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Manuscript Draft

Manuscript Number:

Title: A new fermented beverage from sugarcane (Saccharum officinarum L.) molasses: analysis of physicochemical properties and antioxidant capacity, and comparison with other industrial alcohol products.

Article Type: Research paper

Keywords: Antioxidant capacity; Mead; Honey; Sugarcane molasses; Phenolics.

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Abstract: A new alcoholic beverage made from sugarcane (Saccharum officinarum L.) molasses was studied and compared with analogous products. Three honey meads and two beverages obtained from sugarcane molasses were analysed for alcohol content, acidity, pH and reducing sugars. Extracts were obtained using a rotary evaporator, and tested for antioxidant capacity and total content of phenols, tannins and flavonoids. Antioxidant capacity was measured by DPPH, ABTS, DMPD and FRAP assays. Total phenol content was measured by the Folin-Ciocalteu test. Total tannin and flavonoid contents were measured by colorimetric methods based on (+)-catechin equivalents. The correlation between antioxidant capacity and total phenols was determined. The results obtained showed that the physical and chemical characteristics of sugarcane molasses mead were similar to those of beer. The sugarcane molasses meads had a higher antioxidant capacity than the honey-based ones, from which we conclude that the sugarcane molasses product is a new and interesting alternative.

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Highlights:

1. A new sugarcane molasses beverage was studied/compared with analogous products
2. The sugarcane molasses meads had a higher antioxidant capacity than the honey ones
3. The values of phenols are higher in the sugarcane meads than in the honey ones
4. The sugarcane mead is statistically different from similar alcoholic beverages
5. The sugarcane molasses product is a new and interesting alternative

A new fermented beverage from sugarcane (*Saccharum officinarum L.*) molasses: analysis of physicochemical properties and antioxidant capacity, and comparison with other industrial alcohol products

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ABSTRACT: A new alcoholic beverage made from sugarcane (Saccharum officinarum L.) molasses was studied and compared with analogous products. Three honey meads and two beverages obtained from sugarcane molasses were analysed for alcohol content, acidity, pH and reducing sugars. Extracts were obtained using a rotary evaporator, and tested for antioxidant capacity and total content of phenols, tannins and flavonoids. Antioxidant capacity was measured by DPPH, ABTS, DMPD and FRAP assays. Total phenol content was measured by the Folin-Ciocalteu test. Total tannin and flavonoid contents were measured by colorimetric methods based on (+)-catechin equivalents. The correlation between antioxidant capacity and total phenols was determined. The results obtained showed that the physical and chemical characteristics of sugarcane molasses mead were similar to those of beer. The sugarcane molasses meads had a higher antioxidant capacity than the honey-based ones, from which we conclude that the sugarcane molasses product is a new and interesting alternative.

KEY WORDS: Antioxidant capacity, mead, honey, sugarcane molasses, phenolics.

1. Introduction

Mead is an ancient alcoholic beverage made from honey and water (Kahoun, Řezková, Veškrnová, Královský, & Holčapek, 2008; Mendes-Ferreira et al., 2010). It contains at least 7% ethanol and many other compounds, including sugars, acids, vitamins, antioxidants and minerals. Reflecting its origin, the chemical composition of mead is similar to that of honey. The quality of a mead depends on its parameters and on the content of certain compounds such as reducing sugars, organic acids and phenolic compounds (Švecová, Bordovská, Kalvachová, & Hájek, 2015). The composition and phenol content of mead are influenced by many different factors, including fermentation, storage and maturation (Wintersteen, Andrae, & Engeseth, 2005).

Although honey is the main ingredient of mead, another raw material, with similar characteristics, sugarcane molasses, can also be used to brew mead.

Sugarcane (*Saccharum officinarum L.*) is a major source of sugar, together with beet (*Beta vulgaris L.*) (Maurício Duarte-Almeida, Novoa, Linares, Lajolo, & Inés Genovese, 2006). Molasses, the thick, dark syrup obtained as a byproduct from the processing of sugar cane and sugar beet into sucrose, consists of fermentable carbohydrates (sucrose, glucose and fructose) and non-sugar organic materials (betaine and other amino acids; minerals and trace elements; vitamins, especially of the B-group, etc.). Although molasses is mainly used as a supplement for livestock feed and as a source of carbon in fermentation processes, for example, for the production of ethanol, it is also a traditional sweetener and colourant in cakes. Molasses is generally regarded as nutritionally safe (Valli et al., 2012).

In southern Spain, an important sugarcane industry became established in the provinces of Malaga and Granada in the first decade of the 20th century. Sugarcane contains flavonoids and other phenolic compounds, derived from naringenin, tricin, apigenin and luteolin (Smith & Paton, 1985; Williams, Harborne, & Clifford, 1974), with antioxidant properties (McGhie, 1993).

Various studies have analysed the properties, antioxidant capacity and related properties of honey mead (Kahoun et al., 2008; Mendes-Ferreira et al., 2010; Švecová et al., 2015), and those of sugarcane molasses, considered as such (Asikin et al., 2018)but to our knowledge none have examined the case of a fermented beverage obtained from sugarcane molasses as the main raw material. It should be taken into account that the composition and content of phenolic compounds in honey meads and other fermented beverages are influenced by the ingredients used, the production process followed, the storage conditions, etc. and these factors are likely to affect sugarcane molasses meads, too (Kahoun, Řezková, & Královský, 2017).

In the present study, we analyse the physical and chemical characteristics and the antioxidant capacity of a new fermented beverage made from sugarcane molasses and from each of two yeast strains (one typically used for beer and the other for wine). We then compare these findings with those obtained for analogous alcoholic beverages. Thus, four different meads were crafted, using sugarcane molasses and honey, and their antioxidant capacities compared, together with those for other alcoholic beverages (beer and wine).

2. Materials and methods

2.1. Mead and sample preparation

Sugarcane molasses and honey for mead production were acquired in local commercial establishments in Granada (Spain). The commercial meads used as controls ("semdry") were also acquired from a company from Granada (Spain). *Saccharomyces cerevisiae* yeast, variety Safbrew™ S-33, was acquired from Fermentis and *Saccharomyces bayanus* yeast, variety Bioferm Killer, was acquired from Brouwland (www.brouwland.com). The meads were brewed following the method described below.

Two batches of four meads were prepared, two made from honey and two from sugarcane molasses. In each case, 500 mg of honey or sugarcane molasses were added to 1.5 L of water in a stainless steel cooking pot. The mixture was heated to 80 ºC, and this temperature was maintained for 10 minutes for pasteurisation to take place. The mixture was then cooled using indirect cold water for nine minutes, until the temperature of the pasteurised mixture had fallen to 35 ºC. The yeasts (*S. cerevisiae* and *S. bayanus*) were reconstituted following the manufacturer's recommendations, i.e. placing 7 g of each yeast in 0.3 L of water at 35 ºC for ten minutes. Then, the pasteurised dilutions of honey and sugarcane molasses were each poured into two one-litre glass bottles. 0.15 L of the reconstituted yeasts were added, one type for each bottle, and the bottles were then sealed with airlocks to keep them airtight. The fermentation process was controlled for 18 days, until the CO_2 bubbling in the bottles ceased. When the fermentation had concluded, the bottles were stored at 4 ºC until needed for analysis. Figure 1 shows a summary of the sample preparation.

The mead samples were labelled according to the variety of yeast used, the raw material and the batch number, as follows: SCH1 (*S. cerevisiae* Honey batch 1), SBH1 (*S. bayanus* Honey batch 1), SCS1 (*S. cerevisiae* Sugarcane batch 1), SBS1 (*S. bayanus* Sugarcane batch 1), SEMDRY (Commercial honey mead), SCH2 *(S. cerevisiae* Honey batch 2), SBH2 (*S. bayanus* Honey batch 2), SCS2 (S. *cerevisiae* Sugarcane batch 2) and SBS2(*S. bayanus* Sugarcane batch 2).

2.2. Equipment

Electronic weighing scale (Mettler AE 2000, precision 0.0001 g), mixer (Vortexer, Cleaver Scientific Ltd), high resolution spectrometer SYNAPT G2 HDMS Q-TOF. Waters, Lambda 25 UV/vis spectrophotometer (Perkin-Elmer®, Madrid, Spain), Orion pH-meter.

2.3. Chemicals

All chemicals used were analytical reagent grade, unless otherwise stated. Folin–Ciocalteu phenol reagent was obtained from Merck (Darmstadt, Germany). Gallic acid, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and N, N-dimethyl-p-phenylenediamine (DMPD) were supplied by Sigma–Aldrich (Milan, Italy). Sodium nitrite, aluminium chloride, catechin, vanillin, sodium acetate 3-hydrate, anhydrous sodium carbonate, ferric chloride 6-hydrate, orthophosphoric acid and Rebelein Vinikit were supplied by Panreac (Barcelona, Spain). The 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) for the FRAP method was obtained from Fluka Chemicals (Buchs, Switzerland).

2.4. Alcohol content (% V/V), acidity, pH and reducing sugars

Alcohol content was determined using a pycnometer, as recommended by the European Brewery Convention (1975). Acidity and pH were determined following the methods described by the American Society of Brewing Chemists (1942).

Reducing sugar content was measured using the Rebelein method described in European Council regulation 1234/2007. Specifically, 2 mL of mead sample were mixed with 10 mL of cupric solution and 5 mL of alkaline solution from the Rebelein kit. The mixture was heated and maintained at boiling point for three minutes. The resulting solution was then cooled and mixed with 10 mL of potassium iodide, 10 mL of sulphuric acid and 10 mL of starch solution. The final solution was titrated with thiosulphate solution until it turned yellow.

2.5. Extraction conditions

The extracts were obtained as follows (Socha, Gałkowska, Robak, Fortuna, & Buksa, 2015), 25 mL of mead sample were concentrated in a rotary evaporator in order to remove the alcohol. The resulting solution was then diluted to the primary volume with distilled water.

The sample was adjusted to pH = 2 with HCl solution and then saturated with NaCl. The solution obtained was extracted three times with ethyl acetate, using 25 mL of the solvent. The ethyl acetate fraction was then collected and evaporated to dryness in a vacuum rotatory evaporator. The dry residue after evaporation was dissolved in 5 mL of methanol.

2.6. Total content of phenols, flavonoids and tannins

Total phenol content was determined using a modified version of the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965). 2.5 mL of deionised water and 500 µL of Folin-Ciocalteu reagent were added to an appropriately diluted mead methanolic extract. The mixture was allowed to stand for five minutes, after which 2 mL of a 10% aqueous Na_2CO_3 solution were added. The final volume was adjusted to 10 mL. The samples were allowed to stand for 90 minutes at room temperature before measurement at 700 nm versus the blank, using a spectrophotometer. The total phenol content is expressed as gallic acid equivalent (mg gallic acid/L mead), using the gallic acid calibration curve.

Total flavonoid content was determined using a colorimetric method (Maietti et al., 2012). To 50, 100 and 200 µl of mead phenolic extract respectively, 2 mL of deionised water, 150 µL of 5% NaNO2 solution, 300 µL of 10% AlCl3 solution, and 1 mL of NaOH 1N were added. The final volume was adjusted to 5 mL with deionised water and the absorption was measured at 510 nm versus the blank. The amount of total flavonoids is expressed as (+)-catechin equivalents (mg (+)-catechin/L) through the calibration curve of (+)-catechin.

144 The content of total condensed tannins was determined using a partially modified colorimetric
145 method (Broadhurst & Jones, 1978). 3 mL of vanillin and 1.50 mL of HCl were added to 100 or 250
146 µL of mead phenolic extract. The final volume was then adjusted to 10 mL with methanol, and the
147 absorption was measured at 500 nm. The amount of total condensed tannins was expressed as
148 (+)-catechin equivalents (mg (+)-catechin/L) using the (+)-catechin calibration curve.
149
150 2.7. Antioxidant capacity (TEAC): DPPH, ABTS, DMPD, FRAP methods
151 The DPPH assay was performed by adding a suitable dilution of the methanol extract to the
152 DPPH coloured radical (Brand-Williams, Cuvelier, & Berset, 1995). Absorbance was measured at
153 515 nm every 15 minutes for one hour until equilibrium was reached. In each of the methods ap-
154 plied, the extract methanolic dilution that gave a linear response was determined. The absorbance
155 signal measured was translated into antioxidant activity by using Trolox as a standard antioxi-
156 dant.
157 For the ABTS assay, the radical was generated using potassium persulphate (Pellegrini, Visioli,
158 Buratti, & Brighenti, 2001). The solution was diluted with ethanol until absorbance reached 0.70
159 at 734 nm. Once the radical had formed, 2mL of $ABTS^{\bullet+}$ were mixed with 100 µl of appropriately
160 diluted mead methanolic extract and the absorbance was measured at 734 nm once per minute
161 for 30 minutes (Samaniego Sánchez et al., 2007). The absorbance signal measured was translated
162 into antioxidant activity by using Trolox as a standard antioxidant.
163 The DMPD radical was generated using the following method (Fogliano, Verde, Randazzo, &
164 Ritieni, 1999). Once the radical had formed, 1.0 mL of DMPD-radical were mixed with 50 µL of
165 appropriately diluted mead methanolic extract and the absorbance was measured at 505 nm until
166 equilibrium was reached. The absorbance signal measured was translated into antioxidant activ-
167 ity by using Trolox as a standard antioxidant.

168 The FRAP method (Benzie & Strain, 1996), was applied as soon as the radical had formed, when
169 1.5 mL of FRAP-radical were mixed with 200 µL of appropriately diluted mead methanolic extract
170 and the absorbance was measured at 593 nm once per minute for 10 minutes. The absorbance sig-
171 nal measured was translated into antioxidant activity by using Trolox as a standard antioxidant.
172
173 2.8. Statistical Analysis
174 The statistical package Statgraphics® Centurion XVI (v16. StatPoint Technologies, Inc.) program
175 was used to interpret the data obtained. Duplicate batches were prepared from each mead and all
176 of them were measured three times in each assay. Values were expressed as means ± standard de-
177 viation (SD). One way ANOVA, Kruskall-Wallis, t-test, Mann-Whitney or Wilcoxon tests were
178 used according to characteristics of the sample population. Pearson's correlation coefficients were
179 calculated. Differences of $p<0.05$ were considered significant. A multivariate cluster analysis and
180 discriminant analysis were also performed.
181
182 3. Results and discussion
183 3.1. Alcohol content (%v/v), acidity, pH and reducing sugars
184 Table 1 summarises the results obtained from the mead samples, revealing no significant differences
185 ($p>0.05$) in the alcohol content of meads between batches. The same happens for meads made from the
186 same raw material but different types of yeasts. However, there are significant statistical differences
187 ($p<0.05$) between meads made with different raw material and between semdry. *S. cerevisiae* yeast
188 yielded lower acidity and higher pH values than *S. bayanus*, both in the honey meads and in those made
189 from sugarcane molasses. When *S. cerevisiae* yeast was used instead of *S. bayanus,* a smaller quantity of
190 reducing sugars was measured. However, the influence of raw material on the pH and sugar values of
191 meads and semdry can be observed ($p<0.05$), but not for acidity values ($p>0.05$).

Table 2 compares the values obtained for the mead samples and for beer and wine (the latter values as reported in the literature). The alcohol content of sugarcane molasses and honey meads was similar to that of a high fermentation beer or a low fermentation wine (Mitić et al., 2014) as was to be expected from the superposition of the alcohol contents of beers and wines; the meads were in the middle of this combined range.

The acidity assays performed showed that the pH and acidity values for sugarcane molasses meads were more similar to those of beer than to those of wine (Ceppi de Lecco, C.; Castillo, 2008). However, the pH results for the honey meads were more similar to those for red wines. From these results, we conclude that sugarcane molasses meads differ from honey meads in terms of acidity, being more similar to beer than to wine in this respect (Ceppi de Lecco, C.; Castillo, 2008; Fogliano et al., 1999).

The honey meads had a higher concentration of reducing sugars than sugarcane molasses, and were similar to wines in this respect, while the results for the sugarcane molasses meads were closer to those for beers. These results confirm that sugarcane molasses meads are different from honey meads, and similar to beers in terms of the content of reducing sugars.

3.2. Total content of phenols, flavonoids and tannins

Table 3 shows the gallic acid and catechin standard curves used to calculate the values presented in Table 4. A very good correlation between all three equations was obtained, meaning that these linear equations are suitable for the purposes of our study.

Table 4 presents the total content of phenols, flavonoids and tannins measured in the samples. No significant statistical differences ($p>0.05$) were found between the two batches of samples for TPC, flavonoids and tannins.

The sugarcane molasses meads had a higher concentration of total phenols (TPC) than the honey meads. Among the sugarcane molasses meads, the *S. cerevisae* yeast produced higher val-

ues for phenols (140.91 ± 7.07 mg GAE/L mead and 171.04 ± 9.57 mg GAE/L mead versus 124.91 ± 4.97 mg GAE/L mead and 142.65 ± 5.14mg GAE/L mead), although the differences are not statistically significant ($p>0.05$). In general, and regardless of the yeast used, sugarcane samples have a higher TPC content ($p<0.001$) than those made with honey (SBH, SCH, Semdry).

The flavonoids content of the sugarcane molasses meads was higher ($p<0.001$) than that of the honey meads (ranging from 47.52 ± 4.10 mg Cat/L mead to 64.2 ± 0.38 mg Cat/L mead versus a range of 1.24 ± 0.07 mg Cat/L mead to 2.91 ± 0.17 mg Cat/L mead). From these values, we conclude that these two beverages differ significantly. As with the total phenol content, slightly higher values for flavonoids were obtained with *S. cerevisae* yeast, although these differences between yeasts, cannot be considered statistically significant ($p>0.05$).

The concentration of tannins in the sugarcane molasses meads was ten times ($p<0.001$) that found in the honey meads (SBH, SCH, Semdry), which highlights the difference between these products. With respect to the yeasts used, unlike flavonoids and TPC, *S. bayanus* generated higher tannin concentrations than *S. cerevisae* yeast, although these differences between yeasts, neither cannot be considered statistically significant ($p>0.05$).

Table 5 summarises the tannin concentration values obtained in our assays for the sugarcane molasses and honey mead samples, together with the corresponding values for beer and wine, according to previous research (Katalinić, Milos, Modun, Musić, & Boban, 2004; Mitić et al., 2014; Šeruga, Novak, & Jakobek, 2011; Tinkiliç & Uyanik, 2001).

The total phenol content (TPC) of commercial beers ranges from 330.41 ± 13.44 mg to 545.32 ± 15.51 mg GAE/L (Mitić et al., 2014). The corresponding range obtained in our assay for sugarcane meads was both lower and narrower, from 124.91 ± 4.97 mg GAE/L to 171.04 ± 9.57 mg GAE/L. For honey meads, these values were lower still, ranging from just 13.37 ± 0.54 GAE/L to 21.80 ± 0.52 GAE/L. These findings support the hypothesis that, in their chemical composition, sugarcane

molasses and honey meads are quite different products ($p<0.05$). The total phenol content of beers is much higher than that of meads, and the differences are even greater between meads and wines.[25] Again, this highlights the existence of considerable differences, both between meads, beers and wines and also between sugarcane molasses and honey meads.

The flavonoid content of wine is greater ($p<0.05$) than that of all other beverages studied (Katalinić et al., 2004) (Table 5), while that of beer is higher ($p<0.05$) than for each type of mead (Mitić et al., 2014). Thus, the following results were obtained: 116.35 ± 4.78 mg Cat/L to 208.58 ± 2.39 mg Cat/L beer versus 47.52 ± 4.10 mg Cat/L to 64.2 ± 0.38 mg Cat/L sugarcane molasses mead.

The tannin content of the different beverages presented a similar pattern to that of the flavonoids, except that the differences between beer and wine were not as pronounced as in the former case (Katalinić et al., 2004; Mitić et al., 2014; Tinkiliç & Uyanik, 2001).

In summary, the total content of phenols, flavonoids and tannins is lower in sugarcane molasses and honey meads than in beer and red wine. Moreover, in every case the values are higher in the sugarcane molasses meads than in the honey meads, highlighting the existence of important differences between these products.

3.3. Antioxidant capacity (TEAC): DPPH, ABTS, DMPD, FRAP methods

Table 6 shows the Trolox standard curves used to calculate the values presented in Table 7. All of them with correlation coefficients (r^2) greater than 0.99. Overall, there are no significant statistical differences between batches ($p>0.05$) for any TEAC method. All TEAC methods show significant statistical differences ($p<0.001$) between meads made with honey versus those made with sugarcane molasses, with the exception of the DMPD method that shows no significant statistical differences ($p>0.05$) between honey and sugarcane molasses meads (Table 7). In general, meads

made with sugarcane molasses have higher TEAC values than ones made with honey ($p<0.001$). In relation to the influence yeasts (*S. cerevisae vs S. bayanus*) on TEAC potential of mead, we found no statistically significant differences ($p>0.05$) between those made with honey or sugarcane molasses. However, and as described above, specifically the DMPD method shows statistically significant differences ($p<0.05$) between meads made with one or other raw material. The fact that DMPD method is always the exception, makes us think that it may not be the most appropriate TEAC method for the measurement of antioxidant potential in meads. This question now found, will be studied in the next section.

Table 8 shows the values obtained in our assays for the mead samples and those reported in the literature for beer and wine (De Clerck, 1957; Mitić et al., 2014; Šeruga et al., 2011; Tinkiliç & Uyanik, 2001; Zhao, Chen, Lu, & Zhao, 2010).

Comparison of the mead values with those for beer (Mitić et al., 2014; Zhao et al., 2010), and wine (Busuricu, F.; Balaban, D.; Popescu, A.; Anghel, 2008; Katalinić et al., 2004; Ma et al., 2014; Šeruga et al., 2011), shows that the DPPH values obtained from sugarcane molasses meads are closer to those of wine ($p>0.05$) than to those of beer ($p<0.05$). As can be seen in Table 8, the DPPH values for wine ranged from 9.2 ± 0.6 mmol TE/L to 37.8 ± 2.8 mmol TE/L. The DPPH and ABTS values for honey meads were higher than for beer but lower than for wine.

According to the FRAP results, sugarcane molasses and honey meads differ substantially. The former present values that are closer to those of beer and wine (60.82 ± 2.43 mmol TE/L to 71.14 ± 0.25 mmol TE/L) while the latter are significantly lower (2.67 ± 0.06 mmol TE/L to 6.67 ± 0.33 mmol TE/L). The values obtained for sugarcane molasses meads are closer to those of beer than to those of wine.

287 In conclusion, the antioxidant capacity of sugarcane molasses meads is different from that of
288 honey meads, but similar to that of beer.

289

290 3.4. Correlation between antioxidant capacity (TEAC) and Total Phenols Content (TPC)

291 Table 9 summarises the correlation between total phenol contents and the different measure-
292 ment methods used (Samaniego Sánchez et al., 2007). A very high positive correlation was ob-
293 served between the total phenols in honey and sugarcane molasses meads and their antioxidant
294 capacity (Pearson's correlation coefficients: 0-9 to 0.99) except for DMPD, in which case only a
295 moderate positive correlation was observed (Pearson's correlation coefficients: 0.4 to 0.69).

296 The DPPH values also presented a very high positive correlation with ABTS and FRAP but there
297 was only a low positive correlation (Pearson's correlation coefficients: 0.2 to 0.39) between the
298 DPPH and DMPD values.

299 The ABTS values obtained were almost perfectly correlated with the ABTS values (r=0.9912) but
300 there was only a low positive correlation (Pearson's correlation coefficients: 0.2 to 0.39) with the
301 FRAP values. Finally, the Pearson's correlation coefficients between FRAP and DMPD were mod-
302 erate and positive.

303 In view of these results, it is confirmed that the DMPD method is not suitable for measuring the
304 antioxidant potential of mead.

305

306 3.5. Multivariate analysis

307 To determine whether the sugarcane molasses mead was different from the honey mead, based
308 on all data obtained, two multivariate analyses were conducted. The first was a cluster analysis
309 using the median method, which produced the dendrogram shown below (Figure 2):

This figure shows that the samples of sugarcane molasses mead, the new product (SCS and SBS) closely resembled each other and constituted a well-defined cluster. Among all other beverages compared, the most similar to this was the honey mead. It is interesting to note that the honey mead obtained in our assay was similar to a commercial product marketed as SEMDRY. In conclusion, the sugarcane mead analysed in this study can be statistically differentiated from similar alcoholic beverages.

To further corroborate the conclusion that the new product can be differentiated from existing beverages, a discriminant analysis was also conducted. This method predicts whether a sample belongs to one group or another, according to the overall data available. The results obtained from this analysis are shown below (Figure 3).

The predictions made by the function achieve a 100% success rate. Thus, all the samples and products differ sufficiently to be individually identifiable and the function correctly classified all the samples from the data supplied.

4. Conclusion.

The sugarcane molasses meads had a higher antioxidant capacity than the honey-based ones and values of phenols, flavonoids and tannins higher in the sugarcane molasses meads than in the honey meads. The sugarcane mead analysed in this study can be statistically differentiated from similar alcoholic beverages. In summary, by applying the method described in this paper we

obtained a new alcoholic beverage, made from sugarcane molasses, which differed significantly both from traditional honey mead and from beer and red wine and it is a new and interesting alternative.

Abbreviations

SCH1, *Saccharomyces cerevisiae* Honey batch 1; SBH1, *Saccharomyces bayanus* Honey batch 1; SCS1, *Saccharomyces* Sugarcane batch 1; SBS1, *Saccharomyces. bayanus* Sugarcane batch 1; SEMDRY, Commercial honey mead; SCH2, *Saccharomyces cerevisiae* Honey batch 2; SBH2, *Saccharomyces bayanus* Honey batch 2; SCS2, *Saccharomyces cerevisiae* Sugarcane batch 2; SBS2, *Saccharomyces bayanus* Sugarcane batch 2; Trolox, Gallic acid, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; TPC, Total Phenols Content; ABTS, 2,2-azinobis-(3-ethylbensothiazoline)-6- sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMPD, N, N-dimethyl-p-phenylenediamine; FRAP, Ferric Reducing Antioxidant Power; TEAC, Trolox Equivalent Antioxidant Capacity; GAE, Gallic Acid Equivalent; Cat, Catechin; TE, Trolox Equivalent.

Acknowledgements

We wish to express our gratitude to Glenn Harding for his assistance in improving the English language version of the manuscript.

Funding

This study is part of Gabino Marín Garcia's PhD thesis, from the Nutrition and Food Sciences Doctorate Program of the University of Granada and it was funded by PAIDI groups Junta de Andalucía AGR141 and AGR279.

Notes

The authors have no competing financial interest to declare.

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Figure captions

Figure 1. Mead brewing process.

Figure 2. Dendrogram of all the samples and beverages studied.

Figure 3. Discriminant function chart of all samples and beverages studied.

Table 1

Alcoholic content, acidity, pH and reducing sugars of the samples[a].

	Sample	% V/V	Acidity (g tartaric acid/L)	pH	Reducing sugars (g/L)
BATCH 1	SCH	8.5 ± 0.1	2.1 ± 0.0	4.04 ± 0.11	14.8 ± 0.25
	SBH	8.5 ± 0.1	3.2 ± 0.1	3.85 ± 0.03	19.0 ± 0.75
	SCS	11.0 ± 0.1	2.5 ± 0.1	4.49 ± 0.18	3.0 ± 0.30
	SBS	11.0 ± 0.1	3.8 ± 0.2	4.31 ± 0.03	3.2 ± 0.21
Commercial honey mead	SEMDRY	9.1 ± 0.1	2.1 ± 0.2	4.40 ± 0.21	25.47 ± 1.27
BATCH 2	SCH	7.5 ± 0.1	2.6 ± 0.4	3.75 ± 0.01	18.1 ± 0.10
	SBH	6.9 ± 0.1	3.8 ± 0.4	3.58 ± 0.01	21.9 ± 0.12
	SCS	9.5±0.1	2.9±0.4	4.42±0.01	2.6±0.31
	SBS	10.0±0.1	3.8±0.0	4.17±0.02	2.9±0.20

[a] Values are the means ± SD (n=3).

Table 2

Comparison of alcoholic content, acidity, pH and reducing sugar of beers, wines and meads.

Beverage	% V/V	Acidity (g tartaric acid /L)	pH	Reducing sugars (g/L)
Beer [a]	3.1±0.1 to 8.9±0.1	2.7	4.2 to 4.8	8.23 to 12.40
Red wine [b]	8.9±0.1 to 16.1±0.1	4.5 to 7.0	2.8 to 4.0	1.80 to 2.62
Honey mead (SCH and SBH)	8.5±0.1 to 11±0.1	2.1±0.0 to 3.8±0.4	3.58±0.01 to 4.04±0.11	14.8±0.25 to 21.9±0.12
Sugarcane molasses mead (SCS and SBS)	9.5±0.1 to 11.0±0.1	2.5±0.1 to 3.8±0.2	4.17±0.02 to 4.49±0.18	2.6±0.31 to 3.2±0.21

[a] (Mitić et al., 2014)

[b] (Ceppi de Lecco, C.; Castillo, 2008; Fogliano et al., 1999)

Table 3

Gallic acid and Catechin standard curves: absorbance at 700, 510 and 519 nm.

Method	Linear equation [a]	r^2	Concentration range
Total phenols Folin-Ciocalteu (700 nm)	$y = 0.10047x + 0.0474$	0,9995	0.25 – 10.00 mg/L gallic acid
Flavonoids (510 nm)	$y = 0.0176x + 0.0016$	0,9986	0.5 – 10.0 mg/L catechin
Tannins (519 nm)	$y = 0.0291x + 0.0089$	0,9969	0.5 – 10.0 mg/L catechin

[a] $y = \mathrm{bx} + \mathrm{a}$, y=absorbance, x=concentration

15 **Table 4**

16 Total phenols by Folin-Ciocalteu method, flavonoids and tannins of samples [a].

	Sample	Total phenols by Folin-Ciocalteu (mg GAE/L mead)	Flavonoids (mg Cat/L mead)	Tannins (mg Cat/L mead)
BATCH 1	SCH	21.80 ± 0.52	2.91 ± 0.17	1.01 ± 0.19
	SBH	20.93 ± 0.81	2.32 ± 0.09	2.32 ± 0.05
	SCS	140.91 ± 7.07	57.28 ± 1.20	10.42 ± 0.20
	SBS	124.91 ± 4.97	47.52 ± 4.10	15.62 ± 0.67
Commercial honey mead	SEMDRY	23.01 ± 0.71	3.04 ± 0.12	2.21 ± 0.44
BATCH 2	SCH	15.73 ± 1.42	1.45 ± 0.04	1.66 ± 0.14
	SBH	13.37 ± 0.54	1.24 ± 0.07	1.66 ± 0.17
	SCS	171.04 ± 9.57	64.2 ± 0.38	15.26 ± 0.46
	SBS	142.65 ± 5.14	53.12 ± 0.33	13.83 ± 0.29

[a] Values are the means ± SD (n=3).

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Table 5

Total phenols by Folin-Ciocalteu method, flavonoids and tannins: comparison between beers, wines and mead samples.

Beverage	Total phenols by Folin-Ciocalteu (mg GAE/L)	Flavonoids (mg Cat/L)	Tannins (mg Cat/L)
Beer [a]	330.41 ± 13.44 to 545.32 ± 15.51	116.35 ± 4.78 to 208.58 ± 2.39	66.36± 2.46 to 77.26± 1.36
Red wine [b]	934.0 ± 34.0 to 3013.0 ± 45.0	1074.17 ± 64.82 to 1840.83 ± 88.39	67.18 to 107.62
Honey mead (SCH and SBH)	13.37 ± 0.54 to 21.80 ± 0.52	1.24 ± 0.07 to 2.91 ± 0.17	1.01 ± 0.19 to 2.32 ± 0.05
Sugarcane molasses mead (SCS and SBS)	124.91 ± 4.97 to 171.04 ± 9.57	47.52 ± 4.10 to 64.2 ± 0.38	10.42 ± 0.20 to 15.26 ± 0.46

[a] (Mitić et al., 2014; Tinkiliç & Uyanik, 2001; Zhao et al., 2010)

[b] (Busuricu, F.; Balaban, D.; Popescu, A.; Anghel, 2008; Katalinić et al., 2004; Tinkiliç & Uyanik, 2001)

Table 6

Trolox standard curves: percentage inhibition at 515, 734, 595 and 593 nm.

Method	Linear equation [a]	r^2	Concentration range
DPPH (515 nm)	$y = 89.5544x + 3.5530$	0,9994	0.05 – 1.00 mM Trolox
ABTS (734 nm)	$y = 0.1905x + 4.2983$	0,9997	10 – 500 mM Trolox
DMPD (595 nm)	$y = 37.06361x + 7.3586$	0,9915	0.05 – 2.00 mM Trolox
FRAP (593 nm)	$y = 4.2463x + 0.0879$	0.9997	0.01 – 0.50 mM Trolox

[a] $y = \mathrm{bx} + \mathrm{a}$, y=percentage inhibition, x=concentration

Table 7

DPPH, ABTS, DMPD and FRAP results of the samples in millimolars TE/L[a].

	Sample	DPPH	ABTS	DMPD	FRAP
BATCH 1	SCH	2.82 ± 0.01	3.24 ± 0.04	7.26 ± 0.18	3.82 ± 0.24
	SBH	2.67 ± 0.01	3.86 ± 0.17	20.91 ± 0.12	3.69 ± 1.64
	SCS	11.50 ± 0.15	58.37 ± 0.69	9.09 ± 0.29	60.82 ± 2.43
	SBS	12.63 ± 0.10	80.29 ± 2.20	8.29 ± 0.36	68.51 ± 0.05
Commercial honey mead	SEMDRY	5.89 ± 0.05	7.73 ± 0.17	18.89 ± 0.48	6.67 ± 0.33
BATCH 2	SCH	2.44 ± 0.03	3.42 ± 0.05	8.01 ± 0.20	4.52 ± 0.17
	SBH	2.51 ± 0.03	3.17 ± 0.11	18,44 ± 0.20	2.67 ± 0.06
	SCS	12.06 ± 0.02	90.15 ± 1.29	9.50± 0.63	71.12 ± 2.66
	SBS	11.89 ± 0.13	81.92 ± 1.13	8.50 ± 0.31	71.14 ± 0.25

[a] Values are the means ± SD (n = 3).

Table 8

DPPH, ABTS, DMPD and FRAP comparison between beers, wines and meads in millimolars TE/L.

Beverage	DPPH	ABTS	DMPD	FRAP
Beer [a]	0.35 ± 0.01 to 0.83 ± 0.01	0.14 ± 0.01 to 0.35 ± 0.01	-	22.99 ± 5.11 to 831.20 ± 3.83
Red wine [b]	9.2 ± 0.6 to 37.8 ± 2.8	7.9 ± 0.4 to 24.2 ± 0.8	5.8 ± 0.3 to 10.2 ± 0.5	22.195± 4.479 to 32.280 ± 4.479
Honey mead (SCH and SBH)	2.44 ± 0.03 to 5.89 ± 0.05	3.17 ± 0.11 to 7.73 ± 0.17	7.26 ± 0.18 to 20.91 ± 0.12	2.67 ± 0.06 to 6.67 ± 0.33
Sugarcane molasses mead (SCS and SBS)	11.50 ± 0.15 to 12.63 ± 0.10	58.37 ± 0.69 to 90.15 ± 1.29	8.29 ± 0.36 to 9,50 ± 0.63	60.82 ± 2.43 to 71.14 ± 0.25

[a] (Mitić et al., 2014; Zhao et al., 2010)

[b] (Busuricu, F.; Balaban, D.; Popescu, A.; Anghel, 2008; Katalinić et al., 2004; Ma et al., 2014; Šeruga et al., 2011)

Table 9

Pearson's correlation coefficients (r) between antioxidant capacity and total phenols.

	Total phenols (Folin-Ciocalteu)	DPPH	ABTS	FRAP	DMPD
Total phenols (Folin-Ciocalteu)	1				
DPPH	0.9602	1			
ABTS	0.9780	0.9669	1		
FRAP	0.9853	0.9788	0.9912	1	
DMPD	0.4674	0.3686	0.4871	0.4198	1

Figure 1
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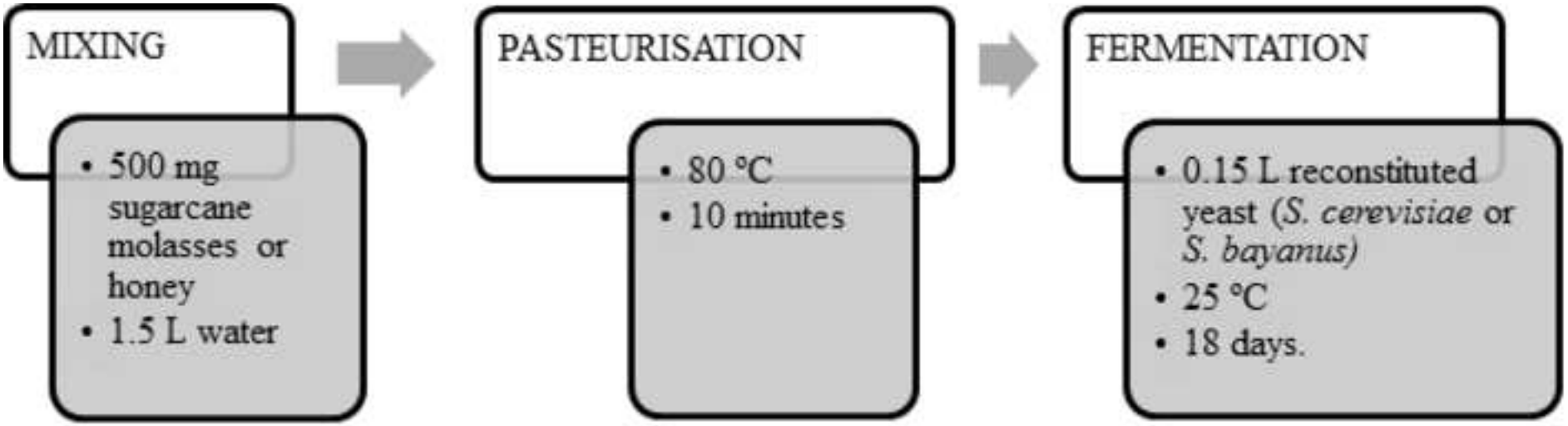

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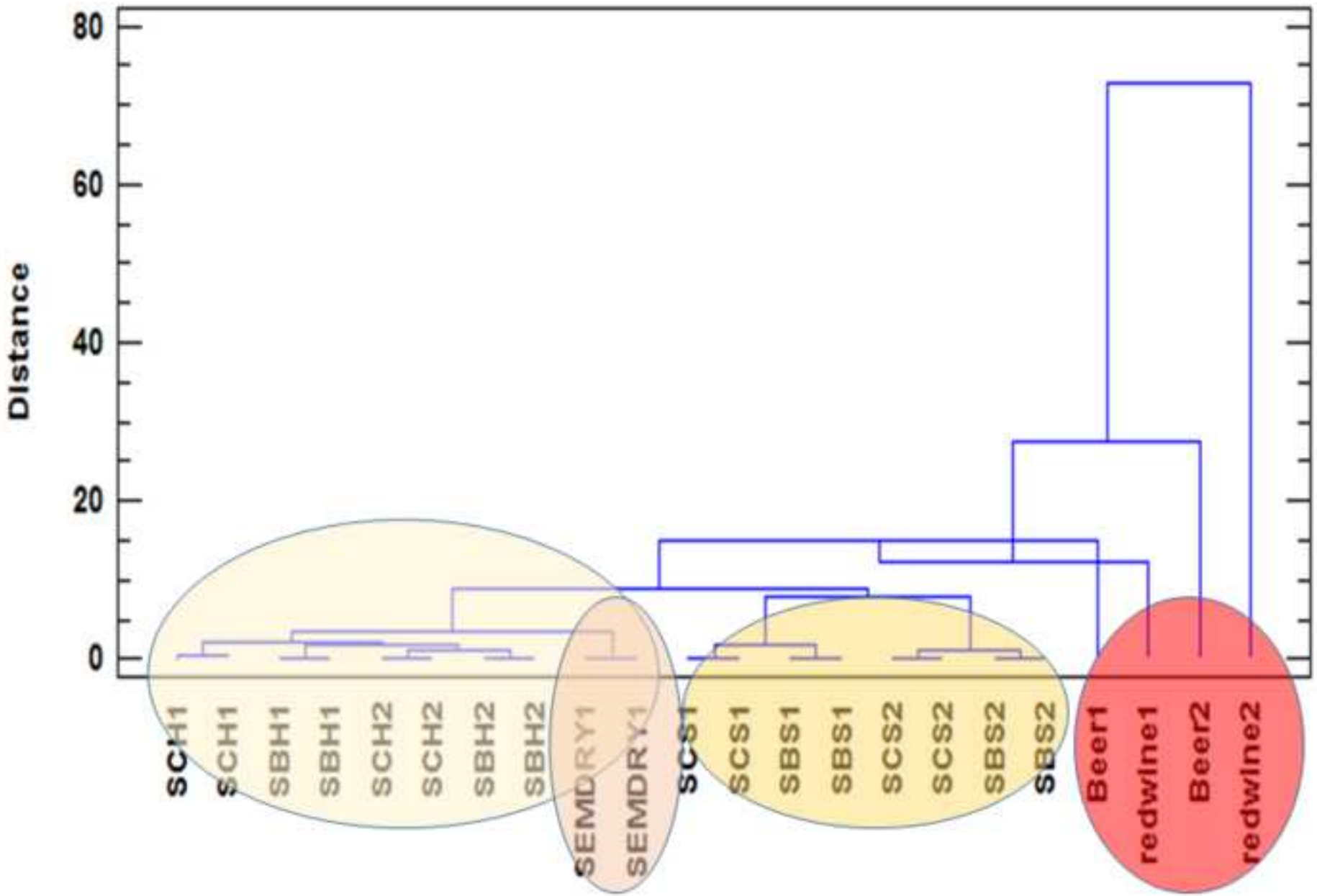

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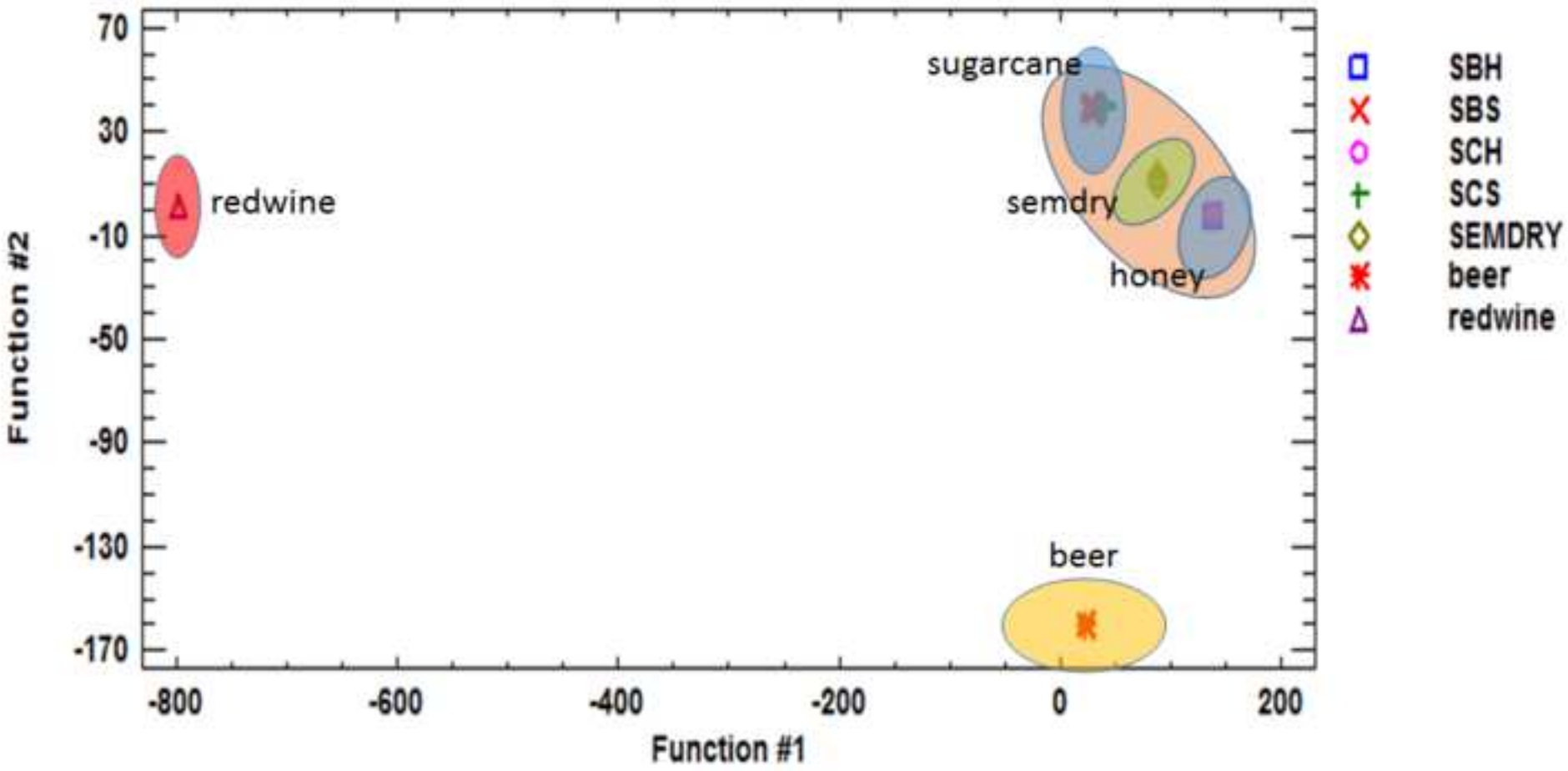

Credit Author Statement

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C. Samaniego-Sánchez: Conceptualization, Methodology, Investigation, Validation, Resources, Writing - Review & Editing. **G. Marín-García**: Conceptualization, Methodology, Investigation, Resources, Formal analysis, Writing - Original Draft, Visualization. **J. J. Quesada-Granados**: Conceptualization, Methodology, Formal analysis, Validation, Writing - Review & Editing, Visualization, Supervision, Project administration.