

1 **DISCRIMINATION BASED ON COMMERCIAL/CRAFT ORIGIN AND ON LAGER/ALE**
2 **FERMENTATION OF UNDILUTED SPANISH BEER SAMPLES- FRONT-FACE**
3 **EXCITATION-EMISSION MATRICES AND CHEMOMETRICS**

4
5
6 **Abstract:** Spanish beer samples of different origin (craft and commercial) and with different type of
7 fermentation (ale and lager) were analyzed employing molecular fluorescence. Excitation-emission
8 matrices were obtained of the bulk samples, employing front-face mode. PARAFAC was performed on
9 the data, obtaining a 6-components model that could be related to the fluorescent components of the beer.
10 A statistical analysis of the scores was carried out, from which it was deduced that the commercial
11 samples are more similar to each other than the craft ones. In addition, ale-fermented samples tend to
12 differ more between them than lager-fermented samples. Finally, an attempt was made to relate the iso-
13 α -acid component to the bitterness values of the samples. This study describes the fluorophores of
14 Spanish beers, and has allowed its qualitative characterization on the basis of their origin, type of
15 fermentation and degree of bitterness.

16
17
18 **Keywords:** beer; EEM; fluorescence; front-face; PARAFAC; chemometrics; ale; lager; craft;
19 commercial

21 1. Introduction

22 Beer is the beverage resulting from the alcoholic fermentation (by means of selected yeasts) of a
23 brewing wort, which transforms the sugars present into ethanol and carbon dioxide. This wort is obtained
24 after aqueous extraction and enzymatic saccharification of ground malt or its extracts, and then, it is
25 clarified and hops and/or derivatives are added, before following a boiling process. Other sources of
26 starch may be used as long as the malt is at least 50% by weight of the raw material (Bamforth, 2005;
27 Boletín Oficial del Estado, 2022; Grupo Mahou - San Miguel, 2021; Makwana & Hati, 2019). Thus,
28 although the brewing process is a complex procedure with several phases, only the four main stages of it
29 will be discussed, to allow the reader an overview of the procedure. These main stages are:

- 30 • Preparation of the malt by controlled germination and roasting of the cereal grains.
- 31 • Aqueous extraction of the malt, which results in obtaining the "sweet wort", rich in sugars and
32 soluble nitrogen compounds.
- 33 • Addition of hops and/or derivatives and boiling of the wort, after clarification and filtration.
- 34 • Alcoholic fermentation of the wort, by means of which the yeasts transform the sugars present
35 into ethanol and carbon dioxide. In the same way, parallel reactions will generate other
36 compounds that will contribute to the aroma and flavour of the beer.

37 Beers are classified according to the type of fermentation they undergo. Although there are many
38 different types of beers, the most basic classification divides beers into *lagers* and *ales*. *Lager* beers (also
39 called bottom-fermented beers) are those that are fermented and matured at a low temperature (6 – 15°C).
40 The yeasts used for their production are usually *Saccharomyces* hybrids, which tend to precipitate at the
41 bottom of the wort. *Lagers* are the most widely consumed beers in the world, accounting for 90% of
42 global consumption during the last years (Callejo et al., 2020; Li et al., 2017; Radonjić et al., 2020). Their
43 organoleptic characteristics preserve the flavour of the raw materials, offering a beverage with a sweet
44 smell from the malt and a fresh taste from the hops. On the other hand, *Ale* beers, also called high-

45 fermented or top-fermented, owe their name to the fact that their fermentation takes place at higher
46 temperatures than *lagers* (temperatures between 15 and 24°C, compared to temperatures of 6 – 15°C
47 aforementioned). This type of beer is also called "top-fermented" because the yeast most commonly used
48 for its production (*Saccharomyces cerevisiae*), does not precipitate, but instead (due to its
49 hydrophobicity) rise up to the surface of the wort with the carbon dioxide gas bubbles, generating, in
50 addition, a surface layer of foam. The taste of these beers is fruitier than the *lagers*, due to the presence
51 of more esters and other secondary flavour and aroma compounds. However, an excessive amount of
52 these compounds would generate a solvent smell, so their concentration must be controlled. Furthermore,
53 there are synergies between the different esters, so, although the detection threshold of each ester is low,
54 they are all important in the final flavor (Callejo et al., 2020; Li et al., 2017; Radonjić et al., 2020; Tang
55 & Li, 2017).

56 Spain is the **third** largest beer producer at European level (2020 and 2021 data), and the **eighth**
57 country at European level in number of breweries (2020 data). With respect to consumption, Spain **ranked**
58 **third** within Europe in 2020 and 2021 (Cerveceros de España y Ministerio de Agricultura Pesca y
59 Alimentación, 2021; Statista, 2021; The Brewers of Europe, 2021). Due to this great socio-economic
60 importance of beer in Spain, there are a large number of companies dedicated to its production. In
61 addition, this importance has encouraged the creation of many small craft breweries.

62 The creation of these craft breweries brings with it a problem regarding the definition of "craft
63 beer", since the conditions for being considered as such vary from one country to another. In Spain, the
64 Official State Gazette defines craft beers as "those beers whose brewing process follows the quality
65 standard, and are produced entirely in the same facility, under the direction of a master brewer or
66 craftsman with demonstrable experience, and giving priority to the human factor over the mechanical,
67 thus obtaining an individualized final result, not produced in large series and following the applicable
68 legislation on craftsmanship" (Boletín Oficial del Estado, 2022). As this definition does not establish the
69 total production that companies can obtain nor the independence of large brewing groups (as does the

70 legislation of other countries), in 2014 the AECAI (Spanish Association of Independent Craft Brewers)
71 was founded. The objective of this association is to define and regulate the craft beer sector, as well as
72 to promote the quality, culture and variety of craft beer, defending the common interests of the sector
73 (AECAI, 2021).

74 **On another note, and with respect to one of the techniques that has been used for the analysis of**
75 **samples of beer,** fluorescence spectroscopy is a non-destructive instrumental technique, with a high
76 sensitivity and selectivity, that provides a lot of information about molecular structure. For these reasons,
77 it has been widely employed for foodstuff characterization. In addition, this technique is fast and easy-
78 to-use, and can be easily implemented in industry (Airado-Rodríguez et al., 2011; Azcarate et al., 2017;
79 Callejón et al., 2012; Carbonaro et al., 2019; Ríos-Reina et al., 2019).

80 Fluorescence spectroscopy can be used in different ways (i.e.: by obtaining spectra or by using it
81 as a detector for other analytical techniques), but one of the most useful ways is the obtention of
82 excitation-emission matrices (EMMs), as they allow a large amount of information to be gathered from
83 the system under study, which can be then analysed using chemometric logarithms. Parallel Factor
84 Analysis (PARAFAC) is the most commonly used second-order logarithm for the decomposition of
85 EMMs, and has been used for several types of food matrices (Airado-Rodríguez et al., 2011; Azcarate
86 et al., 2017; Callejón et al., 2012).

87 The fluorescence of beer has been studied previously and it has been found that it is mainly due
88 to the presence of iso- α -acids (compounds from hops which are responsible for the typical bitterness of
89 beer), different vitamins (mainly group B), aromatic amino acids (in particular tyrosine, tryptophan and
90 phenylalanine) and phenolic compounds (Dramićanin et al., 2019; Fang et al., 2021; Pale et al., 2021;
91 Sikorska et al., 2008).

92 Various authors have used beer fluorescence together with chemometric tools for the
93 characterization and/or classification of different beers. Thus, Sikorska et al. (2004) employed total
94 luminescence and scanning synchronous fluorescence spectra to characterize and differentiate 8 different

95 beer samples. On the other hand, the use of this same method together with the application of Principal
96 Component Analysis (PCA), Linear Discriminant Analysis (LDA) and kNN (Nearest Neighbour
97 Method) allowed the clustering of different beer samples based on different storage conditions (Sikorska
98 et al., 2006). Finally, this same group also achieved the simultaneous analysis of riboflavin and aromatic
99 amino acids in beer by means of front-face fluorescence coupled to different PLS (Partial Least Squares)
100 algorithms (Sikorska et al., 2009). Besides that, Gordon et al. (2017) studied different samples of
101 Australian beer by synchronous fluorescence spectroscopy and EEMs, achieving the classification of the
102 samples based on the brand; while Braga et al. (2021) compared the possible classification of malted and
103 unmalted beer samples using different spectroscopic techniques (i.e.: infrared, UV-VIS and
104 fluorescence), differentiating both types of beer by means of fluorescence spectroscopy associated to
105 Self-Organizing Maps (SOM). Fang et al. (2021), used EEMs and different chemometric algorithms to
106 classify different brands of Chinese *lagers* beers, using three- and four-way data, **comparing the results**
107 **obtained employing each data set**. Finally, Pale et al. (2021) used EEMs and scanning synchronous
108 fluorescence together with PARAFAC, PCA and LDA to differentiate a set of 49 samples, and monitor
109 the effects of their storage at different temperatures and with different exposures to sunlight.

110 **However, to the best of our knowledge, no one has studied the front-face EEMs of Spanish beers,**
111 **and, neither beers from different origins and with different fermentation styles have been studied**
112 **simultaneously. For this reason, the aim of this research has been to analyse and compare the EEMs**
113 **obtained from Spanish beers belonging to different classes (craft and commercial) and with different**
114 **types of fermentation (*ale* and *lager*), in order to be able to describe their fluorescent components, as**
115 **well as classify them into these categories based on their fluorescent profile.**

116

117 **2. Materials and Methods**

118 **2.1 Beer samples**

119 Beer samples employed in this study were purchased from different local shops and stored at room
120 temperature, protected from direct light, until their analysis. A total of 45 samples were analysed, of
121 which 23 were craft beers and 22, commercial beers. On the other hand, 22 samples were *ale*-fermented,
122 while the other 23 samples were *lager*-type. Table 1 lists the samples used, together with their respective
123 information. Before the analysis, the bottles/cans were opened and approximately 10 mL of beer were
124 placed in a beaker. Degassing of the samples was carried out by applying ultrasounds for 5 minutes.

125

126 ***2.2 Instrumentation and software***

127 An Ultrasons bath (reference 3000683) purchased from J. P. Selecta (Spain) was used for degassing
128 beer samples before the EEMs obtention.

129 EEMs were obtained in a Cary Eclipse Varian spectrofluorometer (Agilent Technologies Inc.,
130 USA) connected to a PC by means of a GPIB488 card. The instrument was equipped with two Czerny-
131 Turner monochromators, a constant xenon light source and a photomultiplier tube as detector. Equipment
132 control and data acquisition were carried out employing Cary Eclipse's own software.

133 Data was processed employing MatLab R2016B (The MathWorks Inc., USA). The correction of
134 the Rayleigh dispersion was made employing the EEM_corr routine (Chiappini et al., 2019), freely
135 downloaded from <https://fcb.web1.unl.edu.ar/laboratorios/ladaq/download/>. PARAFAC was carried
136 out using the graphical interface MVC2 (Olivieri et al., 2009), available at [www.iquir-
137 conicet.gov.ar/descargas/mvc2.rar](http://www.iquir-conicet.gov.ar/descargas/mvc2.rar). Statistical analysis of the PARAFAC scores obtained was carried out
138 using XLSTAT software (Addinsoft, France).

139

140 ***2.3 Excitation-emission matrices obtention***

141 The EEMs were obtained from the degassed undiluted beer samples. Measurements were made
142 in a 1.0 cm quartz cell, at room temperature. The excitation wavelength ranged between 250 and 500 nm,
143 with steps of 5 nm; while the emission spectra was registered every 2 nm, between 300 and 600 nm.

144 The excitation and emission slits were set at 5 nm, and the voltage of the lamp was established at
145 600 V. The scan rate employed was 1200 nm·min⁻¹, so each EEM took less than 14 minutes to be
146 completely registered.

147

148 **3. Results and discussion**

149 ***3.1 Optimization of the measurement's conditions and data treatment***

150 Before the measurement of the beer samples, some experiments were made to optimize the
151 instrumental conditions, employing a commercial, *lager* beer. First, an EEM was recorded over a wide
152 range of excitation (200 – 500 nm, each 5 nm) and emission wavelengths (250 – 700 nm, each 1 nm) to
153 find out where the fluorescence of the corresponding beer fluorophores appeared. Slits, acquisition speed
154 and voltage were left at their default values.

155 Once the matrix was obtained, the area with relevant information was checked and the
156 corresponding excitation (250 – 500 nm, each 5 nm) and emission (300 – 600 nm; each 2 nm) wavelength
157 ranges were chosen. The slits and voltage were left at their default values (5 nm for both excitation and
158 emission slits, and 600 V, respectively). The acquisition speed was set to 1200 nm·min⁻¹.

159 After obtaining the EEMs, and although Rayleigh scattering did not directly affect any of the areas
160 of interest in the matrices, the EEM_corr routine was applied to eliminate its presence in order to perform
161 a better analysis of the EEMs. Correction of the first level Rayleigh scattering (Ry1) was done by
162 removing 10 nm, while removal of the second (Ry2) required a width of 13 nm. The interpolation option
163 was used to try to alter the matrix as little as possible.

164

165 ***3.2 Beer samples fluorescence***

166 Under the optimised conditions, the excitation-emission matrices of the 45 beer samples were
167 recorded without any pre-treatment. Figure 1 shows as examples the EEMs obtained from two types of
168 beer (A: craft beer with *ale* fermentation; B: commercial beer with *lager* fermentation). As can be seen,

169 **their shape is very similar, although the intensity of each zone depends on the type of beer analysed.** In
170 any case, there are two distinct zones: a very intense zone between 300 - 600 nm of emission, with a
171 maximum excitation between 400 - 450 nm; and a second, much less intense zone in the range of 350 -
172 400 nm of emission, with a maximum excitation of approximately 275 nm. This shape is similar to those
173 obtained by other authors in previous works (Fang et al., 2021).

174 After visual analysis of the 45 matrices, it was found that the fluorescence intensity of samples 5,
175 18 and 40 was very low (Figure 2). As can be seen in the Figure, the fluorescence intensity of sample 5
176 is 25% of the intensity of the EEMs shown in Figure 1, while the intensity of samples 18 and 40 barely
177 exceed 10% of the fluorescence intensity of the other samples. This great difference with the rest of the
178 samples influenced the chemometric and statistical analyses, making it difficult to differentiate the rest
179 of the samples between them. Therefore, **they were removed and** the chemometric and statistical analysis
180 performed was applied to a set of 42 samples.

181 The low fluorescence intensity of these samples may be due to their specific characteristics. Sample
182 5 was a craft beer with *lager*-type fermentation, which, at the end of fermentation, was mixed with Pedro
183 Ximénez wine, thus altering its composition and, therefore, its fluorescent fingerprint. Sample 18, on the
184 other hand, was a commercial *ale*-type beer, but of a special type (*stout*), which generates darker and
185 more complex beers. Finally, sample 40 was a craft *ale*-beer that used acorns together with barley for the
186 preparation of the malt, which explains the difference in the fluorescent component profile compared to
187 the other samples.

188

189 ***3.3 PARAFAC analysis of the samples***

190 PARAFAC was performed on the matrices to obtain the main components causing the
191 fluorescence of the beer samples. Taking into account that neither the concentration of the possible
192 fluorophores nor the fluorescence spectra can take negative values, the non-negativity constraint was
193 applied in all modes.

194 The selection of the optimal value of components was made bearing in mind the core consistency
195 diagnosis (CORCONDIA), residual analysis (S_{fit}) and the physiognomy of the loadings. The
196 CORCONDIA criterion takes as optimal value the value prior to the number of components that generate
197 a drop in core consistency below 50% (Bro, 1997), while S_{fit} criterion optimize the number of
198 components as the one at which the value of S_{fit} becomes approximately constant (Bro y Kiers, 2003).
199 Taking into account both criterions, and the shape of the loadings, six was selected as the optimal number
200 of components. The contour maps of these components are shown in Figure 3.

201 Taking into account the excitation-emission maxima, the first component can be associated with
202 the iso- α -acid group. These compounds are derived from hops and are responsible for the bitter taste of
203 beer. The second, third and fourth components possess fluorescent characteristics similar to those of the
204 different forms of vitamin B. In particular, these compounds can be related to riboflavin (vitamin B2),
205 niacin (vitamin B3) and pyridoxine (vitamin B6), respectively. On the other hand, sixth component shares
206 excitation-emission wavelengths with the fluorescent amino acids present in beer (i.e.: tyrosine,
207 tryptophan and phenylalanine, represented by the first of them). Finally, the fifth component is the one
208 with the least clear fluorescent characteristics, but could be related to the different phenolic compounds
209 and other fluorescent forms of B vitamins. These components are in agreement with the literature
210 (Dramićanin et al., 2019; Fang et al., 2021; Sikorska et al., 2008).

211

212 ***3.4 Statistical study of the PARAFAC scores***

213 ***3.4.1 Principal Component Analysis***

214 Once the different components were obtained, the scores were statistically analysed. First, a PCA
215 was performed, in order to further reduce the information and obtain the most important fluorophores.
216 Two principal components explained 90% of the variance of the data. Within these, the first Principal
217 Component was mainly influenced by the positive contribution of riboflavin (40.9%), phenolic
218 compounds (18.9%) and iso- α -acids (18.0%) concentration. On the other hand, the second Principal

219 Component was mainly influenced by the positive contribution of the concentration of iso- α -acids
220 (44.8%) and niacin (31.9%). Under these conditions, the resulting score plot can be seen in Figure 4.

221 Although samples were not fully separated into groups based on their class (craft - commercial) or
222 their fermentation type (*ale* - *lager*), some information can be obtained from the PCA. On the one hand,
223 *ale* samples are mainly clustered in the right half of the plot, i.e., positive contribution of Principal
224 Component 1; while *lager* samples appear in the left half of the plot (lower contribution of Principal
225 Component 1). Therefore, it could be said that *ale* fermented beer samples generate a richer profile in
226 riboflavin, phenolic compounds and iso- α -acids than *lager* ones. Also, *ale* samples are more distributed
227 along the plot, indicating a greater difference between them. The *lager* samples, on the opposite, are
228 slightly more clustered.

229 On the other hand, it can also be noted that the commercial samples are more similar between them
230 than the craft samples (blue dots are more grouped than orange ones). This can be explained taking into
231 account one of the characteristics of craft beers, which are sold as more special beers with a greater
232 number of nuances than the commercial ones, due to the specific blends created by each brewmaster.

233

234 **3.4.2 Discriminant analysis**

235 In addition to PCA, a Discriminant Analysis (DA) was performed on the PARAFAC scores. First,
236 two analyses were performed on all data, trying to differentiate the samples based on their class
237 (craft/commercial) and fermentation type (*ale/lager*), respectively. In both cases a single factor was
238 necessary for the classification of the samples, consisting of the positive contribution of the
239 concentrations of riboflavin, niacin and phenolic compounds. The main difference between the
240 differentiating factor in both analyses was that, for the differentiation based on class, the concentration
241 of the other components (iso- α -acids, pyridoxine and amino acids) did not affect; whereas in the

242 differentiation based on fermentation type, these components contributed negatively to the differentiating
243 factor.

244 As can be seen in Table 2, in both cases a good classification rate was obtained in the estimation
245 samples (76.19 - 83.33%), which was reduced when cross-validation was performed (64.29%). It is
246 important to note that, in the differentiation based on class, the commercial samples were better assigned
247 than the craft samples, where there was more confusion. This is in line with what was seen in the PCA,
248 where commercial samples were found to be more similar to each other than craft ones. On the other
249 hand, in the differentiation based on fermentation type, there was not much difference between the
250 assignment of the two types.

251 DA were carried out within each of the categories, too. Thus, in the DA of the samples with *lager*-
252 type fermentation, a 100% correct classification was obtained (both of the estimation sample and after
253 cross-validation) of the samples based on their craft or commercial origin. Similarly, in the DA of the
254 samples of craft origin, samples were fully differentiated on the basis of their fermentation type, with a
255 100% correctness both before and after cross-validation. These results confirm that craft samples are
256 more different from each other than commercial ones, as seen previously in section 3.4.1. Furthermore,
257 greater differences are also observed in the samples with *lager* fermentation, as the *ale* samples could
258 not be differentiated on the basis of their class. This may be due to the fact that the craft yeasts used in
259 *ale* fermentation are very similar to those used in the industry (mainly *Saccharomyces cerevisiae*),
260 whereas there seems to be more variety in the *lager* yeasts (*Saccharomyces* hybrids).

261

262 ***3.5 Correlation between EEMs and beer bitterness***

263 After PARAFAC, and taking into account that Principal Component 1 seemed to represent the iso-
264 α -acid group, it was tried to correlate these data with the IBU values of the different samples (Table 1).

265 IBU (International Bitterness Units) are the unit of measurement for the degree of bitterness of a
266 beer, which corresponds to the milligrams of iso- α -acids per litre. Commonly, IBU are measured by

267 spectrophotometric analysis of the iso-octane acidified extract of the beer, measuring the absorbance at
268 275 nm (Jaskula et al., 2007). In our case, the IBU values of the samples were obtained directly from the
269 brewing companies and/or from websites specialised in compiling information on this type of products.

270 Thus, the values of the Principal Component 1 scores were plotted against the IBU values (Figure
271 5). As can be seen in the Figure, there is not a good correlation between the values, but certain areas can
272 be differentiated. The red circle in the upper area separates craft samples with IPA (Indian Pale Ale)
273 fermentation (average IBU of 70), while the blue circle slightly lower down separates the commercial
274 samples with the same type of fermentation (average IBU of 40.5). As can be seen, the samples with
275 IPA-type fermentation produce much more bitter beers. On the other hand, the average bitterness values
276 of the other beer types are quite similar: the rest of the craft beers with *ale* fermentation (orange circle)
277 have an average IBU of 20, the craft beers with *lager* fermentation (yellow circle) have an average IBU
278 of 22 and the commercial samples with *lager* fermentation (green circle) have an average IBU value of
279 22.5. Although due to this resemblance these samples appear quite mixed in the graph, a slight grouping
280 of samples of the same style can be observed.

281

282 **4. Conclusions**

283 EEMs have been obtained from 45 undiluted beer samples (craft and commercial, *ale* and *lager*
284 fermented), without applying any type of pre-treatment, employing front-face **fluorescence** technique.
285 All the samples analyzed are from Spain and, to the best of our knowledge, this is the first time that the
286 fluorescence of beers with variation in their fermentation type and class has been studied simultaneously.

287 Due to the low fluorescence intensity of three beers, PARAFAC was performed on the remaining
288 42 samples. A 6-components model was obtained, which could be related with the different fluorescent
289 components of the beer, based on the excitation and emission maxima obtained.

290 The PARAFAC scores obtained were statistically analyzed. Firstly, a PCA was carried out, in
291 which, although a total differentiation of the samples was not achieved, it could be concluded that

292 commercial samples are more similar to each other than craft samples, and that samples with *ale*
293 fermentation tend to be more different from each other than those with *lager* fermentation.

294 Then, different DA were carried out. Using the total number of samples, differentiation based on
295 fermentation type and type of fermentation generated a 64.29% success rate after cross-validation. On
296 the other hand, within each category, it was found that samples with *lager* fermentation can be fully
297 classified into craft and commercial. Similarly, craft samples can be fully classified on the basis of their
298 fermentation type. These results confirmed the information obtained by PCA.

299 Finally, taking into account that Principal Component 1 corresponded to iso- α -acids (responsible
300 for bitterness), an attempt was made to correlate its scores with the IBU values of the analyzed samples.
301 Although a good correlation was not achieved, the higher bitterness of the samples with IPA-type
302 fermentation was confirmed, as well as a slight grouping of the samples based on these values.

303 The study of the samples allowed the description of the fluorophores responsible for the
304 fluorescence in Spanish beers, as well as the qualitative differentiation of the samples based on their
305 class, type of fermentation or degree of bitterness.

306

307 **Acknowledgements**

308 Authors are grateful to grant PID2020-112996GB-I00 funded by MCIN/AEI/
309 10.13039/501100011033, and Junta de Extremadura (Ayuda a Grupos GR21048, Proyecto IB20016)
310 both co-funded by European Funds for Regional Development for financial support.

311

312 **References**

- 313 AECAI, 2021. Asociación Española de Cerveceros Artesanos e Independientes [WWW Document].
314 URL <https://aecai.es/> (last access 2022-06-30).
- 315 Airado-Rodríguez, D., Durán-Merás, I., Galeano-Díaz, T., Wold, J.P., 2011. Front-face fluorescence
316 spectroscopy: A new tool for control in the wine industry. *J. Food Compos. Anal.* 24, 257-264.
317 <https://doi.org/10.1016/j.jfca.2010.10.005>
- 318 Azcarate, S.M., Teglia, C.M., Karp, F., Camiña, J.M., Goicoechea, H.C., 2017. A novel fast quality
319 control strategy for monitoring spoilage on mayonnaise based on modeling second-order front-
320 face fluorescence spectroscopy data. *Microchem. J.* 133, 182-187.
321 <https://doi.org/10.1016/j.microc.2017.03.036>
- 322 Bamforth, C.W., 2005. Capítulo 2 - La cerveza, en: *Alimentos fermentación y microorganismos*.
323 Editorial Acribia S.A., pp. 42-47.
- 324 Boletín Oficial del Estado, 2022. Código de la cerveza.
- 325 Braga, F.L., Braga, S., 2021. Fast pattern recognition of malted and unmalted beer: An investigation
326 using FTIR, UV-VIS, fluorescence spectroscopy and chemometrics. *Sci. Agropecu.* 12, 361-367.
327 <https://doi.org/10.17268/SCI.AGROPECU.2021.039>
- 328 Bro, R., 1997. PARAFAC. Tutorial and applications. *Chemom. Intell. Lab. Syst.* 38, 149-171.
329 [https://doi.org/10.1016/S0169-7439\(97\)00032-4](https://doi.org/10.1016/S0169-7439(97)00032-4)
- 330 Bro, R., Kiers, H.A.L., 2003. A new efficient method for determining the number of components in
331 PARAFAC models. *J. Chemom.* 17, 274-286. <https://doi.org/10.1002/cem.801>
- 332 Callejo, M.J., Tesfaye, W., González, M.C., Morata, A., 2020. Craft beers: current situation and future
333 trends. *New Adv. Ferment. Process.* <https://doi.org/10.5772/intechopen.90006>
- 334 Callejón, R.M., Amigo, J.M., Pairo, E., Garmón, S., Ocaña, J.A., Morales, M.L., 2012. Classification
335 of Sherry vinegars by combining multidimensional fluorescence, PARAFAC and different
336 classification approaches. *Talanta* 88, 456-462. <https://doi.org/10.1016/j.talanta.2011.11.014>

337 Carbonaro, C.M., Corpino, R., Chiriu, D., Ricci, P.C., Rivano, S., Salis, M., Tuberoso, C.I.G., 2019.
338 Exploiting combined absorption and front face fluorescence spectroscopy to chase classification:
339 A proof of concept in the case of Sardinian red wines. *Spectrochim. Acta - Part A Mol. Biomol.*
340 *Spectrosc.* 214, 378-383. <https://doi.org/10.1016/j.saa.2019.02.041>

341 **Cerveceros de España, Ministerio de Agricultura Pesca y Alimentación, 2021. Informe socioeconómico**
342 **del sector de la cerveza en España 2021.**

343 Chiappini, F.A., Alcaraz, M.R., Goicoechea, H.C., Olivieri, A.C., 2019. A graphical user interface as a
344 new tool for scattering correction in fluorescence data. *Chemom. Intell. Lab. Syst.* 193.
345 <https://doi.org/10.1016/j.chemolab.2019.07.009>

346 Dramićanin, T., Zeković, I., Periša, J., Dramićanin, M.D., 2019. The Parallel Factor Analysis of Beer
347 Fluorescence. *J. Fluoresc.* 29, 1103-1111. <https://doi.org/10.1007/s10895-019-02421-0>

348 Fang, H., Wu, H.L., Wang, T., Long, W.J., Chen, A.Q., Ding, Y.J., Yu, R.Q., 2021. Excitation-
349 emission matrix fluorescence spectroscopy coupled with multi-way chemometric techniques for
350 characterization and classification of Chinese lager beers. *Food Chem.* 342, 128235.
351 <https://doi.org/10.1016/j.foodchem.2020.128235>

352 Gordon, R., Cozzolino, D., Chandra, S., Power, A., Roberts, J.J., Chapman, J., 2017. Analysis of
353 australian beers using fluorescence spectroscopy. *Beverages* 3.
354 <https://doi.org/10.3390/beverages3040057>

355 Grupo Mahou - San Miguel, 2021. Cervecistas [WWW Document]. URL
356 <https://www.loscervecistas.es/> (last access 2022-06-30).

357 Jaskula, B., Goiris, K., De Rouck, G., Aerts, G., De Cooman, L., 2007. Enhanced quantitative
358 extraction and HPLC determination of hop and beer bitter acids. *J. Inst. Brew.* 113, 381-390.
359 <https://doi.org/10.1002/j.2050-0416.2007.tb00765.x>

360 Li, Q., Wang, J., Liu, C., 2017. Chapter 12 - Beers, en: *Current Developments in Biotechnology and*
361 *Bioengineering: Food and Beverages Industry.* Elsevier B.V., pp. 305-351.

362 <https://doi.org/10.1016/B978-0-444-63666-9.00012-1>

363 Makwana, M., Hati, S., 2019. Chapter 1 - Fermented beverages and their health benefits, en: Fermented
364 Beverages: Volume 5. The Science of Beverages. Woodhead Publishing Limited, pp. 1-29.
365 <https://doi.org/10.1016/B978-0-12-815271-3.00001-4>

366 Olivieri, A.C., Wu, H.L., Yu, R.Q., 2009. MVC2: A MATLAB graphical interface toolbox for second-
367 order multivariate calibration. Chemom. Intell. Lab. Syst. 96, 246-251.
368 <https://doi.org/10.1016/j.chemolab.2009.02.005>

369 Pale, W.-Y., Djiedeu, N., Lissouck, D., Mbogning, W.F., Issac, A., Owono, L.C., Kenfack, C.A., 2021.
370 Impact of temperature and sunlight exposition on locally brewed beers composition revealed by
371 fluorescence spectroscopy coupled with chemometric methods. J. Food Sci. 86, 5175-5187.
372 <https://doi.org/10.1111/1750-3841.15962>

373 Radonjić, S., Maraš, V., Raičević, J., Košmerl, T., 2020. Wine or beer? Comparison, changes and
374 improvement of polyphenolic compounds during technological phases. Molecules 25, 4960-4995.
375 <https://doi.org/10.3390/molecules25214960>

376 Ríos-Reina, R., Ocaña, J.A., Azcarate, S.M., Pérez-Bernal, J.L., Villar-Navarro, M., Callejón, R.M.,
377 2019. Excitation-emission fluorescence as a tool to assess the presence of grape-must caramel in
378 PDO wine vinegars. Food Chem. 287, 115-125. <https://doi.org/10.1016/j.foodchem.2019.02.008>

379 Sikorska, E., Gliszczyńska-Świgło, A., Insińska-Rak, M., Khmelinskii, I., De Keukeleire, D., Sikorski,
380 M., 2008. Simultaneous analysis of riboflavin and aromatic amino acids in beer using fluorescence
381 and multivariate calibration methods. Anal. Chim. Acta 613, 207-217.
382 <https://doi.org/10.1016/j.aca.2008.02.063>

383 Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., De Keukeleire, D., 2006. Monitoring beer
384 during storage by fluorescence spectroscopy. Food Chem. 96, 632-639.
385 <https://doi.org/10.1016/j.foodchem.2005.02.045>

386 Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., De Keukeleire, D., 2004. Fluorescence

387 spectroscopy for characterization and differentiation of beers. *J. Inst. Brew.* 110, 267-275.
388 <https://doi.org/10.1002/j.2050-0416.2004.tb00621.x>

389 Sikorska, E., Khmelinskii, I., Górecki, T., Sikorski, M., 2009. Evaluation of beer aging using its
390 autofluorescence. *Acta Hortic.* 848, 299-306. <https://doi.org/10.17660/ActaHortic.2009.848.31>

391 **Statista, 2021. La industria de la cerveza en España.**

392 **Tang, K., Li, Q., 2017. Chapter 11 - Biochemistry of wine and beer fermentation, en: Current**
393 **Developments in Biotechnology and Bioengineering: Food and Beverages Industry. Elsevier B.V.,**
394 **pp. 281-304. <https://doi.org/10.1016/B978-0-444-63666-9.00011-X>**

395 **The Brewers of Europe, 2021. European beer trends - Beer statistics report 2021.**

396

FIGURE CAPTIONS

Figure 1. Contour maps of two example beers. A) Craft beer with ale-type fermentation. B) Commercial beer with lager-type fermentation.

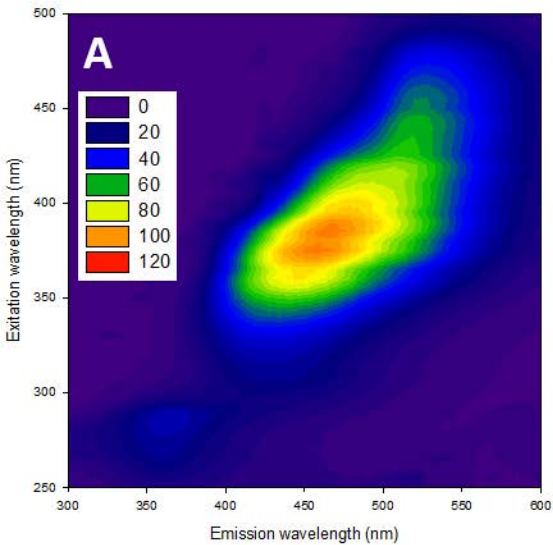
Figure 2. Contour maps of the three samples (5, 18 and 40) removed from the study due to their low fluorescence intensity.

Figure 3. Contours maps of the six components obtained by PARAFAC. Legend is common to all of them (arbitrary units of fluorescence intensity).

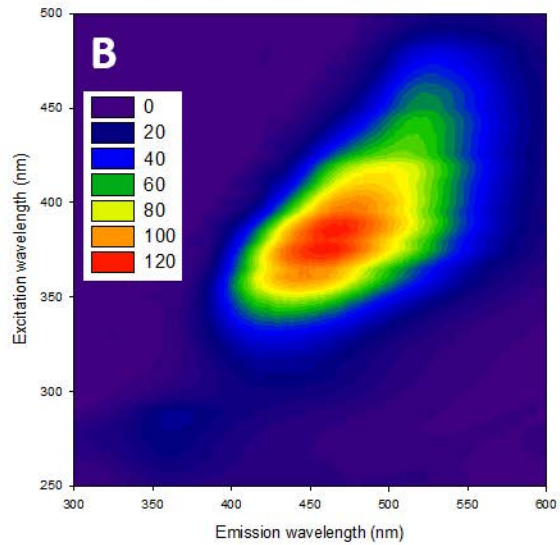
Figure 4. PCA scores plot obtained. Craft samples are marked in orange and commercial samples in blue. Samples with the filled marker are ale fermentation samples, while those with the unfilled marker are lager fermentation samples.

Figure 5. Principal Component 1 versus IBU plot. Craft samples are marked in orange and commercial samples in blue. Samples with the filled marker are ale fermentation samples, while those with the unfilled marker are lager fermentation samples.

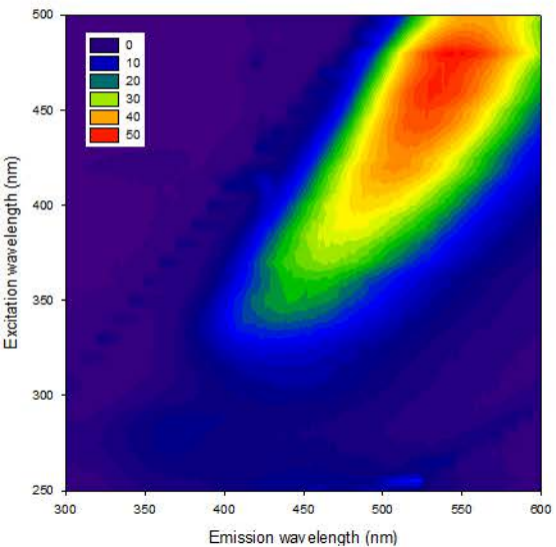
T20



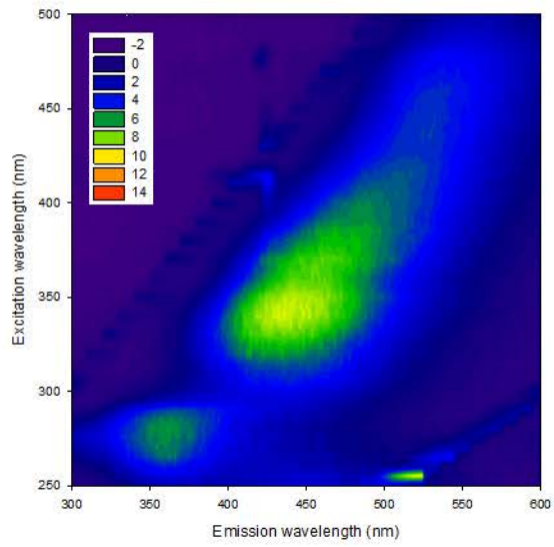
T41



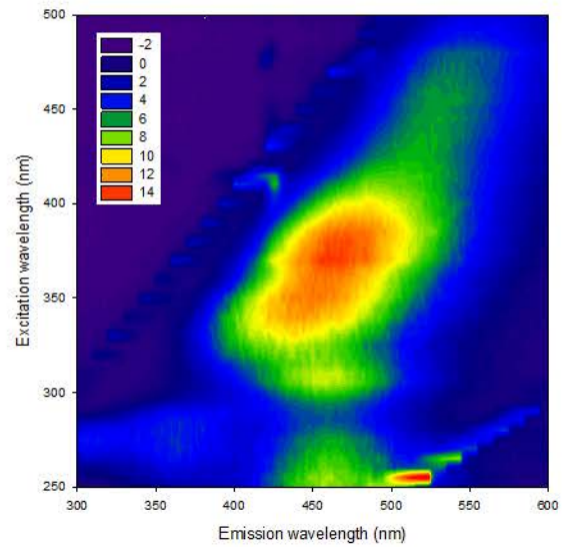
T5



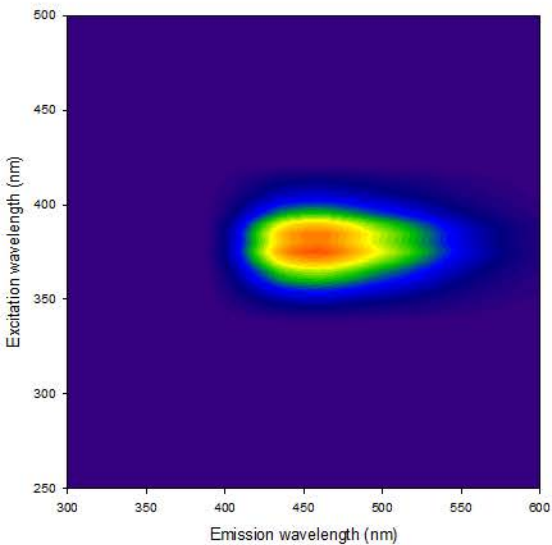
T18



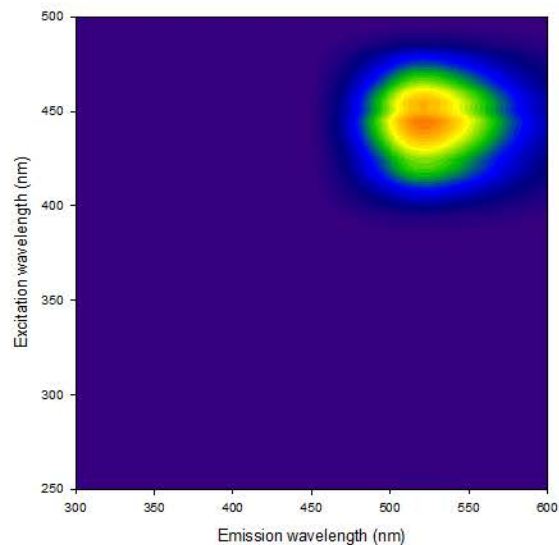
T40



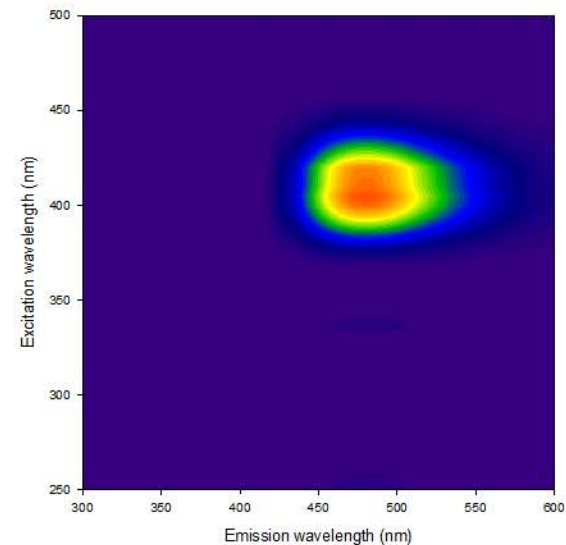
C1



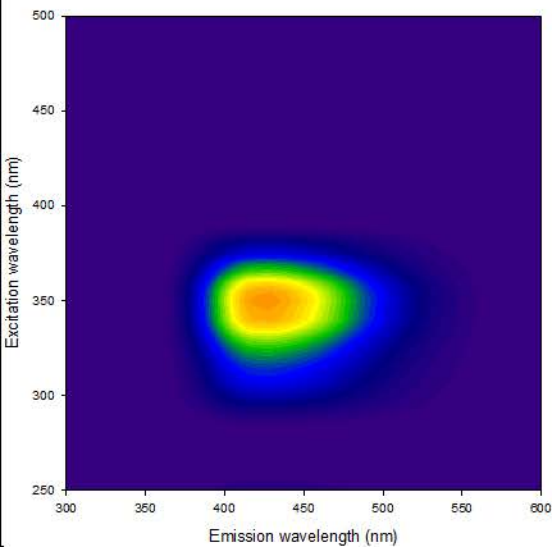
C2



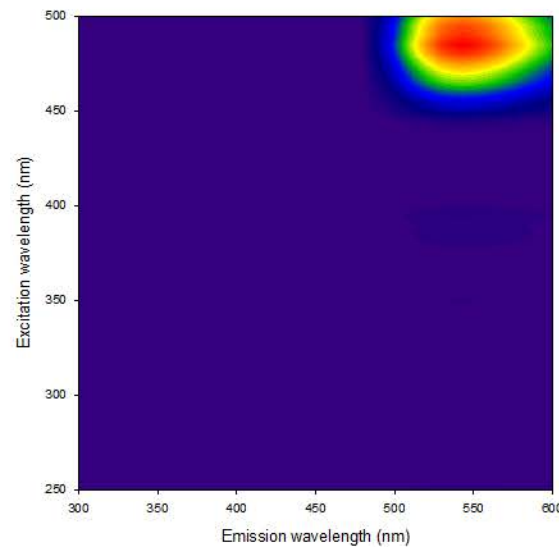
C3



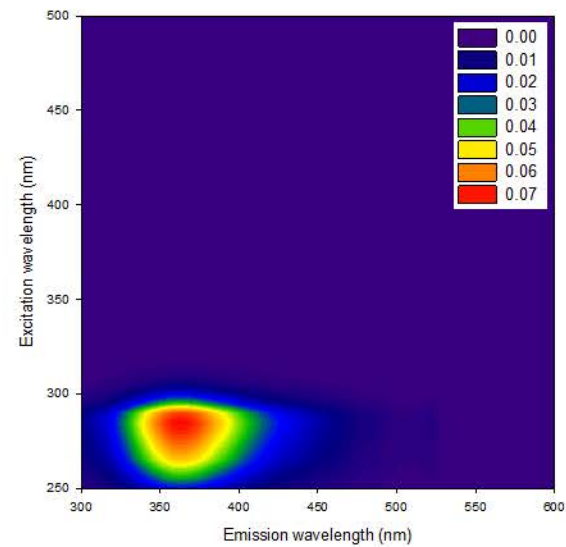
C4

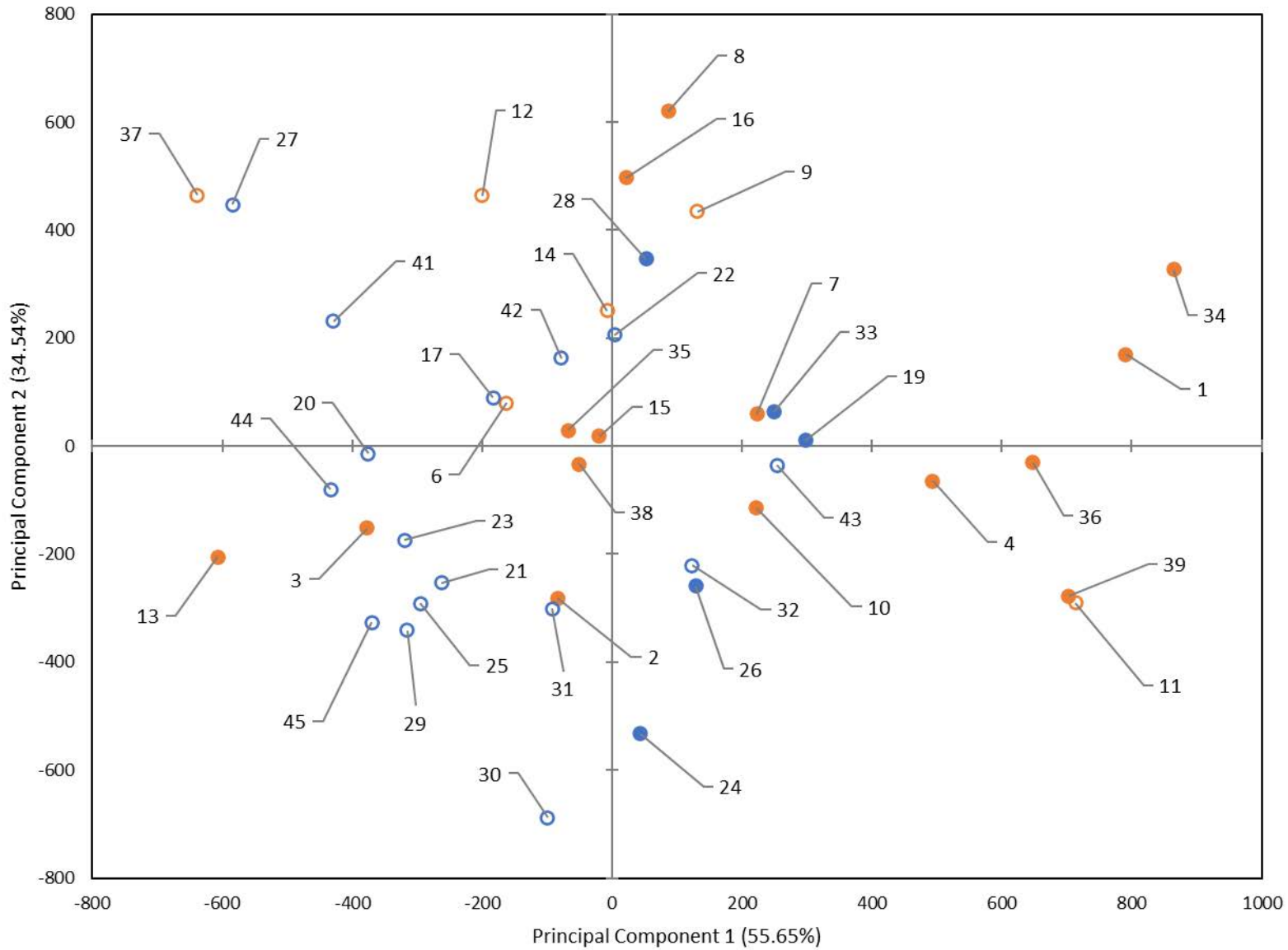


C5



C6





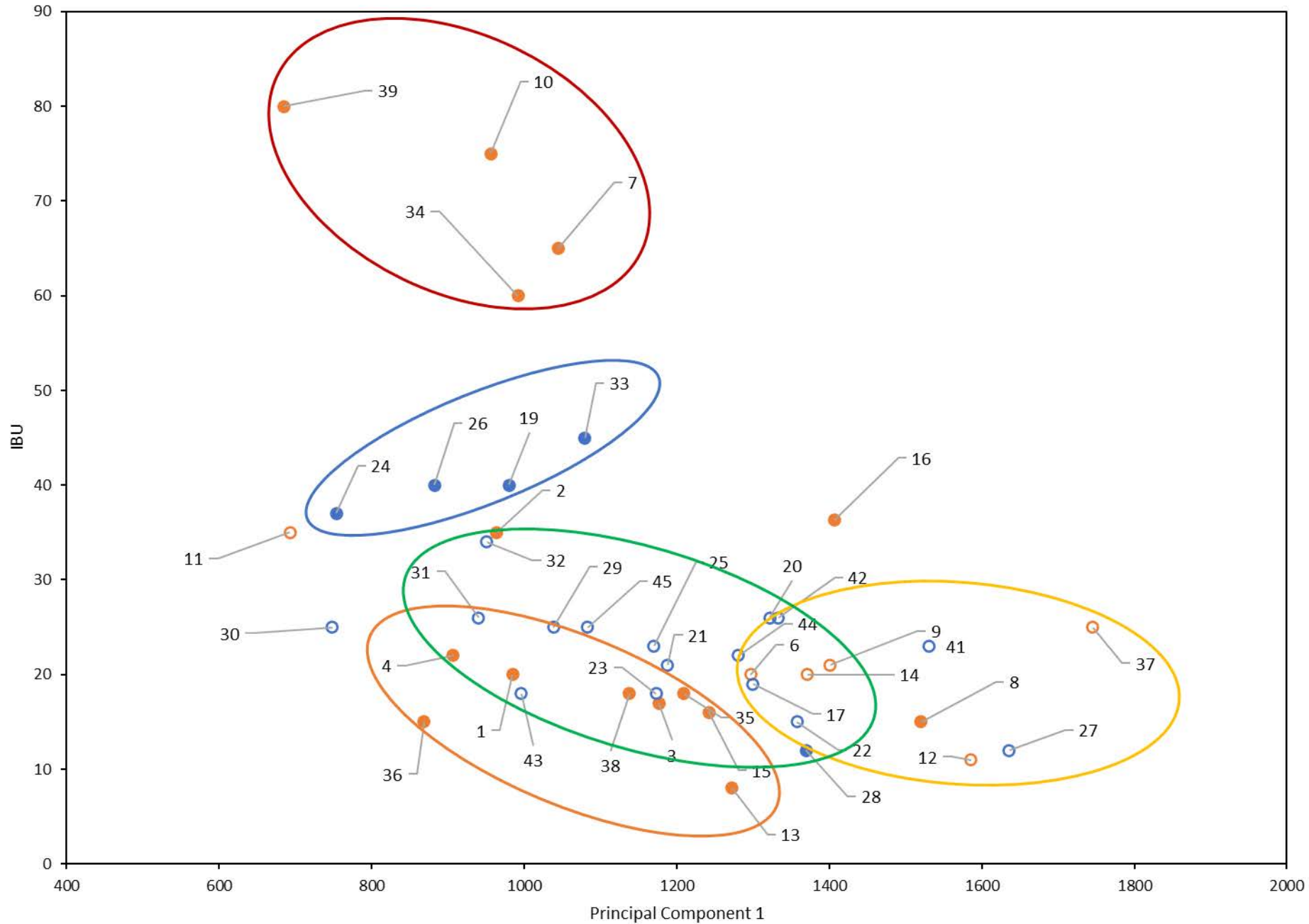


Table 1. Beer samples analysed during the research. IBU (International Bitterness Units) represent the unit of measurement for the degree of bitterness of a beer (mg iso- α -acids per litre).

CRAFT BEERS	Sample	Name	Fermentation	IBU	COMMERCIAL BEERS	Sample	Name	Fermentation	IBU
	1	La Virgen Jamonera	Ale (<i>amber</i>)	20		17	Heineken	Lager (<i>pilsen</i>)	19
	2	La Virgen 360	Ale (<i>pale</i>)	35		18	Guinness Draught	Ale (<i>stout</i>)	45
	3	Califa Rubia	Ale (<i>blonde</i>)	17		19	Cruzcampo Andalusian IPA	Ale (<i>IPA, oat</i>)	40
	4	Califa Morena	Ale (<i>amber</i>)	22		20	Cruzcampo Especial	Lager	26
	5	Dawat Pedro Ximenez	Lager (<i>sweet</i>)	17		21	Amstel Original	Lager	21
	6	Dawat Dry Lager	Lager	20		22	El Águila sin filtrar	Lager (<i>corn</i>)	15
	7	Dawat IPA Citrix	Ale (<i>IPA</i>)	65		23	San Miguel Especial	Lager (<i>pale</i>)	18
	8	Dawat 2 Sunset Ale	Ale	15		24	San Miguel Yakima Valley	IPA (<i>rye</i>)	37
	9	Pergara	Lager (<i>blonde</i>)	21		25	Alhambra Lager Singular	Lager (<i>pale</i>)	23
	10	Albero IPA	Ale (<i>IPA</i>)	75		26	Mahou IPA	Ale (<i>IPA, oat</i>)	40
	11	Albero Doble Malta	Lager (<i>pilsen</i>)	35		27	Budweiser	Lager	12
	12	La Sagra Bohemia	Lager	11		28	Franziskaner Weissbier	Ale (<i>wheat</i>)	12
	13	La Sagra Radler	Ale	8		29	Estrella Galicia Especial	Lager (<i>pilsen</i>)	25
	14	Blanca y Verde Coraje	Lager (<i>pilsen</i>)	20		30	1906 Reserva Especial	Lager (<i>pilsen</i>)	25
15	Guadalquibeer Sevilla	Ale (<i>cream</i>)	16	31	Turia	Lager (<i>Märzen</i>)	26		

16	Río Azul Flora	Ale	36.3	32	Voll Damm	Lager (<i>Märzen</i>)	34
34	Arriaca IPA	Ale (<i>IPA</i>)	60	33	Ambar IPA	Ale (<i>IPA</i>)	45
35	Ballut	Ale (<i>blonde</i>)	18	41	Clásica El Corte Inglés	Lager	23
36	Azarías	Ale (<i>pale</i>)	15	42	Daura	Lager	26
37	Rabiosa Pilsen	Lager (<i>pilsen</i>)	25	43	Daura Märzen	Lager (<i>Märzen</i>)	18
38	Tyris Original	Ale (<i>blonde</i>)	18	44	Cruz del Sur	Lager	22
39	Sevebrau	Ale (<i>IPA</i>)	80	45	Victoria Málaga	Lager (<i>pilsen, rice</i>)	25
40	Cerex	Ale	28				

Table 2. Confusion matrices obtained for the estimation sample and after cross-validation in the DA performed on the scores obtained by PARAFAC.

CLASS DIFFERENCIATION	ESTIMATION SAMPLE				
		<i>Craft</i>	<i>Commercial</i>	<i>Total</i>	<i>%Correct</i>
	<i>Craft</i>	12	9	21	57.14
	<i>Commercial</i>	1	20	21	95.24
	<i>Total</i>	13	29	42	76.19
	CROSS-VALIDATION				
		<i>Craft</i>	<i>Commercial</i>	<i>Total</i>	<i>%Correct</i>
<i>Craft</i>	12	9	21	57.14	
<i>Commercial</i>	6	15	21	71.43	
<i>Total</i>	18	24	42	64.29	
FERMENTATION TYPE DIFFERENCIATION	ESTIMATION SAMPLE				
		<i>Ale</i>	<i>Lager</i>	<i>Total</i>	<i>%Correct</i>
	<i>Ale</i>	17	3	20	85.00
	<i>Lager</i>	4	18	22	81.82
	<i>Total</i>	21	21	42	83.33
	CROSS-VALIDATION				
		<i>Ale</i>	<i>Lager</i>	<i>Total</i>	<i>%Correct</i>
<i>Ale</i>	13	7	20	65.00	
<i>Lager</i>	8	14	22	63.64	
<i>Total</i>	21	21	42	64.29	