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EFFECT OF AGEING ON LEES DISTILLATION PROCESS ON FERMENTED SUGARCANE MOLASSES FOR THE PRODUCTION OF RUM

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Abstract :

The study aimed at evaluating the influence of fermented sugarcane molasses ageing on lees and the distillation process used for the production of rums. Molasses were freshly fermented or 3-months lees aged. Batch (PS: Pot Still) or continuous (CS: Coffey Still) distillation was carried out resulting in four different rum distillates. Gas chromatography and 3D-fluorescence enabled to differentiate rum distillates chemical composition according to the distillation process, regardless of the ageing on lees of fermented molasses. Differences in fluorescent PARAFAC components and volatile acids, acetals and carbonyls contents revealed the predominance of the physicochemical processes driven at the liquid-vapor interface of fermented molasses, generated by the distillation systems. Notwithstanding the distilling conditions, the long chain fatty ester content was significantly higher in the 3-months lees aged condition. Multivariate analysis highlighted that CS rum distillates were chemically more homogeneous than those obtained by PS that preserved the lees effect.

Keywords : sugarcane molasses, ageing on lees, distillation process, aroma analysis, chemical diversity

34 **1. Introduction :**

35 Sugarcane molasses are the viscous end product of sugar companies which is
36 mostly valued as raw material prior to fermentation and distillation for rum
37 production. The choice of yeasts and the conditions of fermentation differentiate
38 molasses wort chemical composition which are revealed later in the
39 characteristics of volatile composition of distillates (Medeiros, de Matos, de
40 Pinho Monteiro, de Carvalho, & Soccol, 2017). During the elaboration of fruit,
41 cereal or plant fermented beverages, a great diversity of microorganisms can be
42 used but the yeast *Saccharomyces cerevisiae* remains the main species generally
43 used (Campos, Silva, Dias, Basso, Amorim, & Schwan, 2010; Walker & Stewart,
44 2016). Additionally in the area of distilled beverages, particularly in whisky
45 production, specific strains of *Saccharomyces cerevisiae* have been selected for
46 their high alcohol content tolerance and their capacities to convert mash sugars
47 into ethanol, carbon dioxide and numerous flavor congeners (Stewart, Hill, &
48 Russell, 2013). In the area of rum production, the inoculation of selected yeasts
49 strains for sugar cane fermentation can be excluded, in favor of the expression of
50 indigenous microbial flora, often associated with rums richer in aromas. For
51 example, the “Rhum Agricole” involves a complex indigenous microbiota made
52 of mixes of yeasts and bacteria, already present in the sugarcane juices.
53 *Lactobacillus* and *Propionibacterium* species have also been shown to remain in
54 sugarcane molasses used for “Rhum Grand Arôme” production (Fahrasmane &
55 Ganou-Parfait, 1998). Another practice used for producing heavy rums consists
56 of adding the “dunder” in the fermenting molasses wort. The “dunder” is the
57 residual creamy vinasse from the previous distillation, made of sugars and dead
58 yeast cells (Fahrasmane & Parfait, 2003; Medeiros, de Matos, de Pinho Monteiro,
59 de Carvalho, & Soccol, 2017). Such ancestral practice could be hazardous with
60 the risk of low alcoholic fermentation yields, unachieved fermentations and the
61 development of spoilage microorganisms. The control of fermentation can be
62 improved by direct inoculation of pure cultures of microorganisms or inoculation
63 of a mother yeasting pre-cultured in a fermenter. In some cases, dried yeasts can
64 be directly added in the washing media (Fahrasmane & Ganou-Parfait, 1998;
65 Murtagh, 2003). The choice of strains impacts the quality of rums. The
66 distinction between the different types of rums, light or heavy rums for instance,
67 can be designed by the choice of inoculated yeast strains belonging to *Saccharomyces*

68 *cerevisiae*, *Saccharomyces bayanus* or *Schizosaccharomyces pombe* (Fahrasmane
69 & Ganou-Parfait, 1998; Medeiros, de Matos, de Pinho Monteiro, de Carvalho, &
70 Soccol, 2017). The quality of the final spirit can be also modulated with a
71 sugarcane fermentation obtained by co-inoculation of a consortium of
72 microorganisms (Duarte, de Sousa, Dias, & Schwan, 2011). Moreover, the presence
73 of yeast lees in the mash could positively impact the spirit's quality, especially for heavy rums
74 (Medeiros, de Matos, de Pinho Monteiro, de Carvalho, & Soccol, 2017; Murtagh,
75 2003). The presence of yeast lees during distillation has been shown to promote
76 different releases in ethyl esters, ethyl hexanoate and octanoate in particular,
77 leading to differences in rum styles (Suomalainen, 1981).

78 Rum technology involves two distillation techniques used all around the world of
79 distilled beverages: the ancestral one with the pot still and the industrial one with
80 coffey still (L. Fahrasmane & Parfait, 2003). In both cases, odorous volatile
81 compounds, concentrated in the final spirit, enabled a classification of the
82 different types of rums according to their level of concentration. Traditional
83 agricultural rums produced from raw sugar cane differ from sugar refinery
84 molasses rums in composition and concentration, generally due to differences in
85 the distillation process (Pigott, 2003). Liebich et al. (1970) identified more than
86 200 flavor compounds in a Jamaican rum using liquid extraction of rum prior to
87 rum analysis by gas chromatography coupled to mass spectrometry, with
88 concentrations reaching 800 ppm, particularly for fused alcohols (Liebich,
89 Koenig, & Bayer, 1970). According to Marse et al. (2004) rum is one of the
90 distilled beverages that has the most of volatile compounds, reaching 550
91 different aromas (Maarse & Van Den Berg, 1994). Some Grand Arôme and heavy
92 rums, often appreciated from rum tasters due to their elevated esters content, can
93 reach concentrations of more than 500 g/hL of pure alcohol (L. Fahrasmane &
94 Ganou-Parfait, 1997). According to Fahrasmane and Ganou-Parfait (2011), the
95 control of the organoleptic quality of heavy rums production remains a big
96 challenge for rum producers and scientists due to the variability in microbiota
97 and the impact of distillation processes. This study presents a quantification of
98 the effect of ageing on lees and the distillation process based on the
99 quantification of chemical differences in the composition of major volatile
100 compounds families and fluorescent components. The discrimination potential of

101 each fermenting and distilling practices in sugarcane molasses rums was
102 evaluated by multivariate statistical analysis.

103 2. Materials and methods

104 2.1. Wort samples and fermentations

105 2.1.1. Sugarcane molasses characteristics and wort preparations

106 Sugarcane molasses were supplied by a French rum company (Compagnie des
107 Indes, Beaune, France). Prior to fermentation, the molasses were diluted with
108 distilled water, in order to obtain 50 kg of diluted molasses characterized by a
109 density of 1.090 at 20°C with a DMA 35 densimeter (Anton Paar, Graz, Austria).
110 The diluted molasses presented a Brix degree of 16 and an initial pH of 4.9. Then
111 16 kg of diluted molasses were poured into three 20 L glass demijohns and
112 supplemented with 30 g/hL of diammonium phosphate (Sigma), 30 g/hL of yeast
113 assimilable nitrogen (Mauriferm Gold, AB Maury, Peterborough, UK). The strain
114 of *Saccharomyces cerevisiae* was Pinnacle MG+ (AB Mauri, Peterborough, UK),
115 packaged in active dry form. The yeast inoculation was applied at the dose of 40
116 g/hL, according to the manufacturer's recommendations.

117 2.1.2. Fermentation processes

118 The fermentations were conducted in demijohns without stirring at room
119 temperature (18-25°C) and monitored in terms of density and temperature.
120 Measures were realized twice per day with a DMA 35 densimeter (Anton Paar,
121 Graz, Austria). Demijohns were weighed with a numeric analytical scale of 35
122 kg (Mettler Toledo, Greifensee, Switzerland).

123 Two series of fermentations were carried out in biological triplicates at three
124 months of interval. After fermentation, the first mashes were left at 4°C in
125 contact with the yeast lees (L: Lees) to age during three months (L1, L2, L3).

126 The second fermentations (F: Fresh) were carried out in triplicates (F1, F2, F3),
127 with the same protocol as previously described, just prior to the distillation. In
128 all cases, yeast lees (fresh or aged) were removed from mashes before
129 distillation.

130 *S. cerevisiae* strain implantations were controlled at the middle of alcoholic
131 fermentation using a PCR interdelta analysis according to a previously published
132 procedure (Legras & Karst, 2003). As illustrated in Fig. S.I.1 all sugarcane
133 molasses were fermented with the same yeast strain.

134 2.2. Distillates samples

135 Two types of distillation: the pot still (*PS*) and the column still (*CS*) were carried
136 on the six samples of fermented sugarcane molasses. Distillation systems used in
137 this study can be viewed in Fig. S.I.2. For that, half of the demijohn content,
138 corresponding to 8 kg was poured into the pot still and 8kg was poured into the
139 column still generating twelve distillates that were used for chemical analyses.

140 2.2.1. Pot Still distillation

141 Pot still distillation was heated directly by flame contact with the copper surface
142 of the 25 L copper still. Two distillations were carried out, the first one leading
143 to the “low wines” and the second one leading to the final white distilled spirit.
144 Volumes and ethanol content of these final distillates were analyzed. For this
145 second pot still distillation, we decided to cut at 50 % of alcohol content for the
146 six wort batches (*PS-F1*, *PS-F2*, *PS-F3*, *PS-L1*, *PS-L2*, *PS-L3*) in order to keep an
147 optimized control of pot still distillation process. The foreshots were removed
148 and corresponded in each case to an approximated volume of 100 mL
149 characterized by an intense solvent olfactive character.

150 2.2.2. Coffey Still distillation

151 Column still distillation was carried out on a 25 L Holstein column (Markdorf,
152 Germany). Temperatures in the boiler, heater, column and deflegmator were
153 automatically measured, with a control of the boiler temperature. Heat was
154 generated by a steam flow in direct contact with the copper still and controlled
155 by a pressure of 150 mbars, enabling to keep a constant temperature of 90 °C
156 inside the still. Temperature, alcohol content and distillate flow rate were
157 automatically monitored online thanks to an infrared detector for the six wort
158 batches (*CS-F1*, *CS-F2*, *CS-F3*, *CS-L1*, *CS-L2*, *CS-L3*). The control of the cooling
159 system was adjusted with an automatic valve. The distillate flow rate was kept

160 constant between 15-20 mL/min and collected as the hearts of the distillation and
161 once passing below 10 mL/min the hearts were separated from the tails. The
162 foreshots were removed the same way as described in the pot still distillation

163 2.3. Chemical analysis

164 2.3.1. Wort and distillate characterization

165 Wort and distillate classical parameters such as ethanol, pH and total acidity and
166 ethanol (only for distillates) were determined according to OIV standardized
167 methods (Recueil des méthodes internationales d'analyse des boissons
168 spiritueuses des alcools et de la fraction aromatique des boissons. OIV 1994).
169 Ethanol content was determined in the mashes at the end of fermentation by an
170 enzymatic method following the manufacturer's instructions (BioSentec®,
171 France).

172 2.3.2. Distillate volatile composition

173 The distillates were also submitted to a targeted analysis of the volatile chemical
174 composition. The liquid extracts (990 mL of distillate sample and 10 µL of octan-3-ol at 1
175 g/L) were analyzed with a Agilent Technology 5975C spectrometry (Shimadzu QP2010+,
176 electronic impact at 70 eV) paired with a Agilent Technology 7890 A gas chromatograph
177 fitted with a split/splitless injector (250°C). The chromatograph was equipped with a capillary
178 column PEG of 30 m × 0.32 mm (J&W Scientific). Film thickness was 0.50 µm. Helium was
179 used as vector gas at a rate of 1.5 mL/min (average velocity of 44 cm/sec). The temperature of
180 the oven was increased from 50°C to 240°C at 5°C/min, and finally held at 240°C for 5
181 minutes. The injection mode was splitless. The analyses were done in triplicate. Spectrometry
182 Selected Ion Monitoring method (SIM method) was used for molecules detection. The mass
183 spectrometer scanned from m/z 29 to 500. The volatile compounds were identified by
184 matching their spectral fragmentation with those provided by the mass spectral library of the
185 National Institute of Standards and Technology (NIST) and the Wiley Registry (WILEY) and
186 by validating with pure chemical standards. Quantification was carried out via an
187 internal standard method by the addition of octan-3-ol to distillates reduced to 50
188 % ethanol (v/v) with ultrapure water prior to injection. Response factors were
189 calculated for volatile compounds from calibration curves obtained by analyzing
190 hydroalcoholic solutions (ethanol 50 %, v/v) made from pure analytical grade

191 standards (SigmaAldrich, Saint Louis, MO) in the ranges 0.05-10 mg/L for
192 phenylethanol, eugenol, ethyl acetate, isoamyl acetate, ethyl lactate, ethyl
193 butanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 1,1-diethoxy
194 ethane, diacetylene, 2-methyltetrahydrofuran-3-one, furfural, propanoic acid, n-
195 decanoic acid, propanoic acid, 2-methyl and octanoic acid and 1-200 mg/L for
196 propanol, 2-methyl-propanol, butanol, 3-methyl-butanol, 2 methyl-butanol. The
197 concentrations of volatile compounds were converted in grams per hectoliter of
198 pure alcohol following CE regulation 2870/2000.

199 2.3.3. Excitation Emission Matrices of Fluorescence (EEMF) of rum distillates

200 All rum distillates were analyzed with an untargeted approach consisting of
201 measuring Excitation Emission Matrices of Fluorescence (EEMF). For that, rum
202 distillates were diluted twenty times with ultrapure water and put in 1 cm path-
203 length quartz cuvette and EEMFs were recorded in a Horiba Aqualog unit,
204 enabling to automatically correct the Rayleigh and Raman scattering and the
205 inner filtering effect and to normalize EEMFs to a quinine sulfate 1 ppm
206 solution.

207 2.4. Statistical analysis

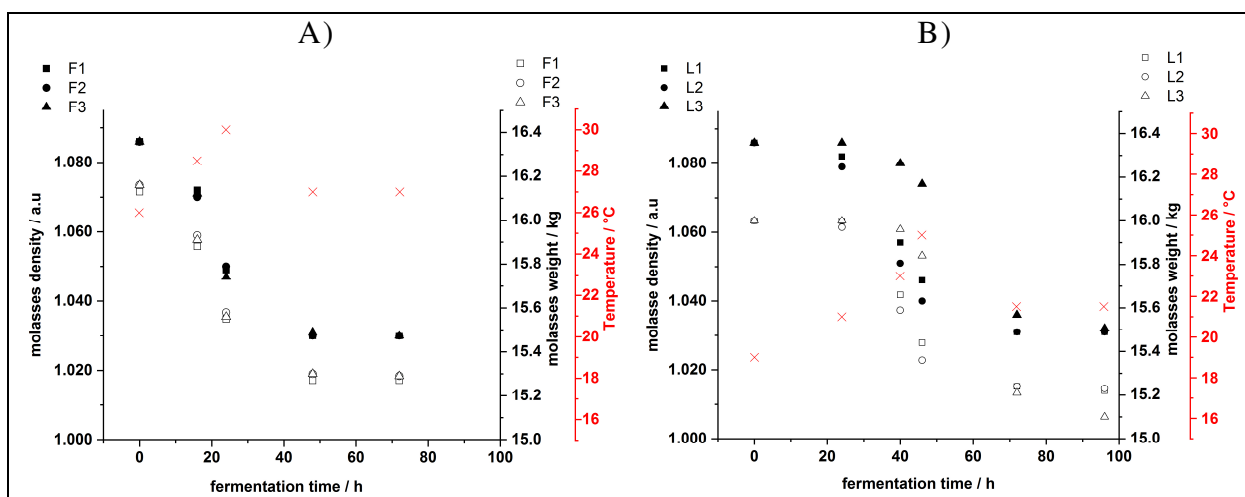
208 Aroma concentrations were statistically analyzed by multivariate analysis using
209 Origin Lab software. PARAFAC model of rum distillates EEMFs was built on
210 home made Matlab software, previously used for wine PARAFAC modeling
211 (Coelho, Aron, Roullier-Gall, Gonsior, Schmitt-Kopplin, & Gougeon, 2015).
212 PARAFAC model was validated by core consistency and split half validation of
213 the dataset. PARAFAC model described each PARAFAC components by their
214 fluorescence intensities at their maximum, represented as Fmax values. Fmax
215 values were used to statistically interpret the distillate fluorescent composition
216 and classify the different rum distillates in function of their elaboration
217 processes.

218 Mean Fmax values of PARAFAC components and mean volatile compounds
219 concentrations were statistically compared with an ANOVA test with an interval
220 of confidence of 95 %, followed by a Tukey's HSD post hoc test to evaluate the
221 impact of yeast lees ageing and distillation practice.

222 3. Results and discussion

223 3.1. Fermentation monitoring

224 The evolutions of the molasses wort density, weight and temperature upon the
225 fermentation stage for the fresh (F) and yeast lees (L) modalities are presented in
226 Fig. 1A and 1B, respectively. Fermentations started at a density of 1.086 and
227 reached a final density of 1.030 for each modality. Molasses weight decreased
228 from 16.1 kg to 15.3 kg for F modalities and from 16.0 kg to 15.1-15.2 kg for L
229 modalities. For F modalities, fermentation started just after the yeast strain
230 inoculation and finished within 48 hours for the three biological replicates (F1,
231 F2 and F3). For L modalities, we observed a lag phase of 24 hours following
232 yeast strain inoculation for L1 and L2. This lag phase was around 40 hours for
233 L3. These delays were probably due to lower non-controlled fermentation
234 temperatures of 20 °C compared to 26 °C for the fresh modality. Fermentation at
235 lower temperatures values affected yeast metabolism by slowing their
236 proliferation in the molasses wort. Nevertheless, the real duration of the alcoholic
237 fermentation for L modalities was comparable to that obtained with F modalities, *ie* 48 hours.
238 Ethanol contents measured at the end of the alcoholic fermentation are specified in Fig. S.I.3.
239 For all modalities, the average ethanol contents presented no statistical differences ($p=0.05$)
240 and were comprised between 6.45 % and 6.80 %, for L and F modalities, respectively. Final
241 pH was measured at 4.5 and 4.6 for (F) and (L) conditions, respectively.



242 Fig. 1. Fermentation monitoring of sugar cane molasses wort density (filled symbols), weight (emptied
243 symbols) and temperature (red cross) for the three biological replicates for (A) fresh (F1, F2 and F3) and (B)
244 3-months yeast lees aged (L1, L2 and L3) modalities.

245 3.2. Rum distillates chemical analysis

246 3.2.1. ~~Aromatic~~ Volatile congeners composition

247 Major volatile congeners concentrations were quantified in each rum distillate.
248 Fig. 2A illustrates a heatmap representation of volatile compounds normalized by
249 the maximum concentration found among the twelve samples per volatile
250 compound and grouped by chemical families (alcohols, esters, acetals, carbonyls
251 and acids). The mean concentrations for CS and PS rum distillates, regardless of
252 the presence/absence of lees on fermented sugarcane molasses are presented in
253 Fig. 2B. The mean concentrations for L and F rum distillates, regardless of the
254 distillation process are indicated in Fig. 2C. Raw concentrations values of
255 individual volatile congeners found in rum distillates are indicated in additional
256 information (Fig. S.I.4).

257 ▪ Distillation process differentiation

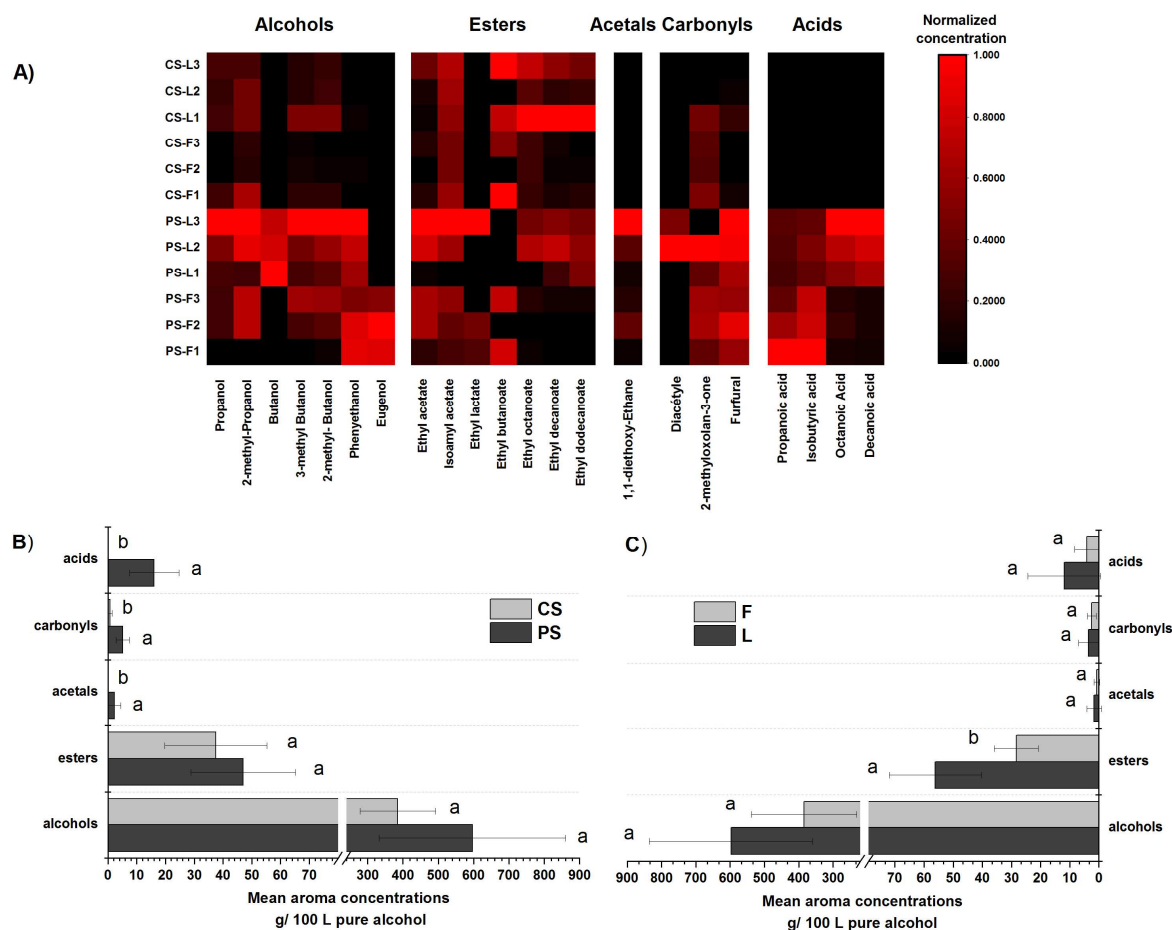
258 First of all, CS and PS rum distillates generated by the two distillation systems
259 presented different normalized concentrations of volatile congeners, particularly
260 for chemical families like acetals, carbonyls and acids and to a lesser extent
261 alcohols (Fig. 2A). Statistical differences were found in PS distillates with higher
262 concentrations in acetals (1,1-diethoxyethane), carbonyls (furfural, diacetyl, 2-
263 methyloxolan-3-one) and acids (propanoic, isobutyric, octanoic, decanoic)
264 compared to CS distillates. No statistical differences were found for alcohols and
265 esters (Fig. 2B). Were also more detected in PS rum distillate, some individual
266 volatile compounds such as 3-methyl-propanol phenylethanol, eugenol, ethyl
267 acetate and ethyl lactate (Fig. S.I.4). Such results have already been pointed out
268 in brandy, cachaça and whisky production (Maarse & Van Den Berg, 1994;
269 Nascimento, Cardoso, & Franco, 2008; Piggott & Paterson, 1994; Simpson,
270 1971). Furfural, already present in sugarcane molasses, is formed by Maillard
271 reaction when using direct heating pot still units (Simpson, 1971). To our
272 knowledge methyloxolan-3-one, a Maillard reaction product already found in rum
273 (Nykänen & Suomalainen, 1983) which has a pleasant coffee note has never been
274 shown to depend on the type of distillation. 1,1-diethoxyethane, conferring a
275 fruity note to the distillate was only present on pot still batches and was not
276 detected in the CS rum distillates, meaning the continuous distillation reduced
277 acetals formation (Piggott & Paterson, 1994). Organic acids were not detected in

278 CS distillates, revealing they were eliminated due to different partitioning of
279 these compounds in the CS column plates, particularly due to the elevated
280 amount of reflux (Maarse & Van Den Berg, 1994). Another plausible reason is
281 these organic acids were more prone to esterification with ethanol leading to
282 higher concentrations of their esterified forms, particularly ethyl hexanoate, ethyl
283 octanoate, ethyl decanoate and ethyl dodecanoate.

284 ▪ **Lees ageing effect after distillation**

285 Interestingly, ester compounds were more present **in rum distillates generated**
286 **from yeast lees aged mashes compared to the fresh mashes, independently of the**
287 **distillation process (Fig. 2A and Fig. 2C). This increase in ester content in rum**
288 **distillate had already been described when lees were directly incorporated into**
289 **the still with a progressive release of their lipophilic content in the wort with the**
290 **temperature increase during the distillation** (Suomalainen, 1981). This abundance
291 in ester compounds was never previously attributed to the lees ageing process on
292 fermented sugarcane molasses. **Only** the 3-months yeast lees aged rum distillates
293 **showed** higher amounts of 1,1-diethoxyethane, diacetyl, octanoic acid and
294 decanoic acid compared to the fresh rum distillates (Fig. S.I.4). Such fatty acids
295 increase after 3-months lees aging has been proposed by Troton et al. (1999) as
296 degradation of membrane compounds from cells (Troton, Charpentier, Robillard,
297 Calvayrac, & Duteurtre, 1989). Nevertheless, propanoic and isobutyric acids
298 were found in higher amounts in fresh fermented rum distillates traducing their
299 preferential accumulation in the distillate after a pot still distillation. ~~With~~ The
300 same tendency **is observed with** compounds like eugenol or furfural, which are
301 more present in fresh fermented rum distillates. As previously mentioned in wine
302 medium, these woody-flavored compounds tend to bind to yeast lees and be less
303 detected in the resulting wines (Chatonnet & Boidron, 1992; Jiménez Moreno &
304 Ancín Azpilicueta, 2007). **This phenomenon could also explain the reduced**
305 **concentration of eugenol and furfural in the rum distillates from 3 months lees**
306 **aged mashes.**

307



308

309 **Fig. 2. (A) Heatmap of aromatic compounds quantified in the rum distillates after Coffey still (CS) and Pot still**
 310 **(PS) distillations from fresh (F) and 3-months yeast lees aged (L) sugarcane molasses fermentations in**
 311 **triplicates. Concentrations are normalized by the maximum concentration per volatile congeners and**
 312 **represented by the color scale from black (0) to red (1). Mean aroma concentrations grouped by chemical**
 313 **families by comparing PS and CS distillation regardless of the type of fermentation (B) and by comparing L**
 314 **and F fermentation regardless of the type of distillation (C). Letters a and b indicate the results of the variance**
 315 **analysis realized performed for each chemical family.**

316

317 3.2.2. Rum distillates EEMF Analysis

318 The chemical composition of rum distillate was assessed by means of 3D
 319 fluorescence spectroscopy in order to strengthen the previous **volatile congeners**
 320 differentiations between the fermentation and distillation modalities. Excitation-
 321 Emission Matrices of Fluorescence of rum distillates elaborated from fresh and
 322 3-months yeast lees aged sugarcane molasses in pot still and coffey still are
 323 shown in **Fig. 3A**. All rum **distillates** present two typical emission areas centered
 324 at 340 nm for 250 and 300 nm of excitation wavelengths. These emissions have
 325 been attributed in other food systems to a great variety of compounds such as
 326 phenolics, furfurals, NADH and Maillard reaction products (Coelho, Aron,

327 Roullier-Gall, Gonsior, Schmitt-Kopplin, & Gougeon, 2015; Elcoroaristizabal,
328 Callejon, Amigo, Ocana-Gonzalez, Morales, & Ubeda, 2016; Ghosh, Verma,
329 Majumder, & Gupta, 2005; Markechova, Majek, & Sadecka, 2014; Matiacevich &
330 Pilar Buera, 2005). The intensity of each emission area was higher in rum
331 distillates from pot still compared to coffee still, regardless of the lees ageing on
332 mashes. For finest discriminations and statistical validation, a PARAFAC model
333 was built based on the analysis of twelve rum distillates samples analyzed in
334 triplicates. The model generated three PARAFAC components (F1, F2 and F3),
335 shown in Fig. S.I.5, enabled to statistically differentiate the effect of distillation
336 process used in the elaboration of rum distillates. Fig. 3B illustrates this
337 differentiation obtained by analyzing each Fmax values of the model. PS rum
338 distillates present higher mean Fmax values of PARAFAC component F1 from
339 12.34 (PS F) to 16.25 (PS L) compared to CS rum distillates (Fmax mean values
340 of 7.07 and 7.59, for CS F and CS L, respectively). CS rum distillates present
341 higher mean Fmax values of PARAFAC component F3 from 9.99 (CS F) to 8.67
342 (CS L) compared to PS rum distillates (Fmax 3 mean values of 3.95 and 5.54 for
343 PS F and PS L, respectively. No statistical differences were found for Fmax
344 values of PARAFAC component F2 for the four rum distillates. This spectral
345 discrimination between batch and continuous distilled liquids by means of
346 PARAFAC components F1 and F3 could be attributed to the influence of volatile
347 compounds mainly present in distillates such as alcohols, esters and acids that
348 affect the chemical environment of intrinsic fluorophores (Sadecka, Urickova,
349 Jakubikova, 2016). Longer wavelength emissive compounds, associated to the
350 statistical PARAFAC component F1, could also be attributed to volatile
351 carbonyls such as furfural, that were analytically measured at higher levels in PS
352 distillates (previously shown in Fig. 2B), coinciding with the observed higher
353 Fmax values of this component. Nevertheless, chemical assignments should be
354 performed carefully due to several overlapping bands originating from different
355 volatile fluorophores present in the total fluorescence spectra of rum distillates.

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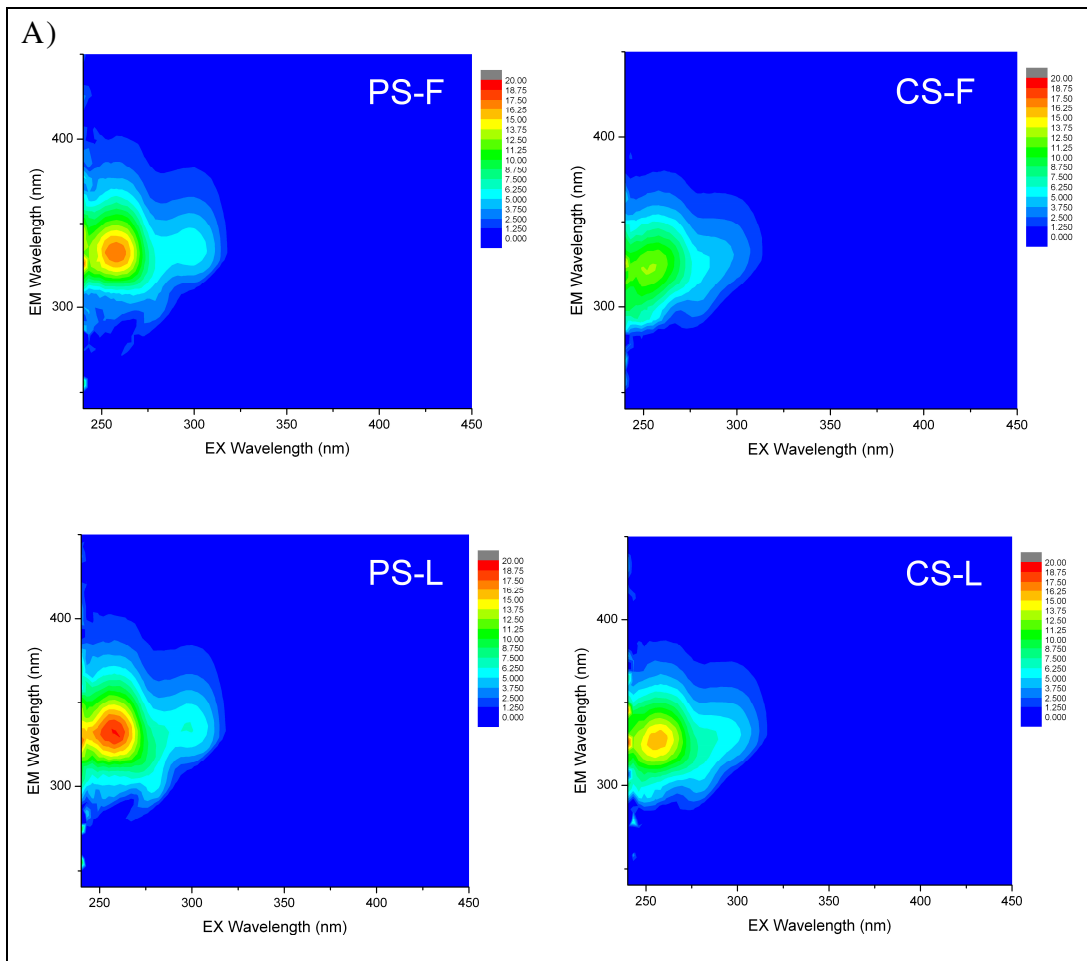
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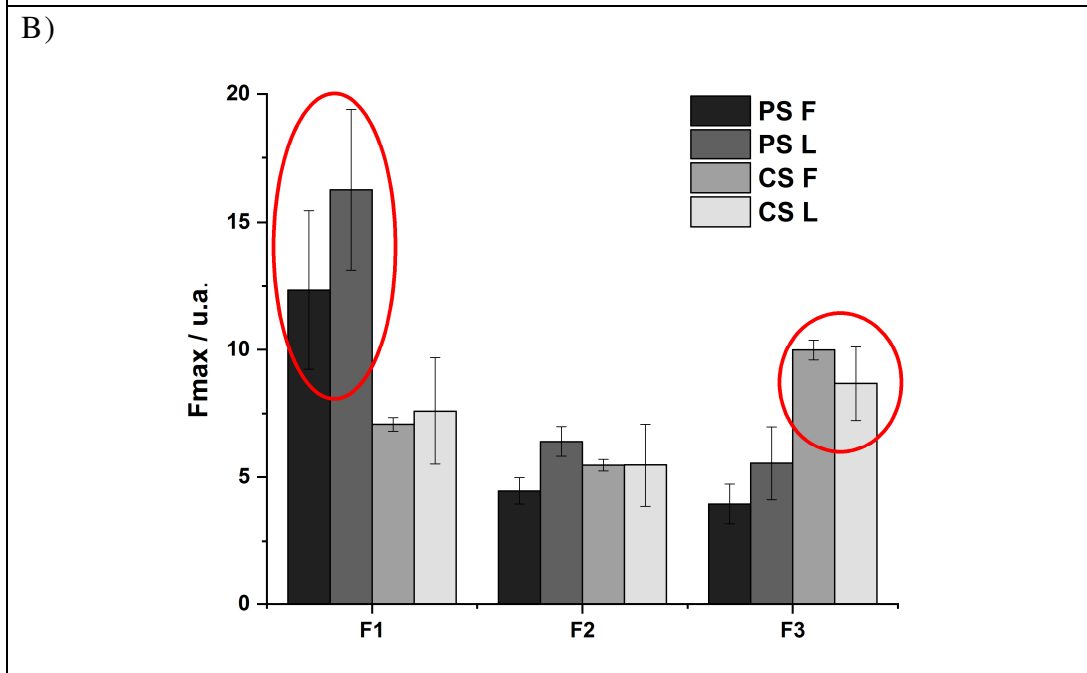
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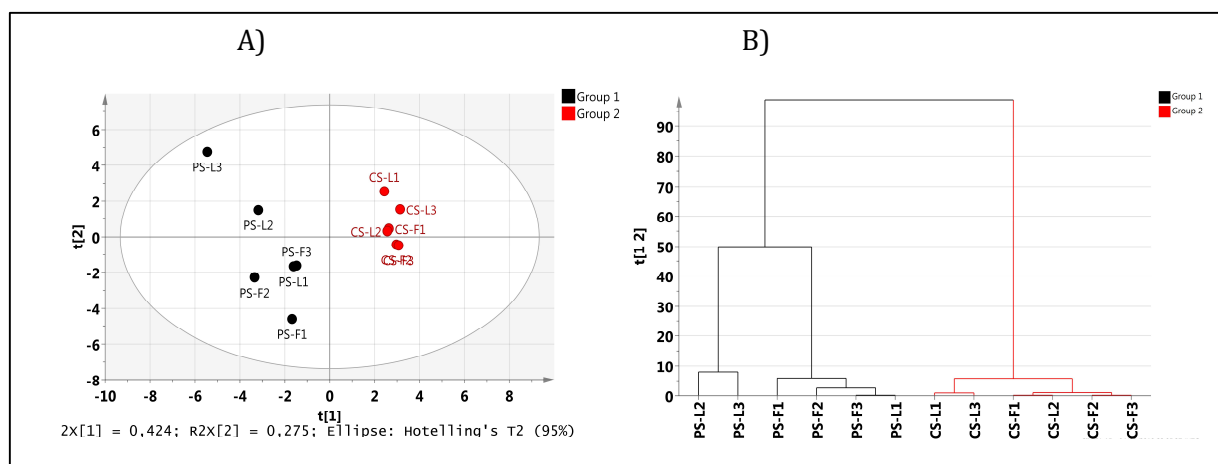
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Fig. 3. Excitation Emission Matrices of Fluorescence of the four rum distillates PS-F, CS-F, PS-L and CS-L (A) and mean F_{max} values of PARAFAC components F1, F2, and F3 of the same four rum distillates analyzed in biological triplicates (B).

385

386 3.3. Impact of the *lees ageing* and distillation practices

387 As rum distillates were differentiated by means of their **volatile congener**
388 composition and their fluorescence fingerprinting, prediction statistic models
389 were built using multivariate approaches by partial least squares discrimination
390 analysis and hierarchical clustering analysis. Results are shown in **Fig. 4** where
391 volatile congeners concentrations and PARAFAC components were used as
392 predicable variables and the distillation type (PS: group 1 or CS: group 2) as
393 dependent variables. **Fig. 4A** illustrates statistically the clear discrimination
394 found between the two types of distillation along the first component $t[1]$
395 regardless of **the treatment of mashes after fermentation**. This PS/CS distinction
396 is essentially driven by **higher Fmax values of PARAFAC component F3** and
397 some long chain fatty esters in C8, C10 and C12 for CS rum distillates and by
398 higher values in Fmax 1, volatile acids, furfural and phenylethanol in PS rum
399 distillates. **Fig. 4B** shows the number of clusters and the level of cluster
400 similarity represented by the Y-axis. It is interesting to notice that CS distillates
401 **presented** closest similarities compared to the PS rum distillates **independently of**
402 **fermented mashes**. In the same way, PS rum distillates **presented** close
403 similarities once they were elaborated from fresh fermented sugarcane molasses
404 whereas the 3-months yeast lees aged one led to a higher discrepancy between
405 the triplicates of rum distillates. **This statistical approach permitted a better**
406 **evaluation of the variability of the distillation process taking into account the**
407 **heterogeneity of fermented sugarcane molasses**. Continuous distillation enabled a
408 **better homogenization of rum distillates whereas batch distillation preserved the**
409 **yeast lees ageing practice on mashes that could be applied or desired by some**
410 **rum producers**.



411

412 **Fig. 4. Statistical discrimination of rum distillates based on their chemical analysis and the way fermentation**
 413 **and distillation was carried measured by a partial least squares discrimination analysis (A) and a hierarchical**
 414 **clustering analysis (B)**
 415

416 4. Conclusion

417 Sugarcane molasses were fermented freshly or yeast lees aged during three
 418 months prior to distillation in order to obtain different styles of rum distillates.
 419 Regardless of the nature of the distillation process, yeast lees ageing led to
 420 higher amounts of ester contents, particularly long chain fatty esters and some of
 421 their precursors like fatty acid in C8 and C10. Once distillation is carried out, pot
 422 still rum distillates differ from coffee still distillates by presenting specific
 423 fluorescence fingerprinting related to their chemical volatile composition. This
 424 study also highlights for the first time that yeast lees ageing practice on
 425 sugarcane molasses coupled to batch distillation could confer a differentiated
 426 rum style whereas continuous distillation tends to minimize its impact.

427

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435 **References**

- 436 Campos, C. R., Silva, C. F., Dias, D. R., Basso, L. C., Amorim, H. V., & Schwan, R. F. (2010). Features
 437 of *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugar
 438 cane beverage, cachaça in Brazil. *Journal of Applied Microbiology*, *108*(6), 1871-1879.
- 439 Chatonnet P., Boidron J.N., & D., D. (1992). Incidence des conditions de fermentations et
 440 d'élevage des vins blancs secs en barriques sur leur composition en substances cédées
 441 par le bois de chêne. *Sc. Alim.* *12* 665-680.
- 442 Coelho, C., Aron, A., Roullier-Gall, C., Gonsior, M., Schmitt-Kopplin, P., & Gougeon, R. D. (2015).
 443 Fluorescence Fingerprinting of Bottled White Wines Can Reveal Memories Related to
 444 Sulfur Dioxide Treatments of the Must. *Analytical Chemistry*, *87*(16), 8132-8137.
- 445 Duarte, W. F., de Sousa, M. V. F., Dias, D. R., & Schwan, R. F. (2011). Effect of Co-Inoculation of
 446 *Saccharomyces cerevisiae* and *Lactobacillus fermentum* on the Quality of the Distilled
 447 Sugar Cane Beverage Cachaça. *Journal of Food Science*, *76*(9), C1307-C1318.
- 448 Elcoroaristizabal, S., Callejon, R. M., Amigo, J. M., Ocana-Gonzalez, J. A., Morales, M. L., & Ubeda, C.
 449 (2016). Fluorescence excitation-emission matrix spectroscopy as a tool for determining
 450 quality of sparkling wines. *Food Chem.*, *206*, 284-290.
- 451 Fahasmane, & Ganou-Parfait. (1998). Microbial flora of rum fermentation media. *Journal of*
 452 *Applied Microbiology*, *84*(6), 921-928.
- 453 Fahasmane, L., & Ganou-Parfait, B. (1997). *De la canne au rhum*. INRA Editions.
- 454 Fahasmane, L., & Ganou-Parfait, B. (2011). Trente ans de travaux en technologie rhumière à
 455 l'Inra-Antilles-Guyane : Trente ans de recherche en technologie des rhums. *Innovations*
 456 *Agronomiques*, *16*, 153-164.
- 457 Fahasmane, L., & Parfait, A. (2003). RUM. In B. Caballero (Ed.), *Encyclopedia of Food Sciences*
 458 *and Nutrition (Second Edition)*, (pp. 5021-5027). Oxford: Academic Press.
- 459 Ghosh, N., Verma, Y., Majumder, S. K., & Gupta, P. K. (2005). A fluorescence spectroscopic study of
 460 honey and cane sugar syrup. *Food Sci. Technol. Res.*, *11*(1), 59-62.
- 461 Jiménez Moreno, N., & Ancín Azpilicueta, C. (2007). Binding of oak volatile compounds by wine
 462 lees during simulation of wine ageing. *LWT - Food Science and Technology*, *40*(4), 619-
 463 624.
- 464 Legras, J. L., & Karst, F. (2003). Optimisation of interdelta analysis for *Saccharomyces cerevisiae*
 465 strain characterisation. *FEMS Microbiology Letters*, *221*(2), 249-255.
- 466 Liebich, H. M., Koenig, W. A., & Bayer, E. (1970). Analysis of the Flavor of Rum by Gas-Liquid
 467 Chromatography and Mass Spectrometry. *Journal of Chromatographic Science*, *8*(9), 527-
 468 533.
- 469 Maarse, H., & Van Den Berg, F. (1994). Flavour of distilled beverages. In J. R. Piggott & A.
 470 Paterson (Eds.), *Understanding Natural Flavors*, (pp. 243-267). Boston, MA: Springer US.
- 471 Markechova, D., Majek, P., & Sadecka, J. (2014). Fluorescence spectroscopy and multivariate
 472 methods for the determination of brandy adulteration with mixed wine spirit. *Food*
 473 *Chem.*, *159*, 193-199.
- 474 Matiacevich, S. B., & Pilar Buera, M. (2005). A critical evaluation of fluorescence as a potential
 475 marker for the Maillard reaction. *Food Chem.*, *95*(3), 423-430.
- 476 Medeiros, A. B. P., de Matos, M. E., de Pinho Monteiro, A., de Carvalho, J. C., & Soccol, C. R. (2017).
 477 16 - Cachaça and Rum. In A. Pandey, M. Á. Sanromán, G. Du, C. R. Soccol & C.-G. Dussap
 478 (Eds.), *Current Developments in Biotechnology and Bioengineering*, (pp. 451-468):
 479 Elsevier.
- 480 Murtagh, J. E. (2003). *Feedstocks, fermentation and distillation for production of heavy and light*
 481 *rums, in: The Alcohol Textbook: A Reference for the Beverages, Fuel and Industrial Alcohol*
 482 *Industries*, .
- 483 Nascimento, E. S. P., Cardoso, D. R., & Franco, D. W. (2008). Quantitative Ester Analysis in
 484 Cachaça and Distilled Spirits by Gas Chromatography–Mass Spectrometry (GC–MS).
 485 *Journal of Agricultural and Food Chemistry*, *56*(14), 5488-5493.

- 486 Nykänen, L., & Suomalainen, H. (1983). *Aroma of beer, wine and Distilled alcoholic beverages:*
487 Springer Netherlands.
- 488 Piggott, J. R., & Paterson, A. (1994). *Understanding Natural Flavors.*
- 489 Piggott, R. (2003). From pot stills to continuous stills: flavor modification by distillation. In T. P. L.
490 K.A. Jacques, D.R. Kelsall (Ed.), *The alcohol textbook 4th Edition.*
- 491 Recueil des méthodes internationales d'analyse des boissons spiritueuses des alcools et de la
492 fraction aromatique des boissons. OIV 1994, 113-114.
- 493 Sadecka, J., Urickova, V., Jakubikova, M (2016). Fluorescence spectroscopy for the analysis of
494 spirit drinks. *Applications of Molecular Spectroscopy to current research in the chemical*
495 *and biological sciences.*
- 496 Simpson, A. C. (1971). Manufacture of brandy. *Processes in Biochemistry*, 6(2), 25.
- 497 Stewart, G. G., Hill, A. E., & Russell, I. (2013). 125th Anniversary Review: Developments in
498 brewing and distilling yeast strains. *Journal of the Institute of Brewing*, 119(4), 202-220.
- 499 Suomalainen, H. (1981). Yeast esterases and aroma esters in alcoholic beverages. *Journal of the*
500 *Institute of Brewing*, 87(5), 296-300.
- 501 Troton, D., Charpentier, M., Robillard, B., Calvayrac, R., & Duteurtre, B. (1989). Evolution of the
502 Lipid Contents of Champagne Wine During the Second Fermentation of *Saccharomyces*
503 *cerevisiae*. *American Journal of Enology and Viticulture*, 40(3), 175.
- 504 Walker, G., & Stewart, G. (2016). *Saccharomyces cerevisiae* in the Production of Fermented
505 Beverages. *Beverages*, 2(4), 30.
- 506