Selection of new yeast starter for cocoa beans fermentation – effect of different *Saccharomyces cerevisiae* strains on the volatile profile and sensory attributes of resulting chocolates

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Abstract

Selection of yeast for cocoa bean fermentation is of growing interest as it is now definitely considered in literature as a tool for cocoa quality improvement[a]. This **long-term process** usually begins in origins by isolating prevailing yeast that gives a resulting high-quality chocolate but can also come from a wide range of other ecosystems such as coffee or grape must. In this work, a strain isolated by Leuven University during spontaneous Malaysian cocoa beans fermentation (**Strain 2**) belonging to the *Saccharomyces cerevisiae* (SC) family was compared to Lalcacao Forte, a commercial strain already available for cocoa application.

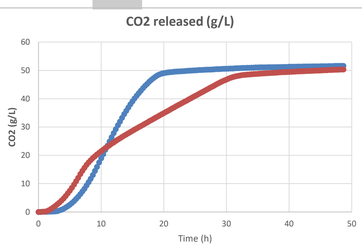
According to selection criterions for this specific type of process, yeasts were screened for their fermentative capacities at a relative high temperature which is essential as cocoa beans fermentation easily reach 35°C within 2 days. Then, **Strain 2** was inoculated with different bacteria species to predict compatibility in the complex cocoa ecosystem. The final step consisted of fermenting Nicaraguan Criollo to evaluate organoleptic properties of the resulting chocolate.

Strain 2 turned out to be well adapted to high temperature condition and compatible with the studied range of bacteria. Resulting chocolate was richer in aromas and its chocolate profile could be qualified as a fine chocolate.

Step 1 : Yeasts phenotyping

Both strains in their Active Dry Form were inoculated (2 g/L) in 2L reactors containing cocoa pulp simulation medium[b] (CPSM) and fermentations were conducted at 35°C. **Strain 2** was compared to the commercial Lalcocoa Forte. CO_2 release was monitored through weigh loss follow-up and yeasts viability was evaluated by recording Colony Forming Unit counted on Sabouraud agar plates.

1.



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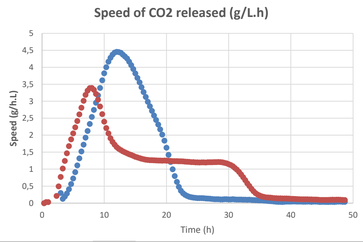
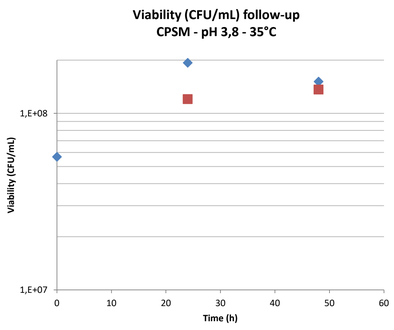


Lalcocoa Forte
Strain 2

3.



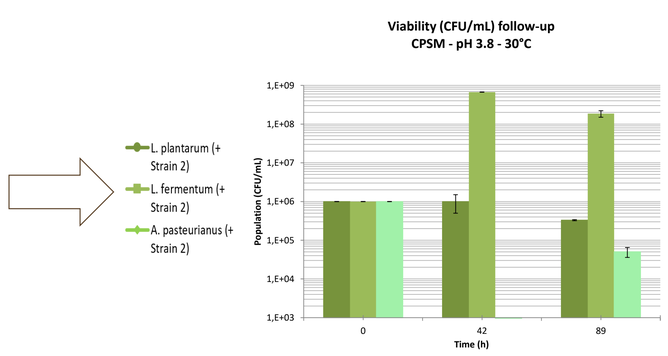
➢ Fermentation kinetics of **Strain 2** was significantly different from Lalcocoa Forte (Cf. 1, 2), reflecting strain dependent **phenotype** in such medium. **Strain 2** showed indeed singular kinetics with a fast exponential phase followed by a linear phase before reaching the stationary phase. **Strain 2** showed then a fair resistance at 35°C since its viability follow-up was close to Lalcocoa Forte's, being over **10^8 CFU/mL** all fermentation long. These observations are likely due to its matrix selection on cocoa bean, making it a suitable candidate for next trials in the selection process.

Step 2 : Yeasts / Bacteria compatibility

In parallel, co-inoculations of **Strain 2** with different bacteria from Lallemand's portfolio were carried out, namely *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Acetobacter pasteurianus* in order to assess compatibility of **Strain 2** with bacteria that are known to prevail in real cocoa beans fermentation[c] resulting to fine chocolate.

Yeast / LAB inoculation rate
Resp. **10^7** and **10^6 CFU/mL**

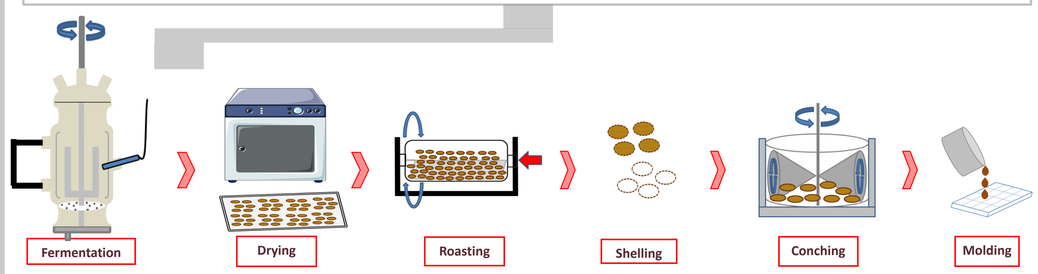
Yeast / AAB inoculation rate
Resp. **10^7** and **10^6 CFU/mL**



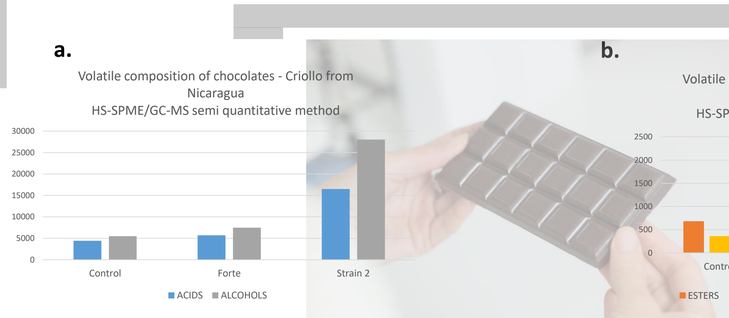
➢ **Strain 2** fermentation kinetics remained **comparable** to its respective condition without bacteria addition (data not shown). Hence, the three species of bacteria screened here did not impact **Strain 2** on its growth/fermentative capacities. *L. fermentum* grew well and *L. plantarum* survived : this latter behavior was expected, hence no inhibition from **Strain 2** was hypothesized. *A. pasteurianus* died off the first two days of fermentation but turned out to **grow** until the fourth despite its very high sensitivity. It could be concluded that **Strain 2** would be friendly to its cocoa environment and wouldn't impede further lactic and acetic acid fermentations.

Step 3 : Validation on lab-scale cocoa beans fermentation

As a final step for selection of starters, a lab-scale solid-state bioreactor has been designed to ferment 1-2 kg fresh cocoa beans. A controlled temperature profile was applied following natural average temperature raise[d] through a double-jacket with water as a heat-transfer. Nicaraguan beans were then fermented 5 days with turning every other day. Lalcocoa Forte and **Strain 2** were inoculated at a 2 g/kg rate, compared to an indigenous fermentation considered as a control. After drying, beans were processed into chocolate and analyzed with HS-SPME-GC/MS(tof) and sensory analyses were performed by a panel of 8 highly trained assessors.



a.



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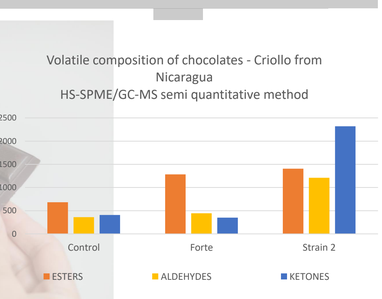

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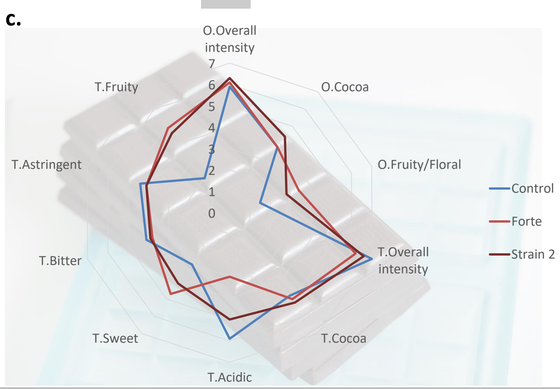

➢ **Strain 2** resulting chocolate was highlighted by its richest composition in 5 of the 5 main cocoa aroma families presented in **a.** and **b.**, esters being as high as Lalcocoa Forte©. Statistically, **Strain 2** was discriminated by its greater chocolate content of 2-phenylethanol (*lilac*, *rose*) and ethyl dodecanoate (*floral*, *clean*), but also contained more lactones (*creamy*) that could be key in sensory. Resulting chocolate had with Lalcocoa Forte the fruitiest odor and taste, **Strain 2** showing a rich red/black berry notes with a particularly balanced profile as would do a fine chocolate.

Future trends

Strain 2, initially isolated for its presence in spontaneous fermentation giving resulting high quality chocolate, well proved its interest for cocoa fermentation application since main microbial criterions were achieved such as resistance to relatively high fermentation temperature of fermentation, as well as compatibility with main cocoa bacteria.

On top of that, chocolate prepared from beans fermented with **Strain 2** showed a fine chocolate profile whereas control was too acidic and considered as regular chocolate.

After **3 years** of lab research, including other cocoa origins fermentations (*not shown*), **Strain 2** confirmed its technological interest giving fruity chocolates, well driven by red berries notes and a particular signature through fatty acid esters high content in chocolates. Trialed in origins and succesfully produced in its active dry form, **Strain 2** is about to become the very first indigenous strain to become commercially available for farmers that are looking for a biotechnological tool to better control their post-harvest processing, upgrading their cocoa quality and improving in the end their income.

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