Physicochemical and sensory (aroma and colour) characterisation of a non-centrifugal cane sugar ("*panela*") beverage

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Abbreviated running header: Odour active volatiles and colour in Colombian non-centrifugal cane sugar beverage from three geographical sources

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Abstract

Non-centrifugal cane sugar (NCS), also called "*panela*", is a high carbohydrate-content food obtained by boil evaporation of the sugar cane juice. This study was undertaken to characterise the physicochemical properties and sensory characteristics of the *panela* beverage at two different concentrations. Evaluation of pH, °Brix, and colour (tristimulus colorimetry) was carried out in all *panela* drink samples. In order to characterise the odour-active volatiles of the beverage, a simultaneous steam distillation-solvent extraction (SDE) method was applied using a mixture of diethyl ether-pentane (1:1, w/w) as solvent. The Aroma Extract Dilution Analysis (AEDA) revealed the presence of six odour-active compounds, being 2-methyl pyrazine and furfural the key aroma compounds. By using PCA (Principal Component Analysis), there were no found any sensory and physicochemical difference regarding the geographical origin of samples.

Keywords: odour-active volatiles; simultaneous steam distillation-solvent extraction (SDE); *Saccharum officinarum* L derivative; non-centrifugal cane sugar (NCS); jaggery.

Chemical compounds studied in this article: 2-Methylpyrazine (PubChem CID: 7976), 2,5-Dimethylpirazine (PubChem CID: 31252), Furfural (PubChem CID: 7362), Propanoic acid (PubChem CID: 1032), 2-Methylpropanoic acid (PubChem CID: 6590), 2-Furanmethanol (PubChem CID: 7361).

1. Introduction

Due to the increasing concern on the negative impacts of excessive refined sugars intake, the global demand for sugar substitutes is continuously expanding. Recent estimates indicate that the sugar substitutes market is around 11.5 billion dollars and it is expected to grow up to 14 billion by 2019 (Markets and markets, 2015). Among the different commercial substitutes, consumers' preference is centered on those from natural origin because they are considered healthier and safer than processed ones. An important natural sweetener in developing countries is the non-centrifugal cane sugar (NCS), a solid unrefined product obtained from the processing of sugarcane juice (FAO, 1994). Depending on the country of origin, it is traditionally known with different names such as "*panela*", jaggery, gur, "*piloncillo*", "*mascavo*", or "*chancaca*".

In general, NCS is produced from the extracted, cleaned, and clarified sugar cane juice, by open evaporation of its water content up to 88-94 ºBrix, obtaining a hot and caramel-like product that solidifies at 18 °C. NCS exhibits a high sucrose content (84.49 ± 5.83 %) and some additional constituents as reducing sugars (glucose and fructose, 7.33 ± 2.81 %), minerals (K 531.26 mg/100 g, Ca 102.62 mg/100 g, P 57.54 mg /100 g), vitamins (vitamin E 55.65 mg/100g, vitamin C 4.23 mg/100 g), organic acids, amino acids, among others (Jaffé, 2015; Guerra & Mujica, 2010). Phenolic compounds with antioxidant activity have been also identified in this sugarcane derivative (Jaffé, 2015; Duarte-Almeida, Salatino, Genovese & Lajolo, 2011).

According with recent reports, world production of NCS is around 172 million tons per year (Foreign Agricultural Service, USDA, 2015), and it is dominated by India (~50%),

Colombia (~17%), and Pakistan (10%). In Colombia, together with coffee, a NCS hot infusion (in water) is one of the most important local drinks. This beverage is commonly known as "*agua de panela*" (i. e. water of panela) and it is used either as source of energy or as a warming up infusion. It is also consumed as a cold refreshing beverage traditionally mixed with limejuice. Because it is a high caloric solution with significant content of salts and vitamins, it is used as rehydrating beverage, and to relief for cold and flu symptoms.

Although this traditional beverage represents an important cultural heritage in Colombia, almost no studies have been done to characterise its physicochemical and sensory properties. According with the only study available in open literature, around thirty volatile compounds were identified by GC-MS in samples treated by Soxhlet extraction with dichloromethane. The main volatile compounds were furans, furanones, 2-acetylpyrrole, and 5-(hydroxy-methyl) furfural; these components are produced by the thermal degradation of carbohydrates via caramelisation or Maillard reactions during the drying of sugarcane juice (Payet et al., 2005).

Worldwide, there is an increasing interest for developing and consuming new added value naturally-based and artisanal-manufactured foods and drinks with protected designation of origin. Thus, the aim of this work was to carry out a physicochemical and sensory (aroma and color) characterization of "*panela*" beverage. This was prepared with NCS samples obtained from three different geographical regions of Colombia at different concentrations, allowing to assess the influence of those variables in the properties of the beverage. The medium-term purpose of this research is to use the obtained data as comparing quality parameters during the development of new processed products from sugarcane juice for the food industry.

2. Materials and methods

2.1. "Panela" samples

Solid "*panela*" blocks of *ca.* 500g, properly labeled with nutritional facts and geographical origin, were purchased at local stores, and used in experiments without further purification. The samples originated from three different geographical regions with different edaphoclimatic characteristic in Colombia, which are the states of major production in Colombia: Santander (northeast), Cundinamarca (center), and Valle del Cauca (southwest).

2.2. Chemicals

Diethyl ether, *n*-pentane, and anhydrous sodium sulfate were acquired from Merck (Darmstadt, Germany). All solvents were freshly distilled prior to use. An *n*-Alkane mix (C_8-C_{26}) was purchased to Laboratory Dr.Ehrenstorfer GmbH, Augsburg, Germany. Pure reference standards of 2-methyl pyrazine, 2,5-dimethyl pyrazine, furfural, propanoic acid, 2-methyl propanoic acid, and 2-furan methanol were were generously supplied by DISAROMAS S.A. (Bogotá, Colombia). γ-Nonalactone (Sigma Chem. Co., St. Louis, MO, USA) was used as internal standard.

2.3. Physicochemical characterisation

"*Panela*" samples were used as purchased to prepare the hot beverage following the traditional method at two different concentrations (25 % and 60 % w/w). In each case, the required weight of "*panela*" was put into a glass beaker and dissolved in 1000 mL boiling water (362 K under local pressure). The solution was maintained under continuous stirring and heating until complete dissolution. Once a homogeneous solution was obtained, heating and stirring were stopped, and the beverage was let cool to room temperature. Residual insoluble solids were removed by filtration before characterization. Further measurements were performed in triplicate to report the mean and standard deviation.

2.3.1. Total soluble solid content and pH

Total soluble solids were determined by using an Atago refractometer (HRS-500, Tokyo, Japan) and the results were expressed as °Brix. The pH of the beverage was determined by using a 370 pHmeter (Jenway, London, UK).

2.3.2. Colour measurement

Colour of different "panela" beverage was determined by using a Cary 5000 UV-Vis-NIR spectrophotometer (Varian, Victoria, Australia) with a 10 mm path length quartz cell. Visible absorption spectrum of each sample was recorded at wavelengths between 380 and 770 nm. CIELAB parameters (L^*, a^*, b^*) were determined by using the CromaLab® software (Heredia, Alvarez, González-Miret & Ramírez, 2004). All measurements were performed in triplicate to report the mean ± standard deviation. The

colour parameters, chroma (C_{ab}*) and hue (h_{ab}), were calculated according to the following equations (Meléndez-Martínez, Vicario & Heredia, 2003):

$$C^*_{ab} = \left[\left(a^*\right)^2 + \left(b^*\right)^2 \right]^{\frac{1}{2}} \quad (1)$$

$$h_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

2.4. Isolation of volatile extract

A Likens–Nickerson type SDE (simultaneous steam distillation-solvent extraction) apparatus (Likens & Nickerson, 1964) was used to extract volatile compounds of the different samples. For the analysis, 800 mL of each sample were loaded in a 2 L round bottom flask, and the extraction was carried out with 500 mL of *n*-pentane-diethyl ether (1:1, *v*/*v*) during 2 h. Each extract was dried over Na_2SO_4 and concentrated to 1.0 mL using a *Vigreux* column.

2.5. Analyses of odour-active volatiles by GC–O (Gas Chromatography–Olfactometry) and Aroma Extract Dilution Analysis (AEDA)

GC-O analyses were performed in a gas chromatograph HP 5890 Series II (Hewlett-Packard, USA) equipped with an FID and operated in split mode (1:10, injected volume, 1 µL). The injection port was set at 230 °C and Helium was used as carrier gas at 1.0 mL/min. Two capillary columns, DB-FFAP and DB-5 (each 30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Chromatographie-Handel Müller, Fridolfing, Germany, and

157 Restek, USA, respectively) were used for the volatile analyses. The column oven was
158 programmed from 40 °C (after 4 min) to 180 °C at 6 °C/min, then at 12 °C/min until 230
159 °C for the DB-FFAP and 300 °C for the DB-5, and finally held them for 10 min at the
160 maximum temperatures. The end of the capillaries were connected to a deactivated Y-
161 shaped glass splitter (Chromatographie Handel Mueller, Fridolfing, Germany), which
162 divides the effluent into two equal parts, one for FID (230 °C) and the other for heated
163 sniffing port (200 °C) by using deactivated fused silica capillaries of the same length (50
164 cm x 0.32 mm i.d.). Sniffing port consisted of a self-made elbow-shaped aluminium tube
165 (80 x 5 mm i.d).

166 Three panelists located the odour-active zones of the SDE extracts by GC-O. Then, the
167 aroma extract was stepwise diluted to obtain 2^n dilutions, and each solution was analysed
168 by GC-O. The odour activity of each compound, expressed as flavor dilution factor (FD),
169 was determined as the greatest dilution at which that compound was still detected by
170 comparing all of the runs (Grosch, 1994). The FD factors obtained by three panelists were
171 averaged.

172

173 *2.6. Gas Chromatography–Mass Spectrometry (GC–MS)*

174

175 GC-MS (EIMS) analyses were carried out on a GC Agilent 7890B gas
176 chromatograph equipped with an ITQ 900 mass selective detector (Agilent Technologies
177 Inc. Wilmington, DE, USA). MS data were recorded between 40-400 u, with an electron
178 energy of 70 eV and processed by Mass Hunter software. Chromatographic conditions were

179 the same that those above-mentioned for GC-FID analyses, and Wiley library was used for
180 compound identification.
181
182 *2.7. Compound identification and quantitation*
183
184 Linear retention indexes (LRI) of the odour-active compounds were calculated by using
185 a mixture of normal paraffin (C_8-C_{26}) as external references. The identification of volatile
186 compounds was completed by comparison of their retention indexes (in the two columns),
187 mass spectra, and odour notes, with those exhibited by standard solutions of volatiles (50
188 µg/mL), if they were available.
189 Quantitative analyses of *panela* beverage odour-active volatiles exhibiting dilution
190 factors higher than 8 was done by the internal standard (IS) method. For this purpose, γ-
191 nonalactone was dissolved in the extraction solvent (100 µg/mL), and added to the panela
192 solution before extraction by SDE. To determine the response factor for each volatile
193 compound, calibration curves were constructed using a series of solutions of varying
194 nominal concentrations containing each analyte (IS:analyte from 1:5 to 5:1), where the
195 slope was assumed as the response factor. An identical amount of the internal standard was
196 added to each solution (IOFI, 2011). All data were obtained by triplicate. The concentration
197 of each analyte was calculated by comparison of GC-FID signals with those of standards,
198 taking into account the relative response factor, according to the following equation:

199
$$[\,]x = \frac{A_x}{A_{istd}} * \frac{\mu g\ istd}{kg\ Panela} * RF \qquad (3)$$

Where [ ]x is the analyte concentration in mg/kg *panela*, A_x is the analyte area, A_{istd} is the internal standard area, and RF is the response factor. Key-aroma compounds were determined based on their OAV (odour activity value = concentration divided by odour threshold; Grosch, 1994) at 60% w/w concentration.

2.8. *Statistical analysis*

Principal Component Analysis (PCA) was applied to analise the data sets of during the physicochemical and sensory characterisation of panela beverages. PCA analysis was applied within XLSTAT version 2015.6.01.24027 (Addinsoft, New York, NY, USA). Similarity maps of images were drawn using the component scores. Interpretation of components was obtained by looking at the linear correlation between the original variables (called loading factors). The relationship between variables is given by the correlation monoplot called score plot. The data of the physicochemical characterisation were analysed by variance and regression analysis and average values were compared using Turkey's test with a probability $p \leq 0.05$.

3. Results and discussion

3.1. Physicochemical characterisation

The pH, total soluble solid content, and colour parameters for each sample of *panela* beverage at different concentrations are presented in **Table 1**. In general, there were no

223 significant differences among pH values of different samples at the same concentration,
224 being the Cundinamarca NCS sample slightly more acid sample than the other two. This
225 was expected because during the traditional evaporation process, alkaline components are
226 added to avoid excessive sucrose hydrolysis. Some organic acids such as, aconitic, succinic,
227 and malic have been detected in NCS (Jaffé, 2015), and they contribute to the acid pH of
228 this beverage. As expected, the total soluble matter content varied accordingly with the
229 evaluated NCS concentrations.

230 The analysis of the colour values showed that the lightness, L^*, decreased with the
231 increase of concentration in Santander ($\Delta L^* = 4.2$) and Valle ($\Delta L^* = 9.7$) samples; in
232 contrast, there was not a significant change in Cundinamarca sample ($\Delta L^* = 1.4$) variety.
233 The sample of Cundinamarca exhibited L^* values slightly lower as well as higher a^* and b^*
234 values than those in the other samples, in agreement with its dark colour. The projection of
235 the points as a function of the geographical origin and beverage concentration onto the (a^*,
236 b^*) diagram, is shown in **Fig. 1**. In this colorimetric tristimulus analysis is shown that all
237 samples are remained nearly to a chromatic axe. The hue (h_{ab}^*) of the samples did not
238 reveal heterogeneity neither a significant dispersion (less than 10°) of the data in the
239 diagram what means that they are similar to each other. In the three NCS samples the b^*
240 value slightly increase with the concentration of NCS, becoming more positive in
241 Cundinamarca sample. The data obtained for Cundinamarca's and Valle's samples are
242 located in the first quadrant of a^*, b^*, and Santander sample in the second a^*, b^* quadrant,
243 because of the slightly variations of a^* value.

The total colour difference (ΔE_{ab}*) at a concentration of 60% (*w/w*) among samples from Cundinamarca and Santander was 21.2, Cundinamarca and Valle was 10.3, and Santander and Valle 11.1. At a concentration of 25% (w/w), those ΔE_{ab}* values were 22.2, 21.2, and 3.6, respectively. These results mean that the samples of Cundinamarca and Santander present a major difference in colour. On the other hand, ΔE_{ab}* values less than 3, are considered as similar in colour because the human eye could not detect any difference.

Colour in NCS is a very variable attribute, which depends on several factors, such as, sugarcane variety, presence of some phenolic compounds, agro-ecological conditions, temperature and time of processing, among others. This is a critical point of quality; thus, in the southwestern region (Valle del Cauca) bleaching agents are normally used because consumers there, prefer paler panela. However, in the case of Santander sample, the factory where it is produced, uses steam instead of direct fumes during the open evaporation, thus reducing oxidation.

3.2. Identification of odour-active volatiles and Principal Component Analysis (PCA)

SDE method was selected as the extraction method because the characteristic NCS flavour is developed after it is dissolved in boiling water. This method has been also uded in the flavour characterization of similar hot beverages such as, coffee or tea (Pripdeevech & Machan, 2011).

AEDA analysis of SDE extracts revealed six odour-active volatile compounds (**Fig. 2**, **Table 2**) among several volatile compounds, as responsibles for the nutty, toasted, sweet, burnt, and rancid odour notes. 2-Methyl pyrazine and 2,5-dimethyl pyrazine exhibited the

267 highest FD values, in all of the samples. Remarkable, 2-methyl pyrazine and furfural were
268 described by the panelists as "*panela*-like" flavoured (i. e. caramel-like). Furfural and 2-
269 furanmethanol are characteristics volatiles produced by sugar (hexose) thermal breakdown,
270 as well as pyrazines. All of them are considered Maillard reaction products, which occurred
271 during the sugarcane juice evaporation to obtain NCS (Fisher & Scott, 1997; Payet, Sing &
272 Smadja, 2005). Payet, Sing & Smadja (2005) had reported that the major volatile
273 constituents of NCS were furans, furanones and 5-hydroxy methyl furfural.

274 The results of the quantitation experiments show the range of the concentration of
275 each odorant at a 60% w/w concentration (**Table 2**). To estimate the aroma potency of the
276 individual *panela* beverage odorants, their concentrations were correlated with the
277 respective odour thresholds, by using the odour activity value (OAV) concept (Schieberle,
278 1995). Based on OAVs 2-methyl pyrazine and propanoic acid were identified for the first
279 time to be key aroma compounds of *panela* beverage.

280 The overall characterization of *panela* samples is summarized in PCA diagrams of
281 **Figure 3A**. The first and second principal components (Factor 1 and Factor 2) explained
282 54.68 % and 31.41 % of variance across the samples, respectively. The factor 1 was a
283 contrast between the º Brix, olour parameters *a** and *b**, and concentration of odour-active
284 compounds vs *L** and pH. The factor 2 seemed to show a contrast between ºBrix, L^*, pH,
285 and concentration of odour-.active volatiles vs *a** and *b**, and furfural (C3). The variable
286 *a** has a high correlation with *b** parameter opposite to *L** value, in agreement with the
287 results showed in **Fig. 1.** Based on quantitative data, none of the odour-active compounds
288 (C1 to C6) allow to discriminate among the samples. This is in contrast with sensory results
289 that showed 2-methyl pyrazine (C2) and propanoic acid (C4) as key aroma compounds, and

exemplify the relevance of sensory analysis. The PCA analysis showed that there is no any tendency to group the data according to the geographical origin of NCS samples (**Fig. 3B**).

4. Conclusions

In this work, a physicochemical and sensory (aroma and colour) characterization of non-centrifugal sugar (*panela*) beverage was carried out. Results showed that panela samples from different geographical origins in Coliombia could not be differentiated by the amount of odour-active volatiles neither pH nor total soluble contents. In contrast colour parameters are a good tool to stablish differences. The identification of odour active volatile compounds in panela beverage was done here for first time; these findings allow to producers to have a sensory tool for quality control of *panela* products. Among three samples evaluated, Cundinamarca sample was different from the other two, but regarding aroma and colour there is no significant differences according to the geographical origin.

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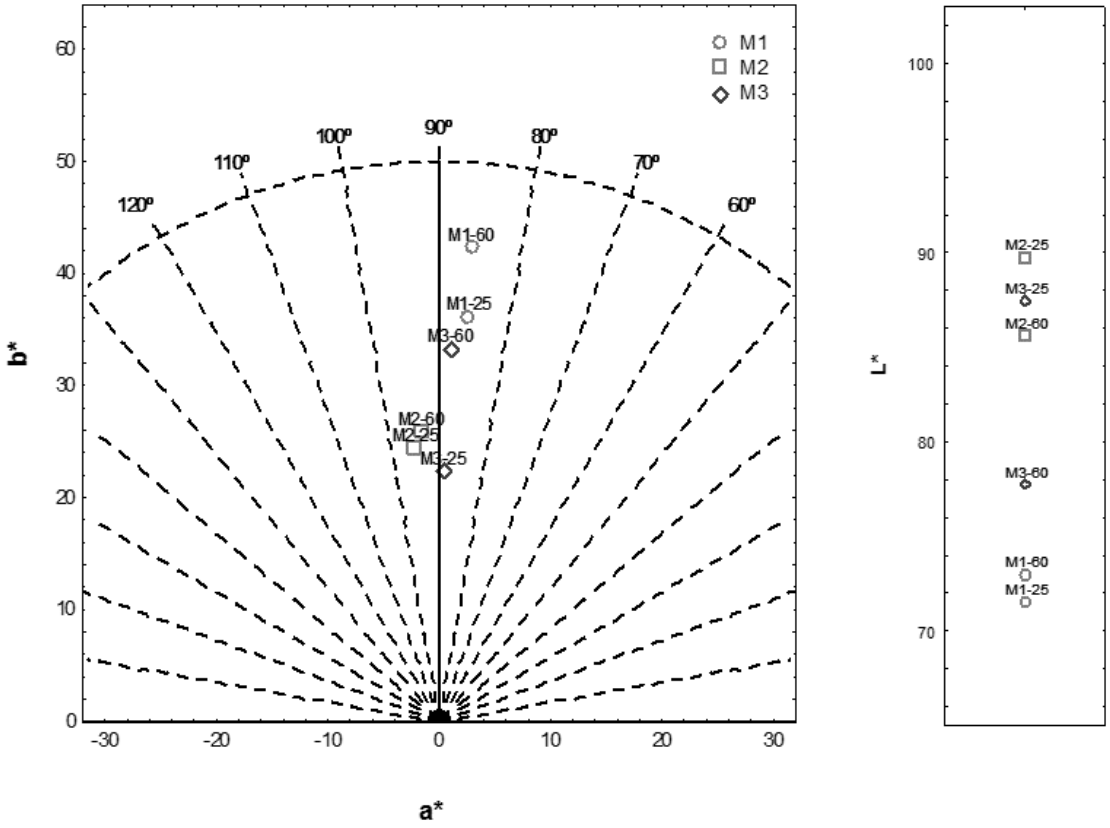
Figure captions

Fig. 1. Localisation on the (*a**, *b**) diagram of colour parameters of panela beverage samples from different geographical origins, obtained at two concentrations, 25% w/w and 60% w/w. Cundinamar ca ○ M1 (M1-60, M1-25), Santander □ M2 (M2-60, M2-25), and Valle del Cauca ◇ M3 (M3-60, M3-25).

Fig. 2. GC analyses on FFAP column of the volatile compounds from panela beverages (60% w/w) obtained by SDE from, A) Cundinamarca's sample, B) Santander´s sample, and C) Valle´s sample. Numbers correspond to Table 2.

Fig. 3. Principal component analysis (PCA) (A) loading plot, (B) score plot for physicochemical parameters measured in panela beverage from different geographical origins (C = Cundinamarca, S = Santander, and V= Valle del Cauca) at two concentrations (25 = 25% w/w, 60 = 60% w/w), B). PH= pH, Brix= °Brix, C_a = *a**, C_b = *b** and C_L = *L**; the concentration of each compound shown in Table 2 correspond to C1 = 2-methylpyrazine, C2 = 2,5-dimethylpyrazine, C3= furfural, C4 = propanoic acid, C5 = 2-methylpropanoic acid, and C6 = 2-furanmethanol.

Figure 1.


M1
M2
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a*
L*
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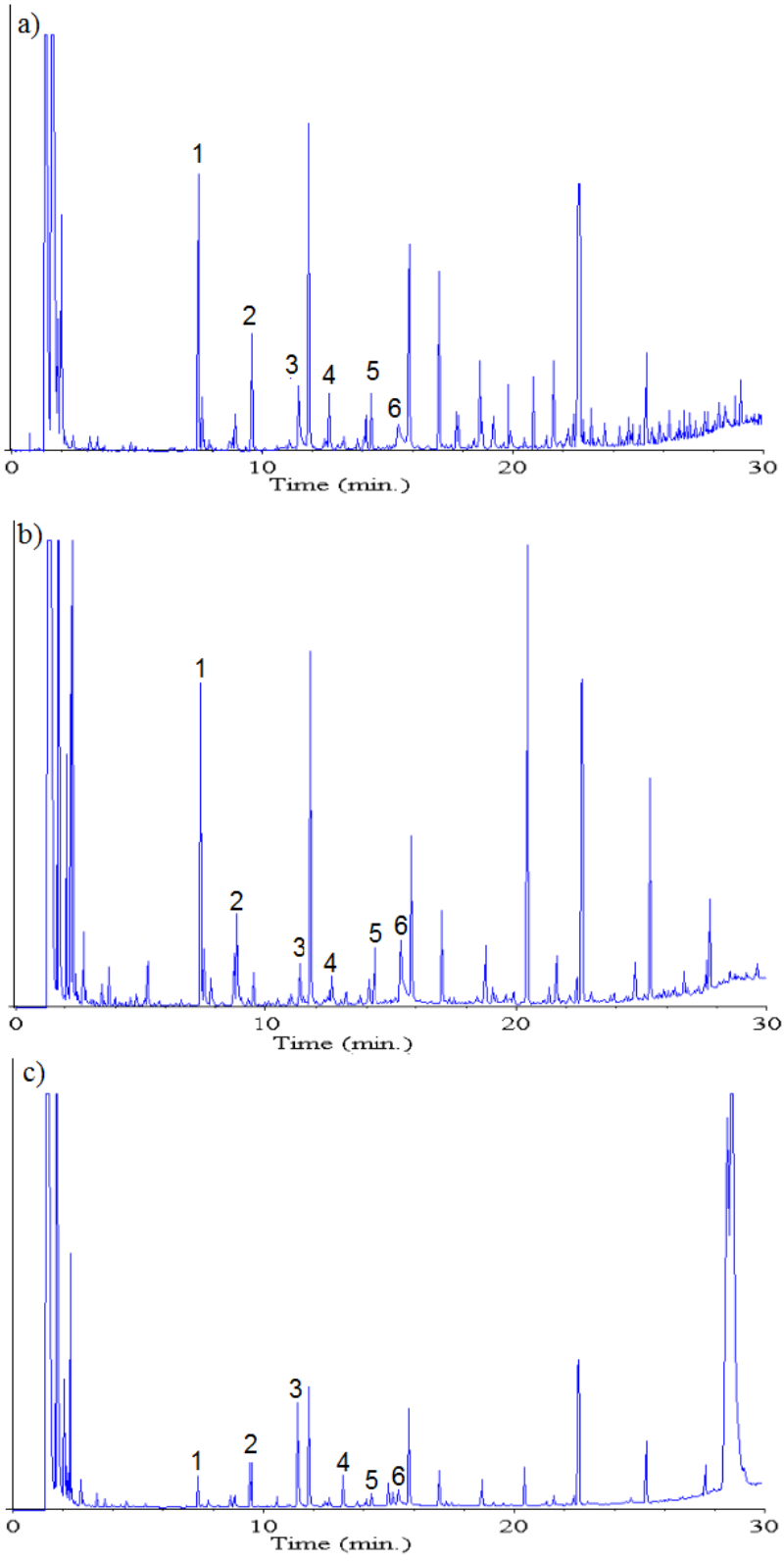
M1-60
M1-25

Figure 2


a)
1
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Time (min.)
b)
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Time (min.)
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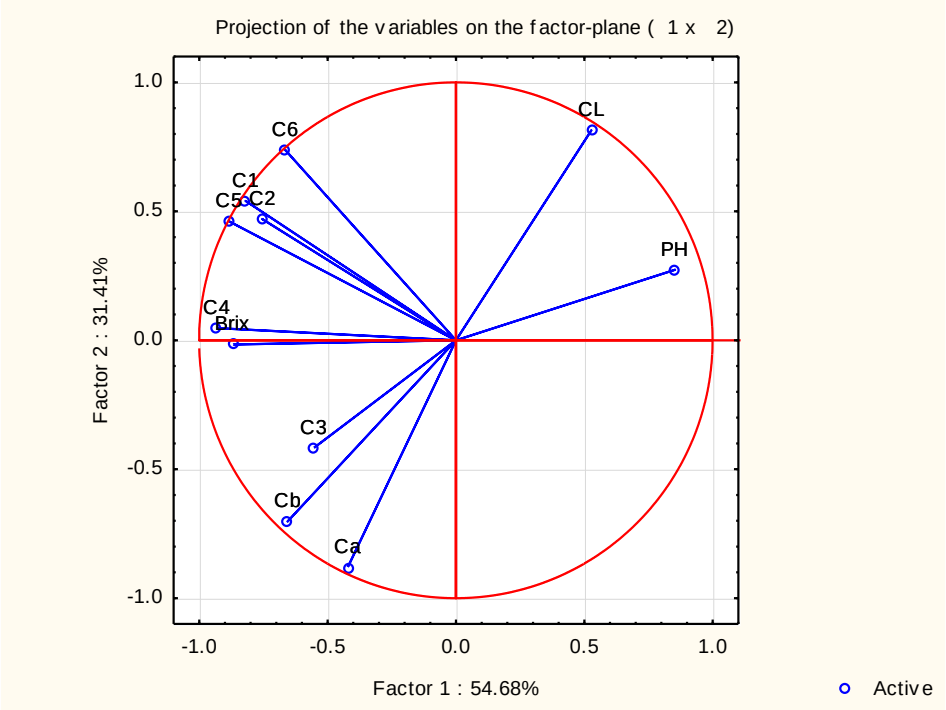
Time (min.)

Figure 3.

A. Loading plot



B. Score

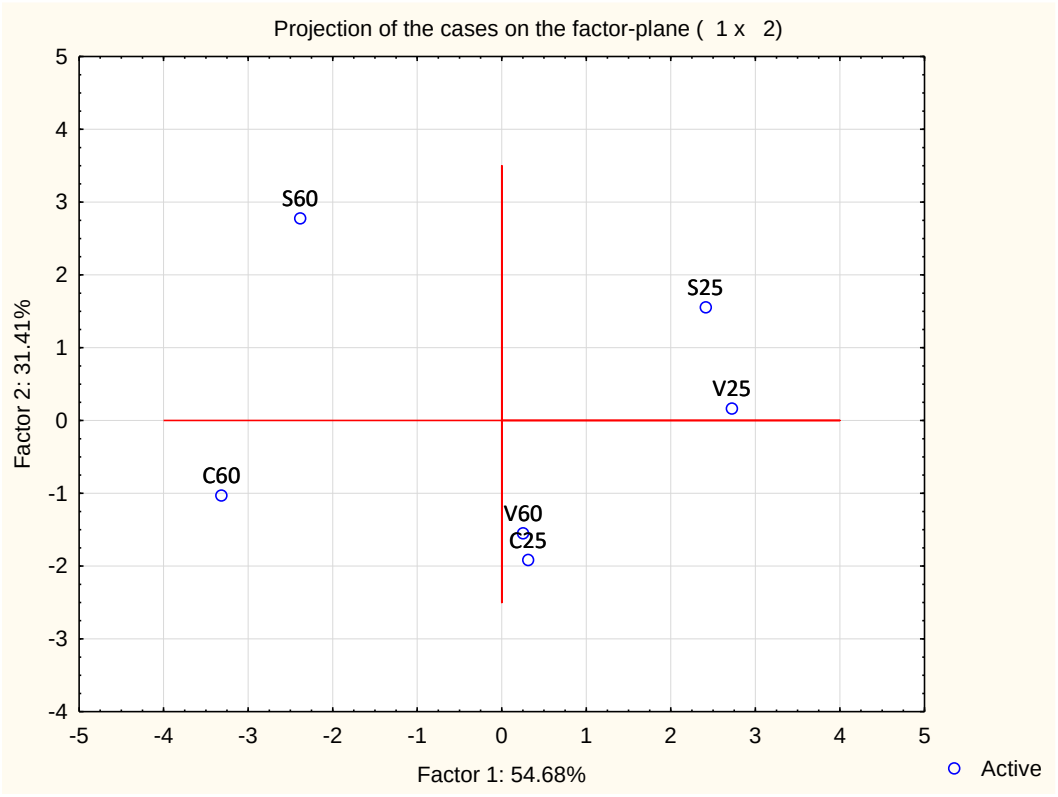


Table 1

Physicochemical characterisation of panela beverage at different concentration and from different geographical regions

Property[a] \ Sample origin		Cundinamarca (C)		Santander (S)		Valle del Cauca (V)	
		Concentration (w/w)					
		25%	60%	25%	60%	25%	60%
Total soluble solids (°Brix)		24.4 ± 0.5^{a}	39.6 ± 5.5^{b}	17.2 ± 1.1^{c}	40.9 ± 3.4^{b}	22.9 ± 1.2^{a}	37.5 ± 5.4^{b}
pH		5.51 ± 0.11^{a}	5.40 ± 0.01^{a}	5.80 ± 0.03^{b}	5.58 ± 0.05^{a}	5.82 ± 0.05^{b}	5.75 ± 0.22^{ab}
Colour parameters	L^*	71.6 ± 4.5^{a}	73.0 ± 2.2^{a}	89.8 ± 1.5^{b}	85.6 ± 2.8^{bc}	87.4 ± 0.8^{c}	77.7 ± 2.9^{a}
	a^*	2.7 ± 1.6^{a}	3.0 ± 0.2^{b}	-2.1 ± 0.2^{c}	-1.5 ± 0.3^{d}	-0.4 ± 0.2^{e}	1.1 ± 0.4^{a}
	b^*	36.1 ± 6.5^{a}	42.3 ± 0.7^{ac}	24.3 ± 3.2^{b}	25.8 ± 3.0^{b}	22.3 ± 2.4^{b}	33.2 ± 1.2^{ac}
	C^*_{ab}	36.2 ± 6.6^{a}	42.4 ± 0.7^{ac}	24.4 ± 3.2^{b}	25.9 ± 3.0^{b}	22.3 ± 2.4^{b}	33.2 ± 1.2^{ac}
	h_{ab}	85.9 ± 2.0^{a}	86.0 ± 0.4^{a}	94.9± 1.0^{b}	93.4 ± 0.7^{b}	91.1 ± 0.7^{c}	88.1 ± 0.6^{a}

[a] All data are the mean of three measurements ± standard deviation. Equal letters in a file means that there are no significant differences based on Tukey test ($p < 0.05$).

Table 2

Odour-active volatiles detected in panela beverage SDE extracts from different geographical regions

No[a]	Odorant[b]	Odour description[c]	RI		FD[d]	Odour Threshold (µg/Kg water)	Conc ± SD (% CV) (µg/Kg panela)[e]			OAV[f]		
			FFAP	DB-5			C	S	V	C	S	V
1	2-Methylpyrazine	Nutty, *panela*-like	1266	801	4096	60[g]	494.9 ± 2.1 (0.4%)	637.2 ± 0.1 (0.0%)	96.1 ± 0.9 (0.9%)	8	11	2
2	2,5-Dimethylpyrazine	Burnt, toasted	1320	889	1024	800[g]	267.2 ± 0.0 (0.1%)	241.9 ± 0.1(0.0%)	247.2 ± 0.9 (0.3%)	<1	<1	<1
3	Furfural	Sweet, *panela*-like	1457	804	64	3000[h]	266.4 ± 0.1 (0.0%)	138.5 ± 0.1(0.0%)	302.1 ± 0.4 (0.1%)	<1	<1	<1
4	Propanoic acid	rancid	1535	714	512	20000[g]	109.8 ± 1.5 (1.4%)	126.3 ± 0.0 (0.0%)	89.4 ± 1.4 (1.5%)	<1	<1	<1
5	2-Methylpropanoic acid	Cooked, rancid	1570	807	64	8100[g]	139.3 ± 0.0 (0.0%)	184.8 ± 0.3(0.2%)	46.1 ± 1.0 (2.2%)	<1	<1	<1
6	2-Furanmethanol	Honey	1660	866	256	8000[g]	81.8 ± 1.9 (2.3%)	162.1 ± 0.9 (0.5%)	27.3 ± 0.7 (2.7%)	<1	<1	<1

[a] Number were consecutively assigned as elution order in FFAP column (Fig. 2).
[b] Odorants were RI (Retention index), odour quality, and mass spectra compared with reference compounds.
[c] Odour quality as perceived at the sniffing port during GC-O.
[d] FD factor = flavour dilution factor
[e] All data are the mean of three measurements ± standard deviation. In all of the cases, correlation coefficient was higher than 0.995 and response. Geographical origin: C = Cundinamarca, S = Santander, V = Valle del Cauca, at 60% w/w concentration.
[f] OAV = odour activity value, concentration divided by odour threshold. This parameter was calculated only for 60% (w/w) beverages.
[g] Leffingwell et al., 2008.
[h] Verschueren (2001).