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1 **Title page**

2 **Transfer kinetics of labeled aroma**
3 **compounds from liquid media into coffee**
4 **beans during simulated wet processing**
5 **conditions**

6

7 **F. Hadj Salem^{1,2,3}, M. Lebrun^{1,2}, C. Mestres^{1,2}, N.**
8 **Sieczkowski³, R. Boulanger^{1,2}, A. Collignan^{1,2},**

9 ¹CIRAD, UMR Qualisud, F-34398 Montpellier, France.

10 ²Qualisud, Univ Montpellier, CIRAD, Montpellier SupAgro,
11 Univ d'Avignon, Univ de La Réunion, Montpellier, France.

12 ³ Lallemand, SAS, 19 rue des Briquetiers, 31700 Blagnac,
13 France.

14

15 **Corresponding author**

16 Corresponding author: Fatma HADJ SALEM

17 Mailing address: CIRAD- international cooperation in
18 agricultural research for development

19 B-16, 73 Rue Jean François Breton, 34398 Montpellier, France

20 Tel.: +33 6 13 26 20 66

21 E-mail : fatma.hadj_salem@cirad.fr

22 **Abstract**

23 The transfer kinetics of three labelled compounds (butanal, 2-
24 phenylethanol, isoamyl acetate) was studied from a liquid
25 medium into the coffee beans during simulated wet
26 processing using four media (M) (M1: contained dehulled
27 beans, M2: contained demucilaginated beans, M3: contained
28 depulped beans, M4: contained depulped beans with
29 yeast).Trials were carried out at 25°C, under agitation and for
30 five time periods (0, 6, 12, 24 and 48 hours), and then the
31 labelled volatiles were analyzed by SPME-GC-MS.

32 The three labelled molecules were transferred into the coffee
33 beans with different mass transfer rates; reaching at 12hrs in
34 the M4, 0.2 ± 0.03 , 11.2 ± 0.66 and $1.3\pm 0.04\mu\text{g/g}$ of coffee
35 respectively for butanal, 2-phenylethanol and isoamyl acetate.
36 The parchment resistance significantly affected the mass
37 transfer of the 2-phenylethanol. Butanal and isoamyl acetate
38 underwent metabolic reactions, which decreased their
39 amount in the coffee beans. Furthermore, an interaction
40 between molecules and the yeast was observed and
41 decreased significantly the butanal's transfer.

42 **Keywords:** Coffee fermentation; aroma compounds; transfer;
43 kinetics; parchment resistance.

44

45 **1. Introduction**

46 Coffee consumption has recorded a significant growth rate
47 over the past 50 years, which has resulted in coffee being
48 ranked as the second most traded global commodity in
49 financial terms (Hameed et al., 2018; Lee et al., 2015; Ramírez-
50 Martínez et al., 2013). Coffee products owe this popularity to
51 their unique sensory and pleasant flavor (Pereira et al., 2019).
52 Thereby, the coffee industry has dedicated its efforts in
53 improving the final beverage quality using roasting and
54 brewing steps (de Carvalho Neto et al., 2018; Lee et al., 2015).
55 Moreover, several studies have highlighted that postharvest
56 processing could have a direct impact on the quality and value
57 of the final product (Joët et al., 2010; Pereira et al., 2017;
58 Selmar et al., 2006).

59 Actually, the postharvest process is a crucial step in producing
60 coffee beans suitable for transport and roasting, and it aims to
61 remove the components surrounding the beans (skin, pulp,
62 mucilage and parchment). There are three main commonly
63 used ways to eliminate these layers: dry processing, wet
64 processing and semi-dry processing (Hameed et al., 2018;
65 Pereira et al., 2017; Silva, 2014).

66 In the dry processing, the whole coffee cherries are sun/air
67 dried until their moisture content reaches 10% to 12%. After

68 drying, the skin and the pulp are removed and, then, the beans
69 are dehulled to obtain the green beans ready for roasting
70 (Pereira et al., 2019). Dry processing offers a coffee with a
71 heavy body and smooth, sweet, and complex cupping quality
72 attributes (Duarte et al., 2010; Hameed et al., 2018; Poltronieri
73 & Rossi, 2016). The wet processing, in contrast, involves more
74 complex steps. First, the skin and pulp of the cherries are
75 removed mechanically, then the depulped beans are
76 fermented in order to degrade the mucilage layer by
77 microbiological activity and finally, the beans are sundried
78 (Pereira et al., 2019). Wet processing provides a lower-bodied
79 coffee, with higher acidity and more aroma than the other
80 processes (Joët et al., 2010; Pereira et al., 2017;). Finally, the
81 semi-dry processing is a transitional system of wet and dry
82 processing, the skin and pulp of cherries are mechanically
83 removed, then, the depulped beans are sun/ air dried until
84 their moisture content reaches 10 to 12% (Ribeiro et al.,
85 2017). Semy-dry processing produces different cup quality,
86 somewhat similar to that of wet-processed coffee with less
87 acidity and somewhat less body than dry-processed coffee.

88 In previous studies (Selmar et al., 2006), it has been shown
89 that the chosen conditions during the post-harvest treatment
90 (wet or dry or semi-dry) could affect the metabolic activities
91 within the beans thus affecting their chemical composition in

92 different ways. While the dry-processed beans contain more
93 simple sugars, the wet-processed beans present a superior
94 concentration of free amino acids. These compounds (simple
95 sugars and amino acids) are important aroma precursors in the
96 formation of aroma compounds during roasting (Bytof et al.,
97 2005; Knopp et al., 2006). Furthermore, fermenting yeast
98 cultures produce many flavor compounds, such as esters,
99 alcohols, aldehydes, ketones, and terpenoids during the
100 mucilage removal process. Accordingly, it has been proposed
101 that these microbial metabolites can diffuse into the coffee
102 beans leading to an intense perception of “floral” and “fruity”
103 aromas and high acidity in the brewed coffee (Pereira et al.,
104 2015; Lee et al., 2017; Mussatto et al., 2011). Based on this
105 observation, many studies were conducted to modulate the
106 coffee aroma quality using yeast starter cultures (Bressani et
107 al., 2018; Pereira et al., 2015; Evangelista et al., 2014; Ribeiro
108 et al., 2017). Moreover, Lee et al., 2017 and Martinez et al.,
109 2019 have shown that fermentation affects green coffee
110 beans’ composition in volatile compounds such as 2-
111 phenylethanol and volatile phenols produced by yeasts, which
112 were preserved after light roasting. However, the direct
113 relationship between the microbial activities during the
114 fermentation and coffee quality is not yet known, but it is
115 assumed that a diffusion process of those metabolites into

116 coffee beans during the fermentation step could occur
117 (Pereira et al., 2019).

118 Therefore, this work was undertaken to assess the existence
119 of a transfer of volatile compounds likely to be produced by
120 the yeast into the coffee beans. For this purpose, three
121 deuterium labelled stable isotope aroma compounds have
122 been selected to track their transfer from the external
123 environment to the coffee beans. Indeed, those compounds
124 do not exist in natural conditions. This type of labelling induces
125 a slight increase in the molecular weight of the labelled
126 compounds without modifying its structural or chemical
127 properties; this mass modification will allow us to detect and
128 identify the labelled molecule in the study medium after
129 analysis by mass spectrometry coupled with a separation
130 method (GC, HPLC) (Zachleder et al., 2018).

131 Furthermore, trials were conducted to study the transfer
132 kinetics of these three labelled molecules and to investigate
133 certain transfer parameters such as grain permeability,
134 parchment resistance and the effect of the presence of yeast
135 on the transfer of these volatile compounds.

136 **2. Materials and methods**

137 **2.1. Plant material, pre-treatment, and sampling of coffee**

138 **cherries**

139 10 kg of fresh coffee cherries (variety *Coffea Arabica*) were
140 purchased from the Tarrazu cooperative (Tarrazu, Costa Rica).

141 Harvested in March 2018, they were frozen at -18 °C to curb
142 any microbiological and enzymatic activity. The frozen samples
143 were transported to France and stored in a cold room at -18
144 °C.

145 To work on homogeneous samples in terms of maturity, the
146 coffee cherries were sorted according to their size and color.

147 Only mature red cherries with 12–14 mm in diameter were
148 selected for the study.

149 Before the experimentation, samples were disinfected by
150 introducing frozen cherries into a chlorinated solution with
151 200 ppm of active chlorine for 10 minutes. Then, they were
152 washed with sterile water and manually depulped under
153 sterile conditions (using a Bunsen burner and sterilized
154 equipment). The removed pulp corresponded to
155 approximately 40 to 45% of the total cherry weight. Then, 10 g
156 of depulped coffee beans were used for each trial.

157 **2.2. Labeled molecules.**

158 The labeled molecules were purchased from CDN-isotopes
159 (Quebec, Canada). These volatile molecules include an

160 aldehyde, butanal ($\text{CH}_3\text{CH}_2\text{CD}_2\text{CHO}$), an alcohol, 2-
161 phenylethanol ($\text{C}_6\text{D}_5\text{CH}_2\text{CH}_2\text{OH}$) and an ester, isoamyl acetate
162 ($(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{OCOCOD}_3$). In order to quantify the labelled
163 volatile molecules, calibration curves were plotted for each of
164 the compounds. First, the stock solution was prepared using a
165 mix of the three volatile compounds dissolved in 2% of
166 ethanol. Afterwards, three dilutions were carried out from this
167 stock solution ($\frac{3}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$).
168 Then, 100 μl of each dilution were introduced into samples of
169 1.5 g of ground coffee beans and weighed in glass vials
170 (repeated 4 times). In addition, to ensure reproducibility,
171 samples were spiked with 100 μL of a solution of benzyl
172 butyrate used as an internal standard (with a concentration of
173 5 $\mu\text{L/L}$), and then, they were analyzed by SPME-GC-MS.

174 **2.3. First experimental approach**

175 To characterize the transfer kinetics of labelled compounds, a
176 stepwise experimental approach was adopted using four
177 different study media with increasing complexity from M1 to
178 M4, a representative schematic of these 4 media is presented
179 in Fig.1.

180 In the first step, the transfer was studied in M1 medium,
181 composed of manually depulped and dehulled coffee beans,
182 and this medium was used to estimate the permeability of the

183 coffee beans to the diffusion of volatile molecules. Afterwards
184 in M2 medium, depulped and demucilaginated coffee beans
185 were used in order to estimate the resistance of the
186 parchment to the transfer of the compounds.

187 Finally, the transfer was studied in M3 and M4 media. The M3
188 medium contained depulped coffee beans and was used as a
189 control medium to evaluate the effect of the presence of yeast
190 that can be observed in the M4 medium. In the latter, the
191 transfer was studied in realistic simulated conditions of wet
192 controlled fermentation, using depulped coffee beans
193 inoculated with the selected yeast LSCC1.

194 The yeast strain used in the M4 media and for the additional
195 tests, *Saccharomyces cerevisiae* LSCC1, was provided by
196 Lallemand SAS (Toulouse, France) in active dry form and
197 belongs to Lallemand's collection of selected coffee yeasts.
198 Before the inoculation, yeast, with a viability of 2.01×10^{10}
199 cfu/g, were rehydrated in distilled water (1/10, p/v) for 30
200 minutes. For the fermentation trials, for 1g of pulped coffee,
201 0.01 mL of rehydrated yeast and 0.99 ml of distilled water
202 were added to submerge the depulped coffee bean.

203 In all trials, coffee beans were submerged in distilled water (10
204 mL/10 g coffee beans) containing a mix of labelled volatile
205 molecules. All compounds were added at the same
206 concentration (40 µg/mL of distilled water). This concentration

207 was determined knowing the natural concentration value of
208 these compounds in fresh coffee beans in order to ensure a
209 concentration gradient in the medium in favor of compound
210 entry.

211 All samples were maintained at 25 °C. Agitation was carried
212 out with an orbital shaker (SSL1-Stuart, UK) at a low speed of
213 120 rpm during the entire transfer period. Five time periods
214 (0, 6, 12, 24 and 48 hours) were chosen to investigate the
215 mass transfer kinetics. All trials were independent and
216 performed in triplicate.

217 At the end of the transfer experiment, samples were washed
218 three times with water to stop the transfer reaction and to
219 remove labeled molecules adhering to the grain surface, and
220 then they were ground and frozen at -80 °C until being
221 analyzed.

222 **2.4. Second experimental approach: additional tests**

223 To properly interpret our results, additional tests were
224 conducted to investigate the interaction between the labelled
225 volatile molecules and the yeast strain LSCC1 used for this
226 study.

227 As for the first experimental approach, 2 mL of a solution
228 containing rehydrated yeast at a concentration of 1 mg/mL
229 and a mixture of the 3 labelled molecules at a concentration of
230 40 mg/mL was prepared, the samples were vortexed for 10

231 seconds to homogenize the mix and then they were analyzed
232 immediately.

233 **2.5. Volatile compounds analyses**

234 • **Extraction by SPME**

235 Coffee bean samples were ground to a fine particle size ≈ 500
236 μm (Retsch-ZM200, France). Volatile compounds of coffee
237 beans were extracted using the SPME (DVB/Carboxen/PDMS,
238 $50/30 \mu\text{m}$, Supelco, USA, solid-phase-microextraction). Ground
239 coffee ($1.50 \pm 0.05 \text{ g}$) was weighed in screw-capped glass vials
240 and mixed with 2 mL of pure water. Subsequently, the vials
241 were placed for 15 min in a thermostatically regulated oven at
242 $50 \text{ }^\circ\text{C}$, to reach sample headspace equilibrium. Then they were
243 subjected to SPME at $50 \text{ }^\circ\text{C}$ for 45 min.

244 • **GC-MS Analysis**

245 The labelled volatile compounds were analyzed in an Agilent
246 6890 gas chromatograph coupled to a mass spectrometer
247 Agilent 5973 (Agilent Technologies, Palo Alto, USA). The SPME
248 fiber was desorbed in a polar column DB-WAX (60 m x 0.25
249 mm, $0.25 \mu\text{m}$ phase film thickness, Agilent J&W GC column,
250 USA) under splitless mode with the injector temperature set at
251 $250 \text{ }^\circ\text{C}$. Hydrogen was used as the gas vector with the flow rate
252 fixed at 1.5 mL/min . The initial oven temperature was $40 \text{ }^\circ\text{C}$ (5

253 min), and then it was increased by 2 °C/min to 170 °C and then
254 by 10 °C/min to 250 °C. The final column temperature was
255 maintained for 10 min.

256 The mass spectrometer was operated at 70 eV in electron
257 impact (EI) ionization mode. The source of ionization heated at
258 230 °C. After the ionization, the molecules were subsequently
259 sorted according to their report mass/charge (m/z), by a
260 quadrupole analyzer maintained at 150 °C sweeping an
261 interval [40 to 350] m/z in SCAN mode. The results set were
262 processed using MassHunter Qualitative Analyses software
263 (version B.08.00).

264 **2.6. Statistical analyses**

265 Means and standard deviations reported were calculated from
266 triplicate trials and they were subjected to ANOVA followed by
267 post-hoc comparison of means by Tukey's test, performed
268 using the Xlstat version 2018.5. Differences were considered
269 significant when the probability value $p \leq 0.05$.

270 **2.7. Transfer rate calculation**

271 The transfer rate R ($\mu\text{g/g/h}$) was calculated using the following
272 formula:

$$273 \quad R = \frac{\Delta C_i}{\Delta t}$$

274 C_i : The transferred masses of labelled molecules in the coffee
275 beans [$\mu\text{g/g}$]

276 t : transfer time (h)

277 i : studied compound

278 **3. Results and discussion**

279 **3.1. Transfer kinetics study**

280 Results of the mass transfer kinetics of the labelled volatiles
281 are shown in fig.2. All trials showed a mass transfer of the
282 labelled volatile compounds into the coffee beans (fig.2).
283 These results confirm previous work by de Carvalho Neto et
284 al., (2017); Pereira et al., (2015); Evangelista et al., (2014); Silva
285 et al., (2013), who postulated that a transfer process could
286 exist of volatile metabolites, that are produced by the yeast
287 during the fermentation, into the coffee beans.

288 The transfer kinetic of the alcohol (2-phenylethanol), studied
289 during the coffee fermentation using the M4 medium (see
290 section 2), showed a significant increase into the coffee beans
291 during the 48 hrs (fig.2) and presented three transfer regimes.
292 During the first hours, the recorded amount showed an
293 exponential increase reaching, at 6 hrs, about 50% of the total
294 transferred amount of 2-phenylethanol. This exponential
295 phase was followed by a deceleration phase noticed by the
296 decrease of the transfer speed, which became constant
297 between 12 hrs and 48 hrs of transfer and represented the
298 “constant rate state” (table 1).

299 In the meantime, the transfer profile of the aldehyde (butanal)
300 and the ester (isoamyl acetate) represented a regime going
301 through a maximum reached at 6 hrs of transfer (fig.2).
302 Moreover, the total content of butanal in the coffee beans
303 showed a significant decrease that was faster than that
304 observed for the isoamyle acetate profile (table 1).Essentially ,
305 a high concentration gradient was maintained to avoid the loss
306 of molecules by the reverse transfer; thereby, this decrease
307 can be explained by a degradation mechanism of these
308 compounds inside the bean. Lee et al., (2016) also showed,
309 during the green coffee fermentation, that some volatiles
310 were degraded and researchers correlated this fact to the
311 metabolism of aroma precursors in green coffee beans by
312 *Rhizopus oligosporus*. Moreover, in their study, Selmar et al.,
313 2006 measured the expression of the germination-specific
314 enzyme isocitrate lyase and they identified that during the first
315 two days of the coffee wet-process, the germination-related
316 metabolism recorded the highest activity throughout the post-
317 harvesting process. Therefore, it can be assumed that after
318 their transfer in the coffee beans, butanal and isoamyle
319 acetate undergo an enzymatic reaction that occurs during the
320 germination. However, 2-phenylethanol seems not to be
321 affected by this degradation process as it's transferred amount

322 into the coffee beans continued to increase by accumulation
323 to reach $15.84 \pm 0.62 \mu\text{g/g}$ after 48 hrs.

324 Indeed, the transfer of volatile compounds is governed by
325 thermodynamic and kinematic parameters related to the
326 composition, the physical state of the matrix and their
327 interaction with the physicochemical properties of the
328 molecules (Voilley et al., 2011). In our case, the molecules
329 were transferred from liquid medium to organic matrix (the
330 coffee bean) by imposing a transfer gradient. According to the
331 physicochemical properties presented in table 2, with a lower
332 molecular weight and a lower LogP, 2-phenylethanol would
333 have an easier transport than the isoamyle acetate. While
334 butanal has the lowest molecular weight and a $\text{LogP} < 1$
335 between the three molecules used, and contrary to what was
336 expected, the 2-phenylethanol represented the highest
337 transfer rate with a transfer speed 16 times faster than that of
338 butanal (during the first 6 hours) (table 1). In their study, Lee
339 et al., (2016) reported an increase of 43% of 2-phenylethanol
340 in the fermented coffee beans compared to the unfermented
341 green coffee beans. Thus, it speculates that the alcohol
342 undergoes an intense diffusion process during the
343 fermentation, which is required to influence the overall coffee
344 quality (Pereira et al., 2019) and this intense transfer cannot
345 be explained exclusively by the physicochemical properties of

346 the compound but maybe by the existence of other pathways
347 such as active transport observed in plants.

348 Previous studies (De Castro & Marraccini, 2006) have reported
349 the existence of metabolite exchanges between the pericarp
350 (pulp) and the endosperm (seed) either by passive transfer or
351 by active transport (i.e. sugars, caffeine). Accordingly,
352 molecules can be transferred into the coffee bean by these
353 two transport pathways, and the assumption of the existence
354 of a specific active transport of the higher alcohol (2-
355 phenylethanol) can explain the reported high rate transfer of
356 this compound.

357 **3.2. Mass transfer resistance study**

358 Labelled compound contents (Butanal, Isoamyl acetate and 2-
359 Phenylethanol) at 12 hrs of transfer, studied in four media
360 (M1, M2, M3 and M4) are visualized in Fig.3.

361 The transferred amount of 2-phenylethanol reached 18.8 ± 1.75
362 $\mu\text{g/g}$ in the green coffee beans (M1), while this amount was
363 significantly lower in the green coffee beans with parchment
364 (M2) (Fig.3.c.). Accordingly, and as assumed by Lee et al.,
365 (2015) and Ramírez-Martínez et al., (2013), the parchment
366 induces a potential mass transfer resistance. However, the
367 quantified amounts in the three media M2, M3 and M4 were

368 quite close, 11.5 ± 2.5 , 12.7 ± 1.4 and 11.2 ± 0.66 $\mu\text{g/g}$,
369 respectively with no significant difference, thereby, pointing
370 out that the presence of mucilage and/or yeast and
371 fermentation mechanisms do not have a significant effect on
372 the transfer rate of 2-phenylethanol.

373 Furthermore, the isoamyl acetate content, in all conducted
374 trials (in the M1, M2, M3 and the M4 media), ranged between
375 0.8 ± 0.42 and 1.3 ± 0.04 $\mu\text{g/g}$ (fig.3.b). Based on statistical tests,
376 this variation showed no significant difference (fig.3.b). Thus, it
377 can be concluded that the parchment and the mucilage have
378 no influence on the transfer rate of this molecule. The
379 physicochemical changes occurring during the fermentation
380 do not affect the mass transfer of this ester either.

381 With regards to butanal, no significant differences in the
382 concentration of this aldehyde were observed in the media
383 M1, M2 and M3 (fig.3.a), which was around 0.6 $\mu\text{g/g}$. Again,
384 the parchment did not show any resistance to the transfer of
385 this molecule. However, the butanal content quantified in the
386 M4 medium (with yeast) was significantly lower, 0.2 ± 0.03 $\mu\text{g/g}$
387 (fig.3.a). In contrast to the alcohol and the ester, the presence
388 of *Saccharomyces cerevisiae* LSCC1 leads to a decrease of the
389 butanal transferred mass. In their study, Chalier et al., (2007),
390 showed that an interaction might exist between aroma

391 compounds and mannoprotein isolated from *Saccharomyces*
392 *cerevisiae* strains, which reduces their volatility; the nature of
393 the observed interaction has not been characterized for the
394 targeted molecules in this study, but they explain in their
395 study that this interaction is linked to the physico-chemical
396 properties of the compound on the one hand and those of the
397 mannoproteins constituting the walls of the yeast cells on the
398 other hand. Hence, it can be assumed that the yeast, used in
399 the M4 medium, affected the volatility of butanal. In this
400 context, trials were conducted to verify the yeast LSCC1
401 retention effect on the three molecules studied (section 2.4.).

402 Measurements of interaction between yeasts and the three
403 aroma compounds are presented in fig.4. As expected, the
404 volatility of butanal was significantly affected by yeast. A
405 decrease of up to 90% of this aldehyde content was observed
406 when compared to the medium without yeast (NY) (fig.4.a).
407 The explanation could be an adsorption of the molecule on the
408 yeast cell walls. This interaction between yeast and butanal
409 could induce a slower transfer of this compound into coffee
410 beans during fermentation (fig.3.a).

411 In contrast, isoamyl acetate did not significantly interact with
412 yeast (fig.4.b). Chalier et al., (2007) reported that the strength
413 of interactions between aroma compounds and yeast cell walls

414 depends on the physicochemical nature of the volatile
415 compound.

416 In addition, the 2-phenylethanol volatility was significantly
417 affected by yeast, and, in contrast to butanal, the amount
418 increased by 30% compared to the medium without yeast. In
419 their study, Comuzzo et al., (2011) observed that 2-
420 phenylethanol did not interact with *Saccharomyces cerevisiae*
421 cell walls. However, the yeast strain used in our study, was
422 known to produce different aroma compounds, especially
423 higher alcohols. Thereby, the 2-phenylethanol concentration
424 probably increased in the medium increasing the volatile
425 fraction in the headspace.

426 Thus, volatile compound transfer, governed by a concentration
427 gradient, undergoes several deviations induced by complex
428 physicochemical interactions between the molecule and the
429 different transfer media. This affects their transfer from
430 mucilage to the coffee beans during the fermentation.

431 The parchment, which is of a fibrous structure that is very
432 close to wood (Ramírez-Martínez et al., 2013), shows a
433 physical resistance and consequently, it acts as a molecular
434 filter. Only 2-phenylethanol was held back by this barrier
435 related to its conformation (table 2), whereas, the labelled
436 aldehyde and ester seemed not to be retained by the

437 parchment. This fibrous layer is a natural coffee seed
438 protection against digesting enzymes from the gut of
439 frugivorous animals, and keep the coffee seed available to
440 germination-related metabolism after harvesting, which is
441 initiated by the pulp removal (De Castro & Marraccini, 2006;
442 Selmar et al., 2006). Hence, during the coffee wet treatment,
443 the biochemical composition of the coffee beans undergoes
444 several metabolic reactions (Patui et al., 2014; Selmar et al.,
445 2006) which occur simultaneously to molecule transfers. These
446 reactions can induce their disappearance but, at the same
447 time, the appearance of other products. In our study, it seems
448 that only 2-phenylethanol was not affected by these reactions.
449 Thus, from a broad perspective, it would be interesting to
450 study the mechanism of these reactions and transfer into the
451 coffee beans and evaluate their impact on the aromatic profile
452 of the final product. Moreover, it is well known (De Castro &
453 Marraccini, 2006) that the existence of active transport may
454 accelerate the transfer rate of specific molecules into coffee
455 beans during the wet processing. In our case, this could
456 explain the high transfer rate of 2-phenylethanol reported
457 throughout the kinetic study.

458 Even if the higher alcohols are known for their higher sensory
459 threshold (Dzialo et al., 2017), 2-phenylethanol seems to be an
460 interesting aroma compound by its high transfer rate into the

461 coffee beans during fermentation. Thereby, in their review,
462 Pereira et al., (2019) postulate that using yeast strains with
463 important higher alcohol production can enhance the sensory
464 quality of the coffee beverage, by providing floral notes
465 (Pereira et al., 2019).

466 However, some volatile compounds can interact with yeast,
467 inducing thereby a significant decrease of their transfer rate.
468 During our study, it seems that butanal was highly retained by
469 the yeasts *Saccharomyces cerevisiae* LSCC1, while, the 2-
470 phenylethanol and the isoamyl acetate were not affected.

471 **4. Conclusion**

472 The volatile transfer into the green coffee beans during
473 fermentation was highlighted and the kinetic study of three
474 labelled volatiles was established. Throughout their transfer,
475 the aroma compounds were influenced by several factors,
476 such as the parchment resistance, the interaction with the
477 yeast cell walls and the degradation reactions into the coffee
478 beans. The 2-phenylethanol seemed to be an interesting
479 aroma compound as it had the highest transfer rate compared
480 to butanal and isoamyl acetate in spite of its retention by the
481 parchment.

482 Thus, it was possible to conclude that all these volatile
483 compounds that can be produced by yeast during

484 fermentation could be transferred into the coffee beans with
485 different rate and concentration. This fact can enable the
486 coffee industry to modulate the coffee aromatic profile by
487 controlling the fermentation step. In the future, it will be
488 necessary to follow the fate of these transferred molecules
489 throughout the processing steps (drying, roasting, and
490 brewing) in order to evaluate the impact of the transfer of
491 volatile compounds on the quality of the final product using a
492 sensorial study.

493

494

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Experimental media

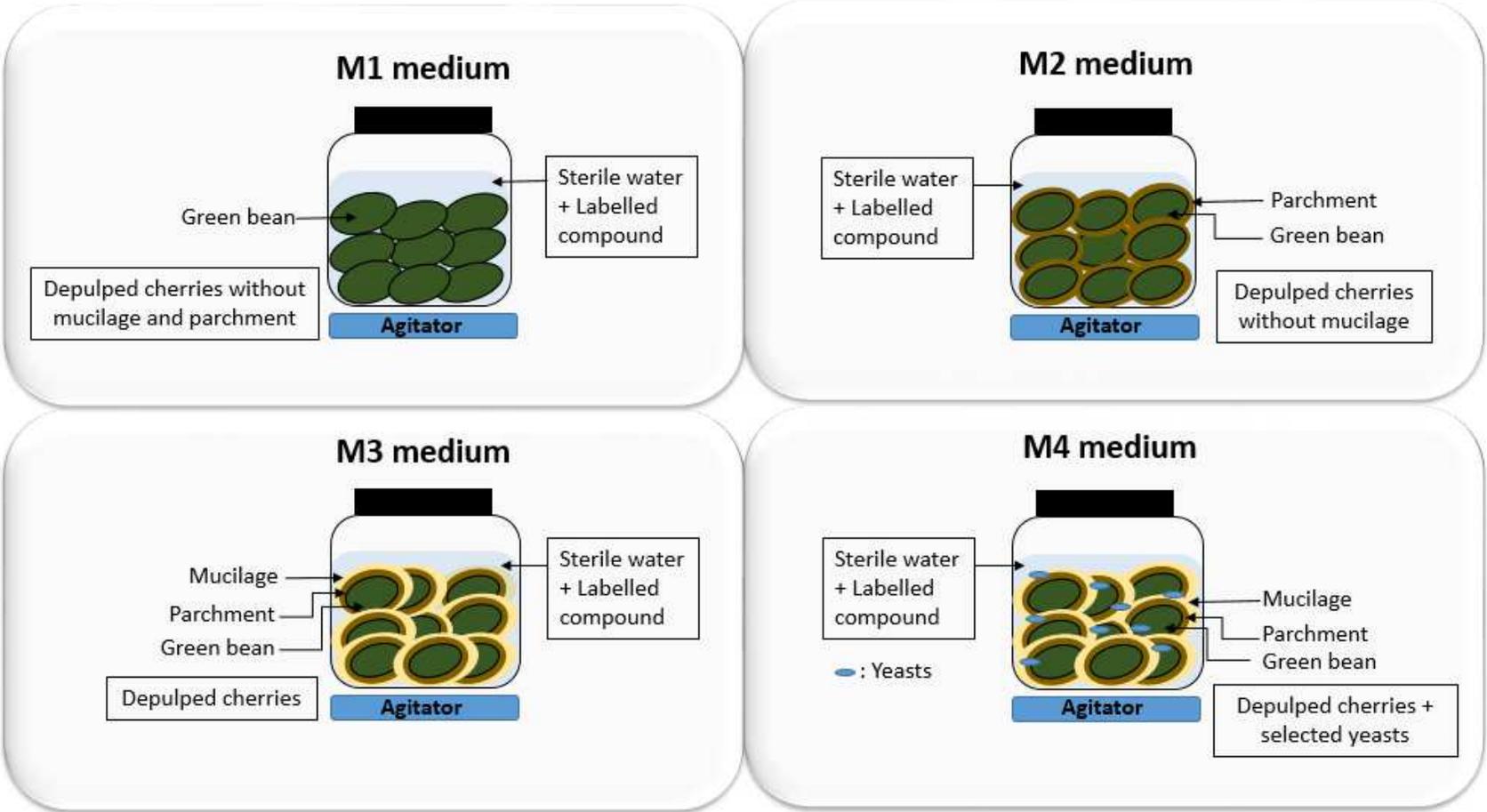


Fig.1. Schematic representation of the media used to study the transfer kinetics of labelled compounds

Transfer kinetics

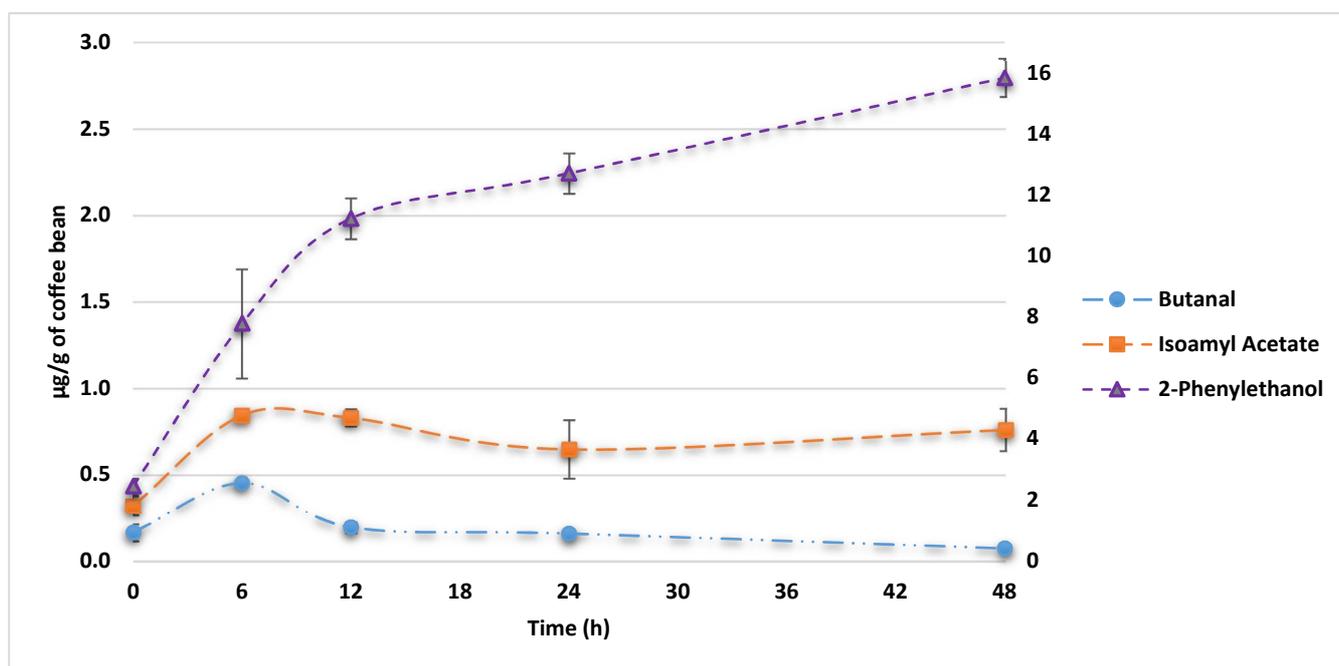


Fig.2. Mass transfer kinetics of labeled compounds into depulped coffee beans (M4) during wet-process treatment. Right vertical axis for 2-phenylethanol and left vertical axis for butanal and isoamyl acetate.

Mass transfer resistance

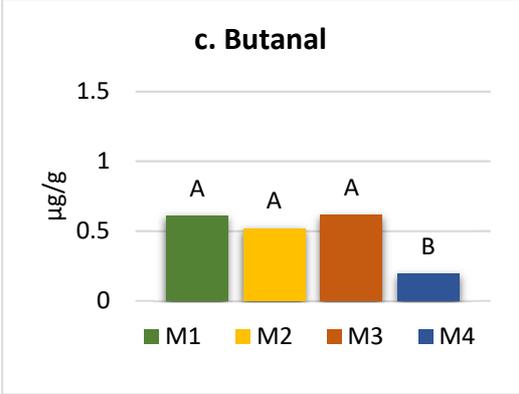
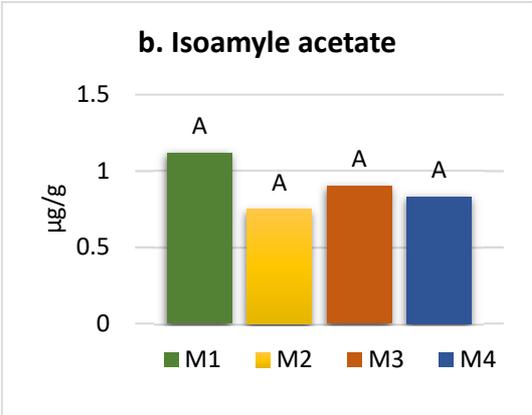
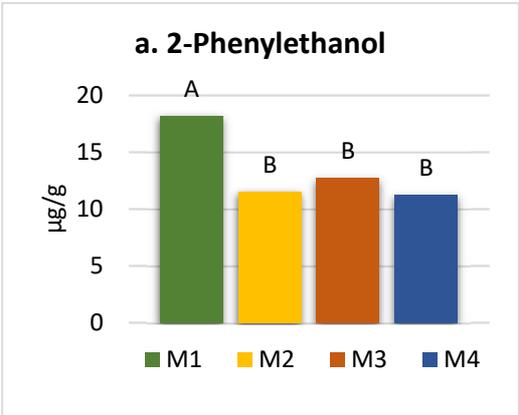


Fig.3. Labeled compound content in coffee beans after 12h of transfer. M1: coffee beans, M2: demucilaginated coffee beans, M3: depulped coffee beans, M4: fermented depulped coffee beans. Different labels (A-B) indicate that means significantly differ at $p < 0.05$ (based on Tukey test).

Yeast effect on the mass transfer

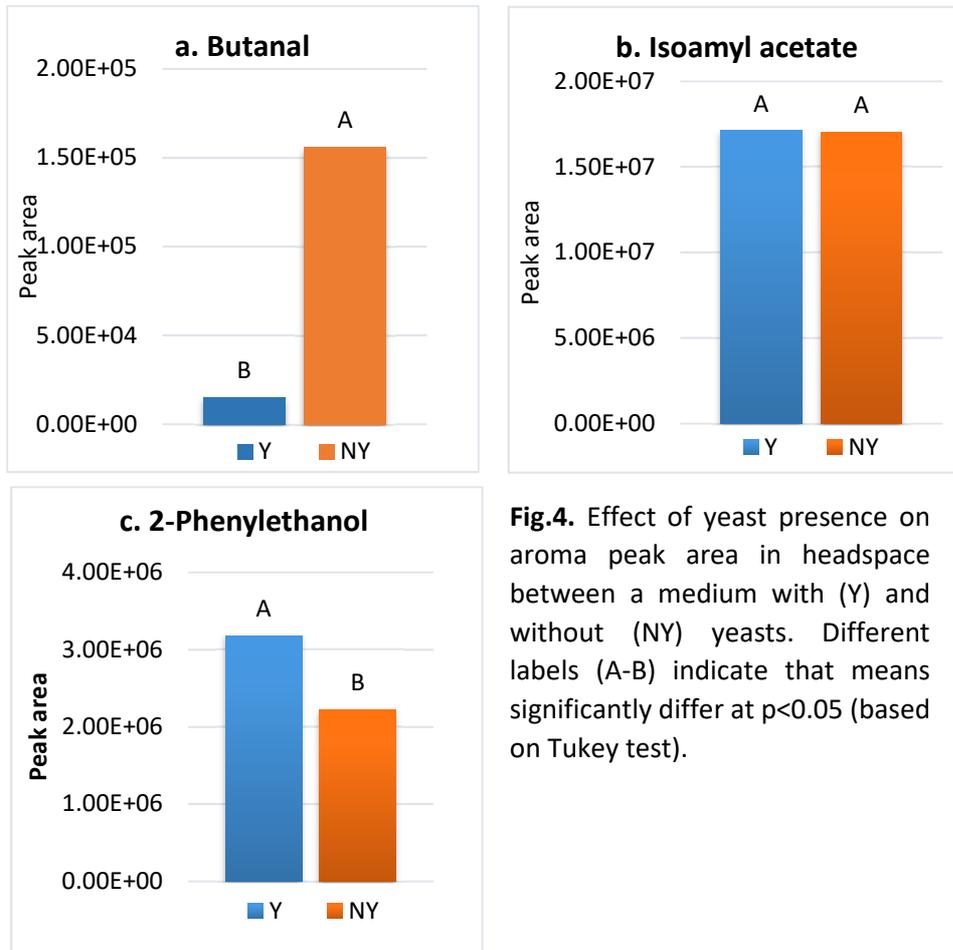


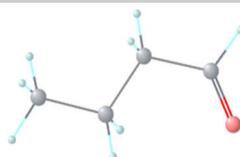
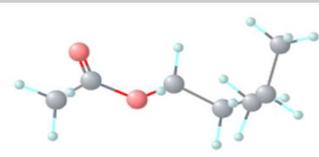
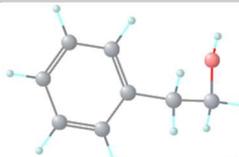
Fig.4. Effect of yeast presence on aroma peak area in headspace between a medium with (Y) and without (NY) yeasts. Different labels (A-B) indicate that means significantly differ at $p < 0.05$ (based on Tukey test).

Table 1. Transfer speed of labeled compounds calculated from the kinetic study.

Compound/time	Transfer rate ($\mu\text{g/g/h}$)			
	0-6h	6-12h	12-24h	24-48h
Butanal	$0,045 \pm 0,005^a$	$-0,038 \pm 0,005^b$	$-0,004 \pm 0,001^c$	$-0,004 \pm 10^{-4}^c$
Isoamyl acetate	$0,088 \pm 0,008^a$	$-0,002 \pm 3 \cdot 10^{-3}^b$	$-0,015 \pm 0,02^b$	$-0,008 \pm 0,009^b$
2-Phenylethanol	$0,757 \pm 0,16^a$	$0,453 \pm 0,06^{ab}$	$0,173 \pm 0,06^b$	$0,161 \pm 0,01^b$

Different labels (a-b) indicate that means significantly differ at $p < 0.05$ (based on Tukey test).

Table 2. Physicochemical properties of the studied molecules (PubChem, 2019)

	Butanal	Isoamyl acetate	2-Phenylethanol
Molecular weight (g/mol)	72,1	130,19	122,17
Topological Polar Surface Area (A)	17,1.10 ²	23,3.10 ²	20,2.10 ²
Solubility in water (mg/L at 25°C)	71000	2000	22200
Density (g/cm³)	0,803	0,876	1,02
LogP* (hydrophobicity)	0,88	2,25	1,36
Conformation			

*LogP: partition coefficient, more the LogP>1, more the molecule is hydrophobic.