Is transfer of aroma compounds produced by yeast during cocoa bean fermentation influenced by the tissue bean structure?

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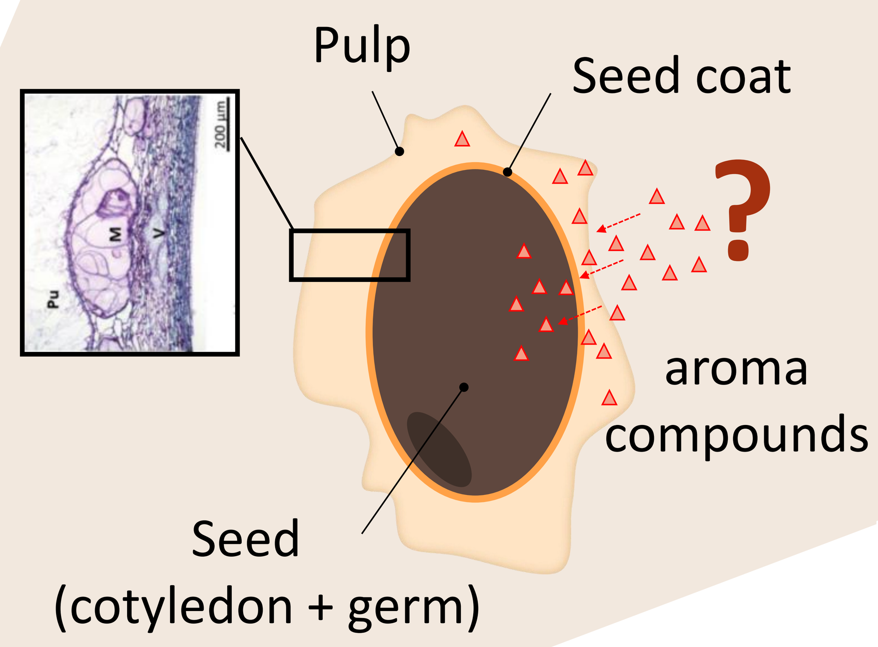
Introduction & objectives

Fermentation is the first post-harvest operation in chocolate processing. Without yeasts, lactic and acetic bacteria fermentative activities, some **aroma compounds** would not be found in chocolate. These observations have led researcher to identify solutions to **optimize** the fermentation step. One focus has been done on **starters** ability to produce specific aroma compounds that could then be found in chocolate, and thus induce **specific flavor qualities**. However, there is still a lake of information regarding the transfer of aroma compounds produced by yeasts and their **diffusion** into the **cocoa seed**.



Our aim is to prove that aroma compounds, produced by yeast during cocoa bean fermentation, are diffused from the pulp to the seed.

Material & methods

4 labelled and **2 non-labelled volatile compounds** were chosen *(Fig. 1-2): ethyl acetate-d3, ethyl octanoate-d15, linalool-d5, 2-phenyl ethanol-d5, delta-decalactone, beta-damascenone.* In all media (**M_1, M_2, M_3**), 10 g of disinfected **beans** are **submerged** in 6 ml of a solution prepared beforehand *(i.e. mix of 40 µg/ml of each molecule)*. Media are then **stored** at **36°C** during **3h to 120h** time period **without agitation** *(Fig. 1)*. After time transfer period, samples are washed, the seed coat and pulp are removed to keep only the seeds. Seeds are frozen with liquid nitrogen, ground and kept at -20°C until being analyzed.
Labelled and non-labelled volatile compounds are determined by **spme-gc/ms**.

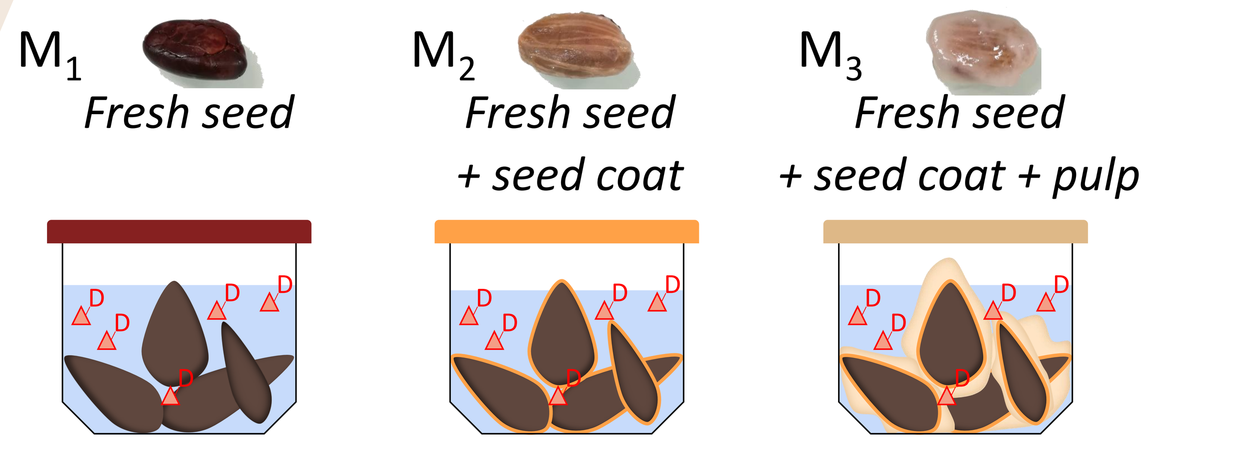


Fig. 1: experimental design. Symbol ▲-D refers to volatile compounds. Media are stocked from 3h to 120h at 36°C. All trials are made in triplicate and are independent.

Results & discussion

- **All 6 volatile** compounds **transfer**, with different transfer rates *(Fig. 2)*.
- Maximum **concentration** reached **depends** on the **volatile compound** and **time** *(e.g. 7 µg / g for ethyl acetate at 6h vs. 36 µg / g for linalool at 24h in M_1)*.
- The **seed coat** and **pulp** can induce a mass transfer **resistance**.

- For all media, concentrations **increase exponentially** as function of time, potentially due to the **concentration gradient** between the external solution and the seed. This phase is followed by a concentration **decrease**, more important in M_1, which could be explained by a **reverse transfer** in M_1, that is not observed in M_2 or M_3. The **seed coat** and **pulp** seem to act as **physical barriers** and reduce a reverse mass transfer.

- 3 trends can be highlighted with regards to the tissue bean structure:

(1) **Significant effect** of the **seed coat** and **pulp** over time: *ethyl octanoate*. The seed coat and pulp reduce respectively about 70% and 90% ethyl octanoate amount transferred *(Fig. 2b)*.

(2) **Significant effect** of the **seed coat** during the exponential phase: *ethyl acetate, linalool, beta-damascenone.* Linalool and beta-damascenone final concentrations (120h) reached in M_3 are significantly higher *(Fig. 2c and 2d)*. High degradation of ethyl acetate concentration over time *(Fig. 2a)*.

(3) **Significant effect** of the **pulp** during the exponential phase: *2-phenyl ethanol, delta-decalactone*. Final concentration (120h) reached in M_3 is significantly higher *(Fig. 2e and 2f)*.

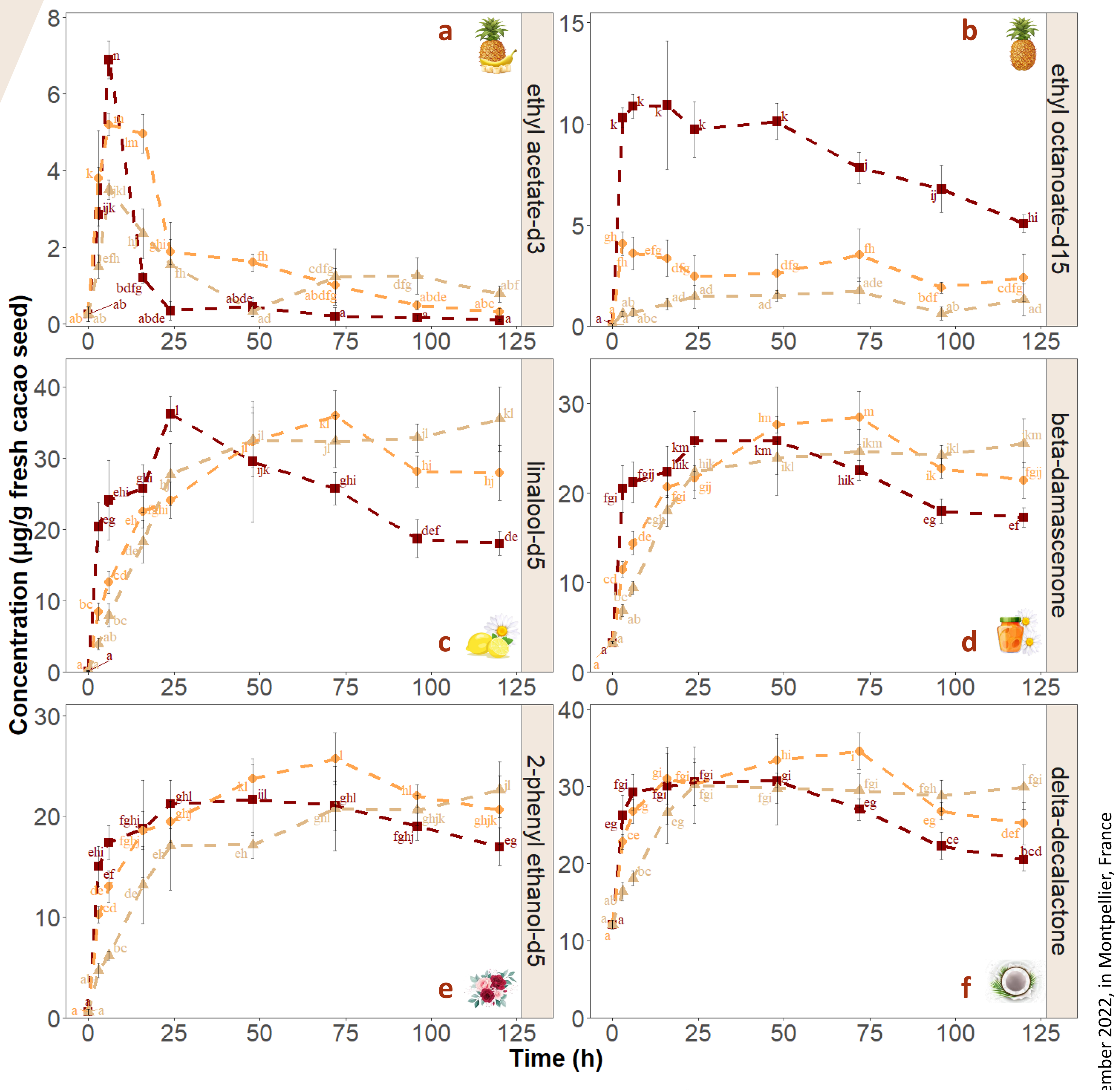


Fig. 2a-f: volatile compounds concentration (µg/g) as a function of time (h) in fresh cacao seeds from media M_1 (-■-), M_2 (-◆-), and M_3 (-▲-).

Conclusion & perspectives

This work proves that these **aroma compounds, released** by yeast during **fermentation**, will **diffuse** from the external phase to seed. Over time, there is a mass transfer, which could be limited due to seed coat or/and pulp (**mass transfer resistance**) but also due to an **equilibrium** between the **external phase** and **seed** concentration. This equilibrium depends also on the **concentration gradient** of each volatile compound, that could degrade over time. This work opens on innovative perspectives for the development of **new yeast selection criteria**, which would offer additional aromatic potential to cacao. Further works must be extended to the study of aroma compounds transfer during various environmental fermentative conditions linked to the operation unit (*e.g.* temperature, pH, yeast strain…).

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