Title page

Transfer kinetics of labeled aroma compounds from liquid media into coffee beans during simulated wet processing conditions

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Abstract

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

The three labelled molecules were transferred into the coffee beans with different mass transfer rates; reaching at 12hrs in the M4, 0.2±0.03 ,11.2±0.66 and 1.3±0.04µg/g of coffee respectively for butanal, 2-phenyethanol and isoamyl acetate. The parchment resistance significantly affected the mass transfer of the 2-phenylethanol. Butanal and isoamyl acetate underwent metabolic reactions, which decreased their amount in the coffee beans. Furthermore, an interaction between molecules and the yeast was observed and decreased significantly the butanal's transfer.

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

1. Introduction

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

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

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

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

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

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

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

10 kg of fresh coffee cherries (variety *Coffea Arabica)* were purchased from the Tarrazu cooperative (Tarrazu, Costa Rica). Harvested in March 2018, they were frozen at -18 °C to curb any microbiological and enzymatic activity. The frozen samples were transported to France and stored in a cold room at -18 °C.

To work on homogeneous samples in terms of maturity, the coffee cherries were sorted according to their size and color. Only mature red cherries with 12–14 mm in diameter were selected for the study.

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

2.2. Labeled molecules.

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

aldehyde, butanal ($CH_3CH_2CD_2CHO$), an alcohol, 2-phenylethanol ($C_6D_5CH_2CH_2OH$) and an ester, isoamyl acetate ($(CH_3)_2CHCH_2CH_2OCOCD_3$). In order to quantify the labelled volatile molecules, calibration curves were plotted for each of the compounds. First, the stock solution was prepared using a mix of the three volatile compounds dissolved in 2% of ethanol. Afterwards, three dilutions were carried out from this stock solution (¾, ½, and ¼).

Then, 100 µl of each dilution were introduced into samples of 1.5 g of ground coffee beans and weighed in glass vials (repeated 4 times). In addition, to ensure reproducibility, samples were spiked with 100 µL of a solution of benzyl butyrate used as an internal standard (with a concentration of 5 µL/L), and then, they were analyzed by SPME-GC-MS.

2.3. First experimental approach

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

In the first step, the transfer was studied in M1 medium, composed of manually depulped and dehulled coffee beans, 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 x 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

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

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

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

2.4. Second experimental approach: additional tests

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

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

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

2.5. Volatile compounds analyses

- **Extraction by SPME**

Coffee bean samples were ground to a fine particle size ≈ 500 µm (Retsch-ZM200, France). Volatile compounds of coffee beans were extracted using the SPME (DVB/Carboxen/PDMS, 50/30 µm, Supelco, USA, solid-phase-microextraction). Ground coffee (1.50±0.05 g) was weighed in screw-capped glass vials and mixed with 2 mL of pure water. Subsequently, the vials were placed for 15 min in a thermostatically regulated oven at 50 °C, to reach sample headspace equilibrium. Then they were subjected to SPME at 50 °C for 45 min.

- **GC-MS Analysis**

The labelled volatile compounds were analyzed in an Agilent 6890 gas chromatograph coupled to a mass spectrometer Agilent 5973 (Agilent Technologies, Palo Alto, USA). The SPME fiber was desorbed in a polar column DB-WAX (60 m x 0.25 mm, 0.25 µm phase film thickness, Agilent J&W GC column, USA) under splitless mode with the injector temperature set at 250 °C. Hydrogen was used as the gas vector with the flow rate fixed at 1.5 mL/min. The initial oven temperature was 40 °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 (µg/g/h) was calculated using the following
272 formula:

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

274 C_i : The transferred masses of labelled molecules in the coffee
275 beans [µg/g]

t : transfer time (h)

i : studied compound

3. Results and discussion

3.1. Transfer kinetics study

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

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

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

into the coffee beans continued to increase by accumulation to reach 15.84±0.62 µg/g after 48 hrs.

Indeed, the transfer of volatile compounds is governed by thermodynamic and kinematic parameters related to the composition, the physical state of the matrix and their interaction with the physicochemical properties of the molecules (Voilley et al., 2011). In our case, the molecules were transferred from liquid medium to organic matrix (the coffee bean) by imposing a transfer gradient. According to the physicochemical properties presented in table 2, with a lower molecular weight and a lower LogP, 2-phenylethanol would have an easier transport than the isoamyle acetate. While butanal has the lowest molecular weight and a LogP<1 between the three molecules used, and contrary to what was expected, the 2-phenylethanol represented the highest transfer rate with a transfer speed 16 times faster than that of butanal (during the first 6 hours) (table 1). In their study, Lee et al., (2016) reported an increase of 43% of 2-phenylethanol in the fermented coffee beans compared to the unfermented green coffee beans. Thus, it speculates that the alcohol undergoes an intense diffusion process during the fermentation, which is required to influence the overall coffee quality (Pereira et al., 2019) and this intense transfer cannot 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 phenyethanol) 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 µg/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 µg/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 µg/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 µg/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 µg/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

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

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

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

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

4. Conclusion

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

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

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

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Experimental media

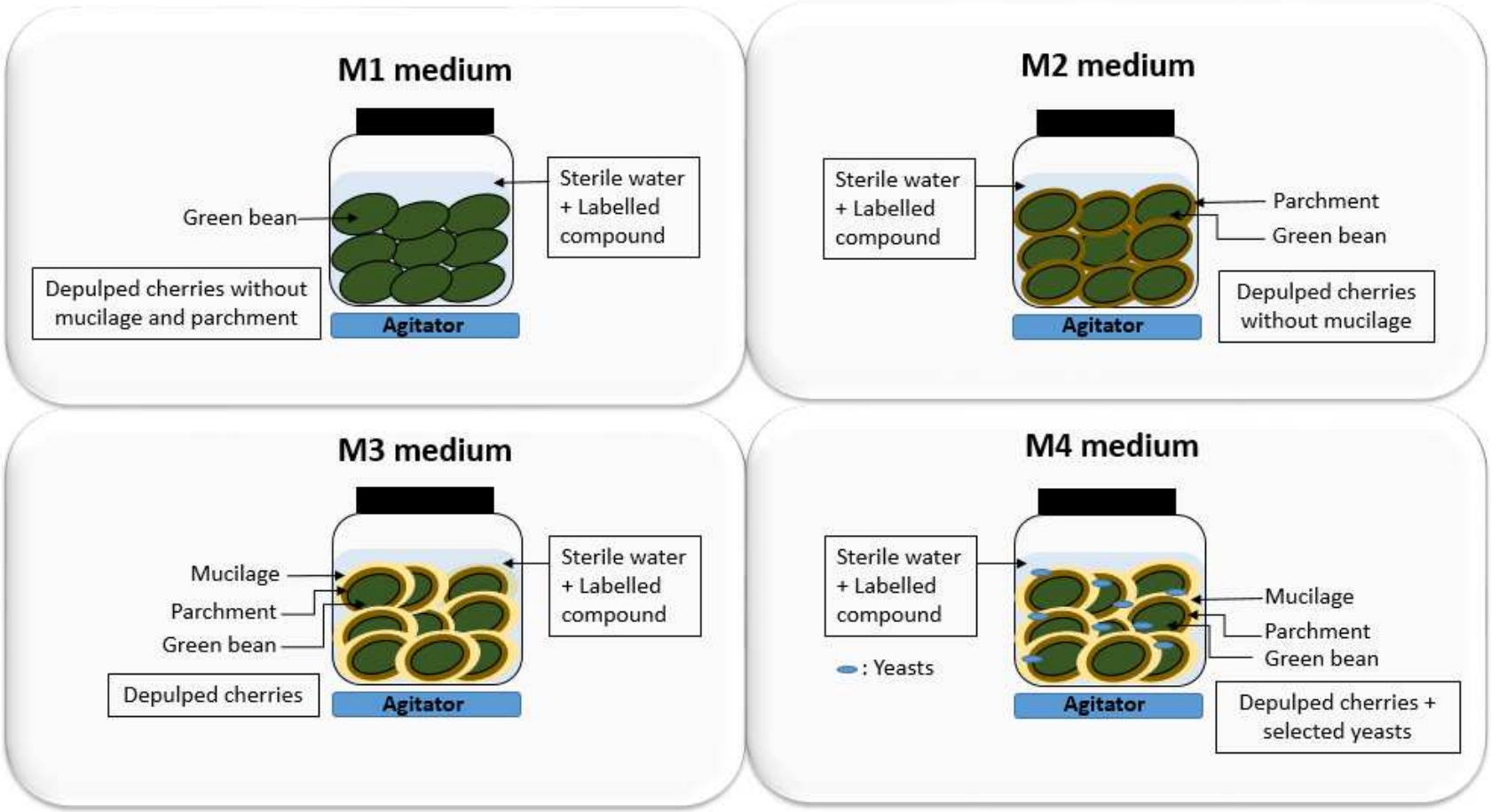


Fig.1. Schematic representation of the media used to study the transfer kinetics of labelled compounds

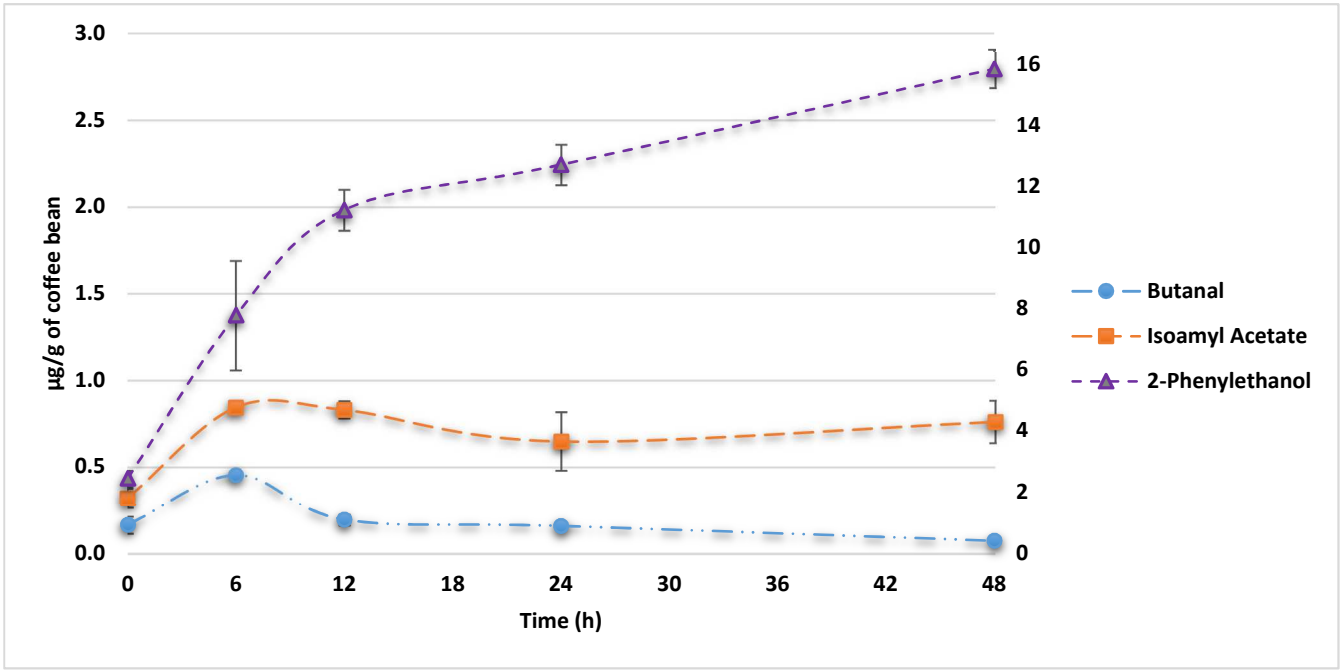


Fig.2. Mass transfer kinetics of labeled compounds into depulped coffee beans (M4) during wet-process treatment. Right vertical axis for 2-phenylethanol and left vertical axis for butanal and isoamyl acetate.

Mass transfer resistance

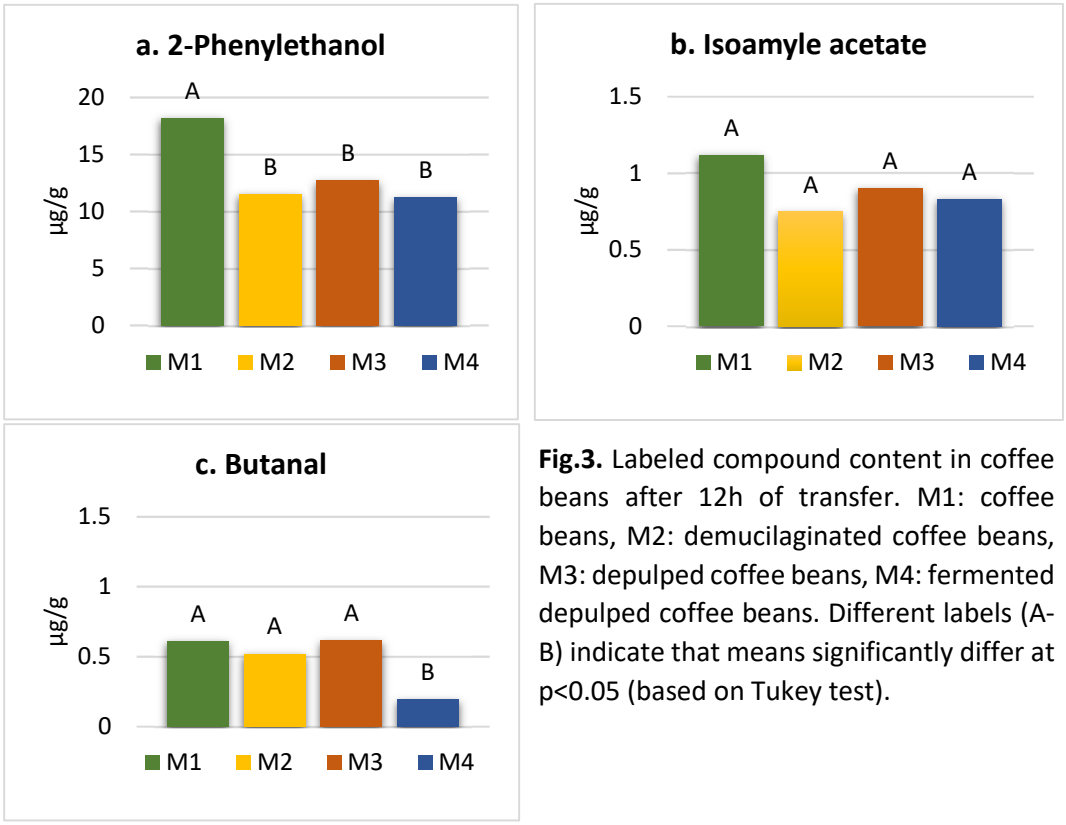


Fig.3. Labeled compound content in coffee beans after 12h of transfer. M1: coffee beans, M2: demucilaginated coffee beans, M3: depulped coffee beans, M4: fermented depulped coffee beans. Different labels (A-B) indicate that means significantly differ at $p<0.05$ (based on Tukey test).

Yeast effect on the mass transfer

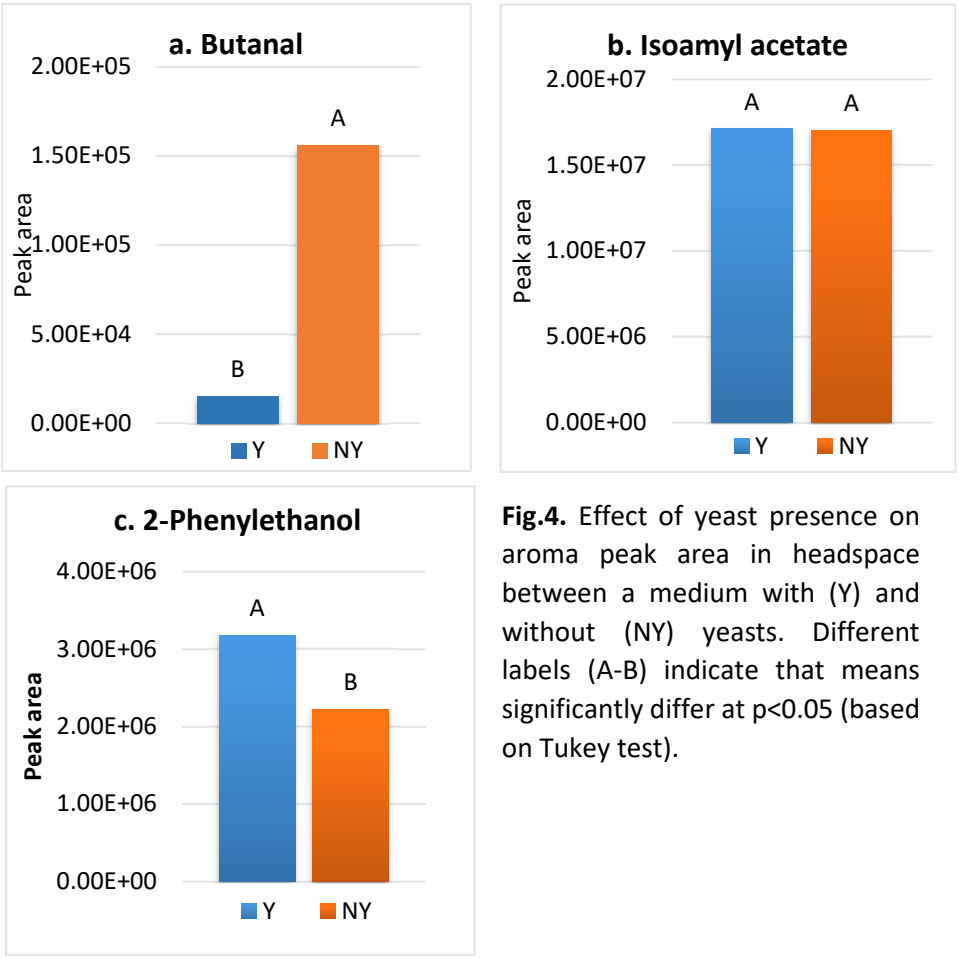


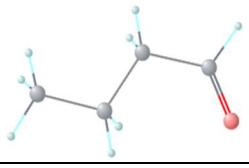
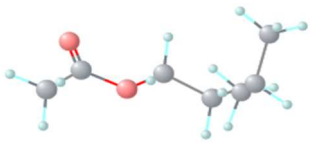
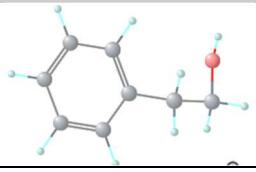
Fig.4. Effect of yeast presence on aroma peak area in headspace between a medium with (Y) and without (NY) yeasts. Different labels (A-B) indicate that means significantly differ at $p<0.05$ (based on Tukey test).

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

Compound/time	Transfer rate (µg/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	$0,088 \pm 0,008^{a}$	$-0,002 \pm 3.10^{-3\,b}$	$-0,015 \pm 0,02^{b}$	$-0,008 \pm 0,009^{b}$
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/cm^3)	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.