.3	3.4	3.1	30.3
39	3.6	0.4	2.8	1.6	1.4	2.6	5.8	4.3	2.1	1.8	5.2	2.5	5.1	4.5	3.4	20.3
40	3.9	0.5	4.6	3.7	2.6	3.5	4.9	4.7	3.4	3.3	3.9	4.4	3.6	4.4	4.1	42.5

Dark beers: 1-6; Pale beers: 7-34; Alcohol free beers: 35-40.

IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence; [IAA]: total concentration of iso-α-acids

Table 2. Average values, standard deviation and ANOVA of valued variables in the types of lager beers

Variables	Type of Beer Dark (n=6)	 Pale (n=28)	 Alcohol free (n=6)
IN1	7.36 ±1.46^{b}	3.96 ±1.56^{a}	3.91 ±1.31^{a}
TUR	0.45 ±0.27^{a}	0.39 ±0.18^{a}	0.4 ±0.14^{a}
HOP	2.82 ±1.82^{a}	4.28 ±1.98^{b}	4.26 ±2.28^{b}
MAL	6.06 ±1.89^{b}	3.4 ±1.97^{a}	2.88 ±2.01^{a}
TOS	6.10 ±1.83^{b}	2.96 ±1.12^{a}	2.34 ±1.94^{a}
CAR	5.79 ±2.14^{b}	2.74 ±1.96^{a}	2.78 ±2.12^{a}
IN2	5.64 ±1.57^{a}	4.13 ±2.12^{a}	4.56 ±1.90^{a}
CARB	4.44 ±1.91^{a}	4.58 ±1.96^{a}	4.45 ±2.04^{a}
VIS	5.19 ±1.85^{b}	4.06 ±1.73^{b}	2.61 ±1.78^{a}
AST	4.63 ±2.21^{b}	3.96 ±1.86^{b}	2.94 ±1.97^{a}
SOUR	3.53 ±1.88^{a}	3.8 ±1.76^{ab}	4.05 ±1.79^{b}
BIT	5.49 ±2.14^{b}	5.1 ±1.95^{b}	4.02 ±2.14^{a}
SWE	4.03 ±1.90^{b}	2.87 ±1.63^{a}	3.57 ±1.92^{b}
IN3	5.48 ±1.79^{b}	4.49 ±1.72^{a}	3.87 ±1.88^{a}
PERS	5.49 ±2.09^{b}	4.5 ±1.96^{ab}	3.49 ±2.09^{a}
IAA(mg/L)	55.19 ±11.59^{b}	63.21 ±20.63^{b}	34.01 ±18.28^{a}

Different letters within the same file for each variable mean significant differences ($p < 0.01$).IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence; [IAA]: total concentration of iso-α-acids

3.2. Multivariate Statistical Study of Sensory Data Analysis of Commercial Beers

This section indicates the results obtained when statistical analysis of data during sensory analysis by multivariate methods were performed. The variables used are the ones specified in Table 2, except iso-α-acids concentration.

In order to verify the sensory analysis performed to differentiate the three types of beers tested, the data obtained were subjected to discriminant analysis using the 15 sensory descriptors.

Table 3. Standardized discriminant function coefficient of the 15 variables using the type of beers

Sensorydescriptors	F1(69.38%)	F2 (30.62%)
TOS	1.78489	0.83959
IN1	1.72684	-0.609728
CAR	1.65561	0.814311
PER	-1.36785	-0.462571
VIS	1.05726	-0.856188
IN3	-0.808651	0.901546
SWE	0.878669	-0.480778
AST	0.81369	0.195066
IN2	0.64202	-0.766857
MAL	-0.511332	-0.863273
SOUR	-0.485281	0.205017
CARB	0.478968	-0.551714
BIT	0.422803	-0.348431
HOP	0.339474	-0.5729
TUR	0.0644918	-0.171047

IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence

Figure 1 shows the distribution of the three types of beers in the discriminant space, and Table 3 shows the standardized discriminant function coefficient of the 15 variables. By this method, two discriminant functions were obtained. The first one represents 69.38% of variability and the second function 30.62%. The first discriminant function, mostly associated to colour intensity, smell of caramel, smell of toast and persistence, differentiates pale beers, from dark and alcohol free beers. The second discriminant function, mainly related to the viscosity and the intensity of gustatory sensation differentiates dark beers from alcohol free beers. By means of these two discriminant functions, all the beers of the three types are classified correctly. The analysis of the differences in the means for the discriminant functions studied, confirms the results obtained in the discriminant analysis. The study of dispersion regarding each discriminant function shows that there are no differences in the variances of these functions for the three types of beers.

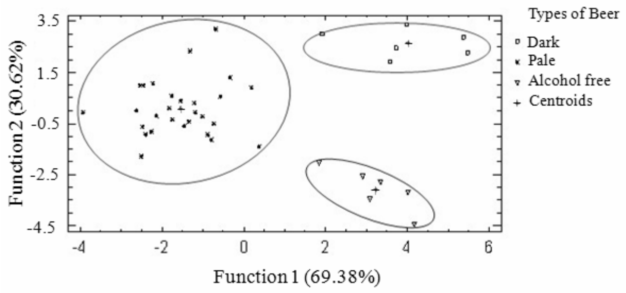

Figure 1. Discriminant function plot for three types of beers using 15 sensory descriptors

Once tested that the sensory parameters studied differentiate the three types of beers, Cluster Analysis and PCA were applied in order to determine whether these parameters are correlated to each other.

Cluster Analysis was performed in order to find natural groupings of correlating variables. Figure 2 represents 4 clusters created from the 679 variables provided by the cluster analysis procedure. The first cluster includes IN1, MAL, TOS, CAR and SWE; the second cluster includes TUR; the third cluster includes HOP, CARB, BIT and SOUR; and the fourth cluster includes IN2, VIS, AST, IN3 and PERS. According to these results, the descriptors included in each cluster are correlated with each other, except turbidity, which is not related to any other descriptor of the sensory analysis.

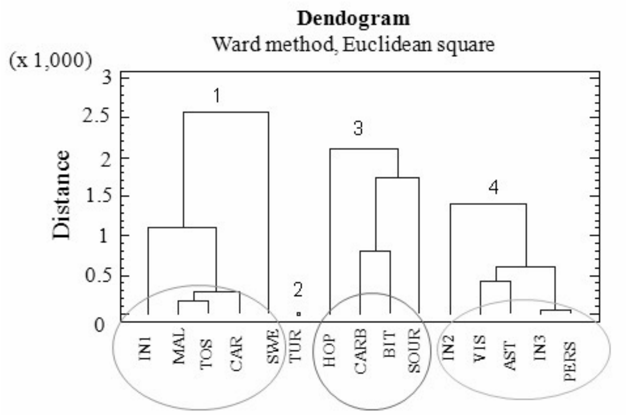

Figure 2. Dendrogram obtained from sensory analysis data of commercial beers using sensory descriptors

In order to corroborate the correlations obtained in the Cluster Analysis, a Correlation Matrix of variables and Principal Component Analysis (PCA) were performed. The PCA analysis seeks to explain data variability through a low number of linear combinations of the 15 variables. Table 4 shows the matrix of correlations between the different descriptors used in sensory analysis. The main components were obtained in these sensory analysis.

Table 4. Correlation matrix between sensory descriptors

	IN1	TUR	HOP	MAL	TOS	CAR	IN2	CARB	VIS	AST	SOUR	BIT	SWE	IN3	PERS
IN1	1														
TUR	0.1	1													
HOP	0.16	-0.05	1												
MAL	0.55	-0.04	0.17	1											
TOS	0.65	0.06	0.11	0.82	1										
CAR	0.53	0.03	0.04	0.78	0.81	1									
IN2	0.51	0.07	0.42	0.55	0.56	0.48	1								
CARB	0.36	0.01	0.52	0.33	0.24	0.26	0.45	1							
VIS	0.58	-0.01	0.35	0.52	0.49	0.44	0.63	0.58	1						
AST	0.49	-0.04	0.43	0.48	0.42	0.33	0.63	0.63	0.78	1					
SOUR	0.24	-0.04	0.52	0.31	0.23	0.24	0.35	0.60	0.34	0.43	1				
BIT	0.47	-0.06	0.5	0.54	0.45	0.40	0.50	0.71	0.7	0.74	0.55	1			
SWE	0.33	-0.06	0.19	0.47	0.43	0.51	0.36	0.34	0.31	0.27	0.33	0.28	1		
IN3	0.54	-0.03	0.39	0.51	0.48	0.40	0.7	0.61	0.77	0.81	0.54	0.76	0.34	1	
PERS	0.53	-0.03	0.38	0.45	0.42	0.32	0.64	0.61	0.76	0.81	0.53	0.74	0.29	0.91	1

IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence

Table 5. Correlation coefficients of main component with sensory analysis descriptors

	Component 1	Component 2	Component 3	Component 4
IN1	0.71	-0.40	-0.16	0.01
TUR	-0.00	-0.16	-0.52	0.66
HOP	0.42	0.60	0.26	0.23
MAL	0.76	-0.48	0.14	-0.05
TOS	0.72	-0.58	0.04	0.01
CAR	0.66	-0.62	0.18	0.05
IN2	0.78	-0.11	-0.08	0.09
CARB	0.69	0.45	0.12	0.19
VIS	0.83	0.08	-0.27	-0.19
AST	0.83	0.26	-0.23	-0.16
SOUR	0.56	0.42	0.40	0.29
BIT	0.82	0.31	-0.03	-0.07
SWE	0.52	-0.29	0.34	0.19
IN3	0.89	0.18	-0.17	-0.12
PERS	0.86	0.25	-0.22	-0.14
Eigenvalues	8.20	2.24	1.19	1.02
% of total variance	51.24	13.99	7.45	6.37
Cumulative % of variance	51.24	65.23	72.69	79.06

IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence

Table 5 contains the loadings for the first principal components with their variances. The main components associated to the PCA are calculated using the values of the matrix correlation. In this case, 4 components with eigenvalues equal to or greater than 1 have been taken into account. The values of the variables are standardized by subtracting their means and dividing by their standard deviations. The correlation of each component can be made with each of the variables using aggregation of variables (descriptors of sensory analysis). The correlation between them is calculated by multiplying the weight of each variable by the value of each component.

The first (PC 1) and second (PC 2) principal components explain 49.82% and 14.88%, respectively, of the total variance. Component 1 accounts for the maximum amount of variability and is positively correlated with all the sensory descriptors, except turbidity. Component 2 is the most responsible for the differentiation, incorporating in its positive part the descriptors related with gustatory sensations -except sweet taste- and in its negative part the colour and smell sensations, except the hop aroma. Components 3 and 4 are mainly correlated with turbidity.

A plot of the results (Figure 3) shows the formation of four groups. Turbidity is not correlated with any other descriptor; it may be due to the insolubilization or precipitation of proteins and carbohydrates during the process of beer brewing or to residual yeast cells from a second fermentation in bottle. The descriptors hop aroma and bitter taste are related with the type and amount of hop used in the elaboration of beers. Astringency, sour taste, persistence, viscosity and intensity are associated with the gustatory sensation. The parameters colour intensity, smell of caramel, smell of toasted, smell of malt, odor intensity and sweet taste show a high degree of correlation as they relate to the feeling and roasted sweet due to the effect of Maillard reaction during malting process and their values are higher in dark beer. The labels of the particular dark beers analysed show that some of them include caramel additive and malt extract which agree with above mentioned sensory perceptions.

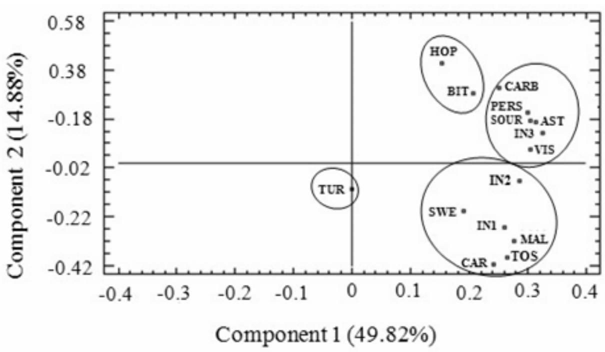

Figure 3. PCA of all sensory descriptors, biplot for the first two principal components

The significant correlations found between the sensory descriptors suggest that it would be necessary to evaluate all of them in order to differentiate the three types of beers. The Stepwise Analysis was applied to the set of descriptors and beers, in order to narrow down the variables, this would allow an accurate differentiation of these types of beers.

In order to limit the number of attributes to be assessed in each sample during the same tasting session, the essential variables required for the differentiation of these three types of beer were determined. Stepwise analysis BMDP was responsible for eliminating 10 of the 15 descriptors. A new discriminant analysis considering only 5 variables showed a correct classification for all the 40 beers analyzed in their corresponding group (Figure 4). As can be seen, a significant weight of those 5 attributes is shown in this differentiation (Table 6).

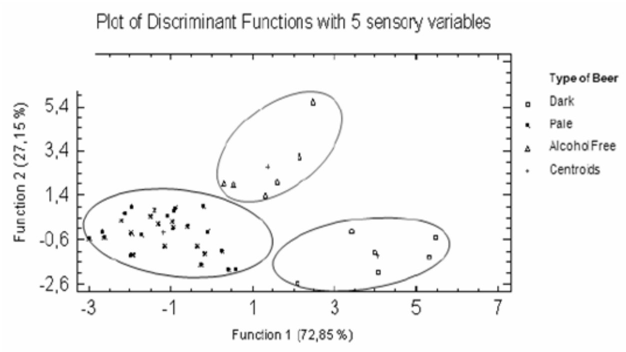

Figure 4. Discriminant function plot for three types of beers using 5 sensory descriptors

Table 6. Standardized discriminant function coefficient of the 5 variables using the type of beers

Sensorydescriptors	F1(72.85 %)	F2 (27.15 %)
IN1	1.34715	0.769059
CAR	1.23198	0.214453
TOS	0.985428	0.746757
PER	-0.762815	-0.0446791
VIS	-0.268117	-0.996996

IN1: Colour intensity; TUR: Turbidity; HOP: smell of Hops; MAL: smell of Malt; TOS: smell of Toasted; CAR: smell of Caramel; IN2: Odour intensity; CARB: Carbonic; VIS: Viscosity; AST: Astringency; SOUR: Sour taste; BIT: Bitter taste; SWE: Sweet taste; IN3: Intensity of gustatory sensation; PERS: Persistence

The first discriminant function shows a greater influence of colour intensity, smell of caramel, smell of toasted and persistence, while in the second function viscosity is the parameter with present more influence.

The dark beers, located in the positive part of the component 1, differ in their higher values of colourintensity, smell of caramel and smell of toasted, and this may be due to the effect of Maillardre action during the malting process. The pale beers, located in the negative part of the component 1 are mainly characterized by their lower persistence. Alcohol-free beers are differentiated by their low viscosity, due to the drying out process which brings not only the loss of alcohol, but also colloidal components[48].

Reducing to five the number of sensory descriptors to differentiate these types of lager beer suggests that the rest of the descriptors are either not needed for the differentiation or correlated with each other, as we have seen in Cluster and PCA analyses. The results obtained allow establishing a very fast and effective sensory methodology to distinguish these three types of beers, and it is a valuable business tool for its simplicity and easy implementation.

3.3. Correlation of Sensory Descriptors and Iso-α-Acids Content in Commercial Lager Beers

In the analysis of the correlations between iso-α-acids concentration and sensory analysis descriptors, only hop aroma, sour taste and bitter taste descriptors have been taken into account because they are those which may be related to the bitter substances derived from the isomerisation of α-acids produced by hops [48]. According to Denise, Baxter and Hughes [49], the threshold of sensory detection of iso-α-acids is about 5 mg/L, concentration widely exceeded in the lager beer evaluates (Table 1).

The results obtained show that there is no correlation between the concentration of iso-α-acids and hop aroma and sour taste sensory variables. However we have found a linear relationship between the concentration of iso-α-acids and bitter taste in dark, pale and alcohol free commercial lager beers (Figure 5). The equations which account for it are:

Black: Bitter taste = 0,023 [IAA] + 4,19 Eq. 1

Pale: Bitter taste = 0,012 [IAA] + 4,35 Eq. 2

Alcohol-free: Bitter taste = 0,007 [IAA] + 3,99 Eq. 3

The equality slopes test provides a non-significative p-value (p>0.05), although a clear tendency can be observed when comparing slopes. Black beers' slope is greater than that of pale beers, and the latter's greater than that of free-alcohol beers. In spite of the randomness in beer samples selection for this work, the results illustrated in Figure 5 clearly show that black beers are associated with higher bitterness values, whilst, free-alcohol beers are associated with lower bitterness values, values which are clearly separated.

The correlations obtained in Figure 5 show the relevance of HPLC data in predicting lager beer bitterness.

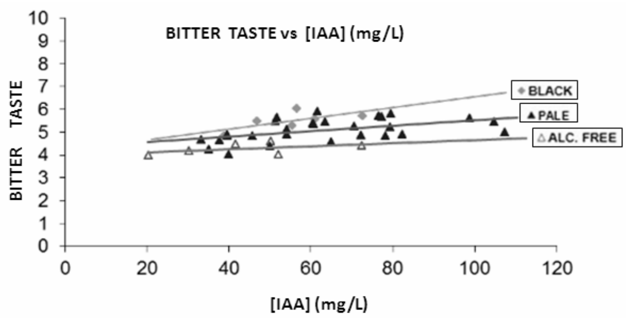

Figure 5. Correlation between the concentration of iso-α-acids and bitter taste

4. Conclusion

This work has allowed not only a reduction in variables to establish a very fast and effective sensory methodology to distinguish these three types of beers, but it also a model to predict an approximate content of iso-α-acids through a simple correlation with bitter taste. Furthermore, this study will allow an approximate estimation of iso-α-acids in other types of beers which would contribute to an effective control of production processes in the brewing industry.

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