

# Supplementary materials

## Analysis of the microbial diversity and population dynamics during the pulque fermentation process.

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**Supplementary file 1. Biomass quantification by dry weight.** Methodology modifies from Mexican Official Norm NMX-V-017-1970 and AOAC for wine analyses.

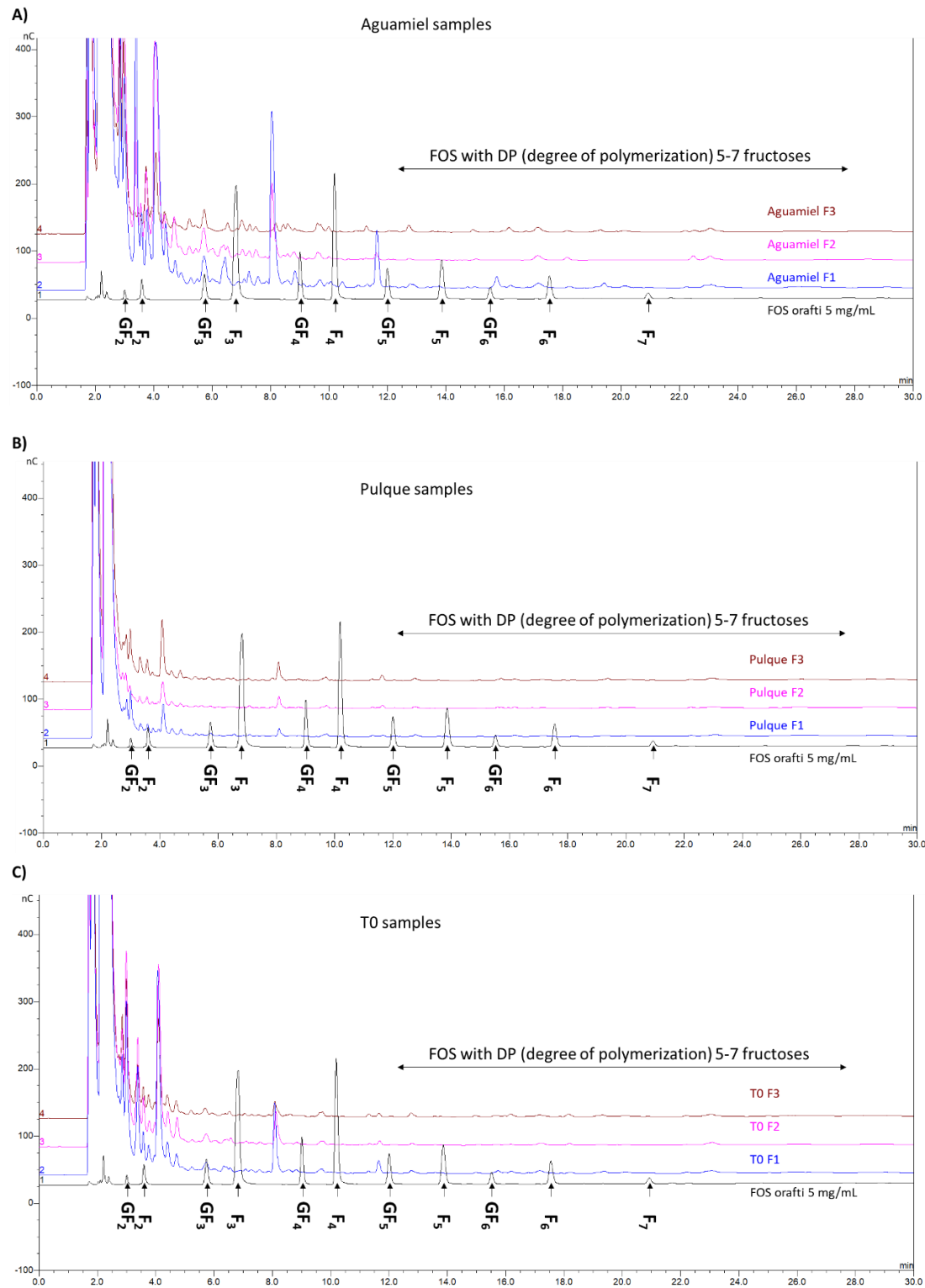
- Wash the 15 mL porcelain crucibles well and place them at constant weight overnight at 95 °C.
- After a minimum of 12 hours, remove the crucibles from the furnace and allow them to cool in the desiccator for 30 minutes.
- Weigh on an analytical balance with an error of  $\pm 0.1$  mg.
- Place 10 mL of sample volume (pulque, aguamiel, fermentation times) in each crucible.
- Allow all the liquid to evaporate in a water bath at approximately 90°C.
- Once the liquid has evaporated as much as possible (until a very viscous syrup is generated), it is left in the oven overnight at 90-95°C for 12 hours.
- Take out and let cool for 30 min in the desiccator and weigh on the analytical balance.
- Ignite the muffle at 575°C
- Place the crucibles in the muffle for 15 minutes, remove, transport in the desiccator and leave to cool for 30 minutes in the same.
- To wash the ashes, place the crucibles in a water bath at approximately 90 °C and place 3 mL of milliQ water in each crucible and allow to consume completely.
- Raise the muffle temperature to 675°C
- Place the crucibles again for 5 hours or until white ashes are obtained (one hour before 5 hours it is recommended to raise the temperature to 100°C).
- After 5 hours, the crucibles are removed and allowed to cool for one hour to later weigh on the analytical balance.

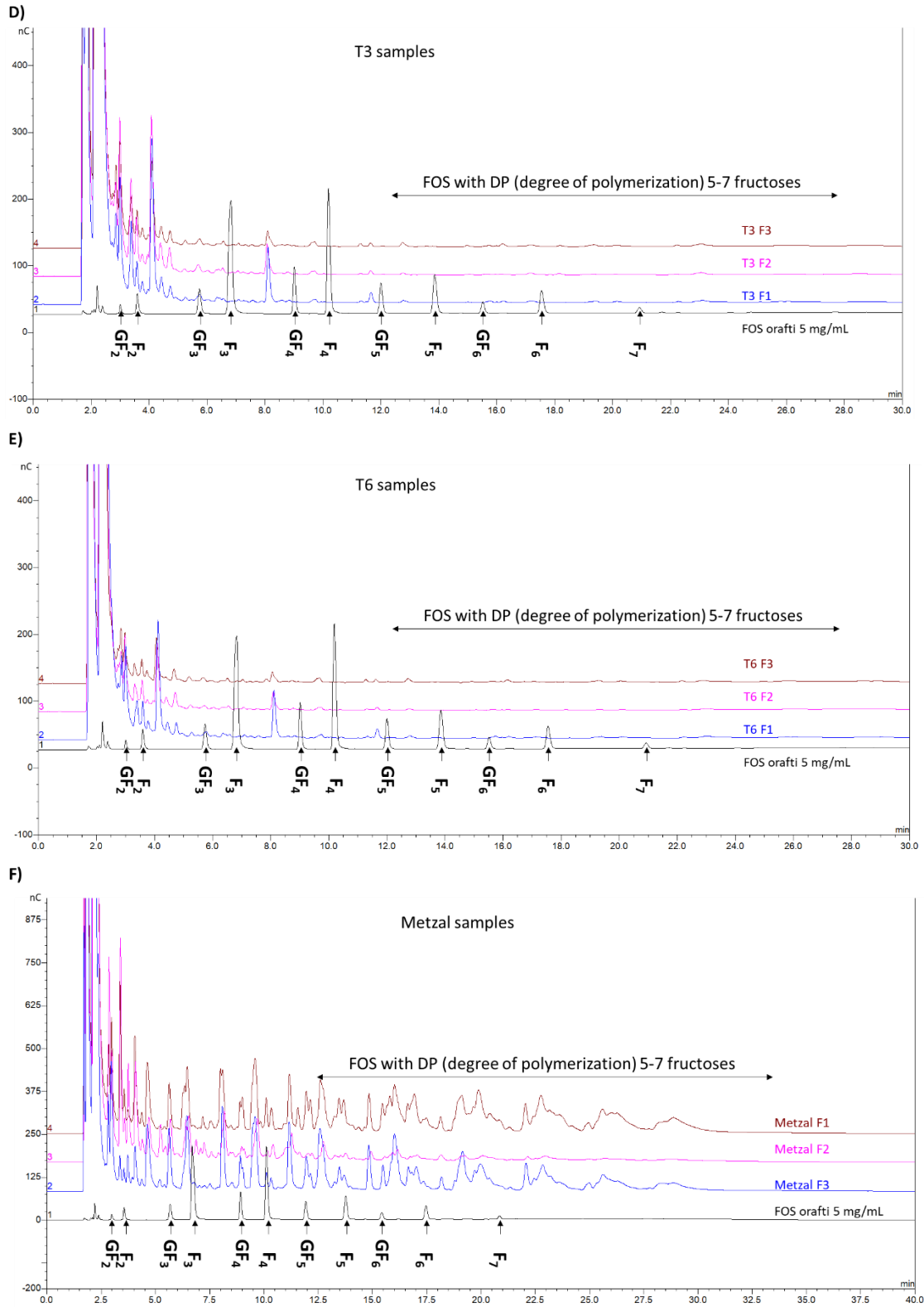
$$Total\ solids = \frac{X_o - X_y}{V} \times 100$$

$$Ashes = \frac{M_o - M_y}{V} \times 100$$

$$Biomass = Total\ solids - Ashes$$

**Figure S1. Fructooligosaccharides identification per fermentation.**





**Figure S1. Fructooligosaccharides identification per fermentation.** A) Aguamiel samples, B) Pulque samples, C) T0 samples, D) T3 samples, E) T6 samples. F) Metzal samples. **GF<sub>2</sub>**: 1-ketose, **F<sub>2</sub>**:

Inulobiose, **GF**<sub>3</sub>: Nystose, **F**<sub>3</sub>: Inulotriose, **GF**<sub>4</sub>: Fructosyl-nystose, **F**<sub>4</sub>: Inulotetrose, **GF**<sub>5</sub>: Ketoheptose, **F**<sub>5</sub>: Inulopentose, **GF**<sub>6</sub>: Ketoheptose, **F**<sub>6</sub>: Inulohexose, and **F**<sub>7</sub>: Inuloheptose.

Table S1. Bacteria taxonomic identification.

| Pulque | T6     | T3     | T0     | Aguamiel | Metzal | Core   | Others |
|--------|--------|--------|--------|----------|--------|--|--------|
| 47.35% | 69.25% | 65.24% | 63.18% | 51.27%   | 56.29% | <i>Zymomonas mobilis</i> subsp.                |        |
| 0.46%  | 0.07%  | 0.14%  | 0.21%  | 1.46%    | 2.23%  | <i>Weissella ghanensis</i>                     |        |
| 7.86%  | 5.32%  | 7.63%  | 7.29%  | 0.73%    | 0.52%  | <i>Acetobacter pasteurianus</i>                |        |
| 2.20%  | 1.59%  | 2.81%  | 3.02%  | 12.91%   | 3.09%  | <i>Lactococcus uncultured</i>                  |        |
| 3.00%  | 2.63%  | 3.36%  | 3.25%  | 5.79%    | 5.12%  | <i>Leuconostoc</i>                             |        |
| 3.00%  | 2.63%  | 3.36%  | 3.25%  | 5.79%    | 5.12%  | <i>Leuconostoc</i>                             |        |
| 0.85%  | 0.58%  | 0.87%  | 0.68%  | 0.04%    | 0.03%  | <i>Acetobacter cibinongensis</i>               |        |
| 0.14%  | 0.05%  | 0.09%  | 0.11%  | 1.67%    | 0.24%  | <i>Obesumbacterium proteus</i>                 |        |
| 0.50%  | 0.29%  | 0.32%  | 0.24%  | 0.24%    | 0.23%  | <i>Lactococcus lactis</i> subsp. <i>lactis</i> |        |
| 0.09%  | 0.05%  | 0.10%  | 0.07%  | 0.01%    | 0.05%  | <i>Gluconobacter albidus</i>                   |        |
| 0.07%  | 0.05%  | 0.06%  | 0.05%  | 0.04%    | 0.04%  | <i>Leuconostoc citreum</i>                     |        |
| 0.10%  | 0.02%  | 0.05%  | 0.04%  | 0.03%    | 0.05%  | <i>Lactobacillus capillatus</i>                |        |
| 0.04%  | 0.02%  | 0.03%  | 0.04%  | 0.02%    | 5.52%  | <i>Allium ampeloprasum</i> (leek)              |        |
| 0.00%  | 0.00%  | 0.00%  | 0.00%  | 0.01%    | 0.33%  | <i>Arachis duranensis</i>                      |        |
| 0.00%  | 0.00%  | 0.00%  | 0.00%  | 0.00%    | 0.08%  | <i>Cyrtopodium macranthos</i>                  |        |
| 0.05%  | 0.02%  | 0.03%  | 0.01%  | 0.00%    | 0.00%  | <i>Drosophila kikkawai</i>                     |        |
| 0.24%  | 0.13%  | 0.10%  | 0.16%  | 0.02%    | 0.00%  | <i>Lactobacillus acetotolerans</i>             |        |
| 0.03%  | 0.01%  | 0.03%  | 0.01%  | 0.01%    | 0.00%  | <i>Lactobacillus brevis</i>                    |        |
| 0.03%  | 0.01%  | 0.02%  | 0.01%  | 0.01%    | 0.00%  | <i>Lactobacillus camelliae</i>                 |        |
| 0.11%  | 0.09%  | 0.07%  | 0.03%  | 0.00%    | 0.00%  | <i>Lactobacillus faeni</i>                     |        |
| 4.68%  | 2.74%  | 2.26%  | 1.96%  | 0.01%    | 0.00%  | <i>Lactobacillus hilgardii</i>                 |        |
| 0.94%  | 0.48%  | 0.42%  | 0.58%  | 0.07%    | 0.12%  | <i>Lactobacillus nagelii</i>                   |        |
| 0.04%  | 0.04%  | 0.06%  | 0.02%  | 0.00%    | 0.00%  | <i>Lactobacillus pentosus</i>                  |        |
| 0.32%  | 0.27%  | 0.28%  | 0.21%  | 0.01%    | 0.02%  | <i>Lactobacillus sanfranciscensis</i>          |        |
| 0.72%  | 0.19%  | 0.24%  | 0.32%  | 0.10%    | 0.22%  | <i>Lactobacillus satsumensis</i>               |        |
| 0.04%  | 0.02%  | 0.03%  | 0.01%  | 0.00%    | 0.00%  | <i>Lactobacillus senioris</i>                  |        |
| 0.50%  | 0.21%  | 0.37%  | 0.29%  | 0.00%    | 0.00%  | <i>Lactobacillus similis</i>                   |        |
| 4.09%  | 1.59%  | 3.10%  | 1.92%  | 0.00%    | 0.00%  | <i>Lactobacillus similis</i> DSM 23365         |        |
| 1.75%  | 1.08%  | 1.04%  | 0.86%  | 0.01%    | 0.01%  | <i>Lactobacillus</i> sp. <i>rennanqilfy19</i>  |        |
| 0.17%  | 0.11%  | 0.12%  | 0.09%  | 0.00%    | 0.00%  | <i>Lactobacillus uncultured</i>                |        |
| 0.07%  | 0.04%  | 0.02%  | 0.02%  | 0.00%    | 0.00%  | <i>Lactobacillus uncultured</i>                |        |
| 16.02% | 10.62% | 7.15%  | 10.36% | 0.09%    | 0.04%  | <i>Lactobacillus uncultured</i>                |        |
| 0.56%  | 0.50%  | 0.52%  | 0.53%  | 0.01%    | 0.03%  | <i>Leuconostoc uncultured</i>                  |        |
| 2.83%  | 0.35%  | 1.10%  | 1.12%  | 13.54%   | 23.56% | <i>Weissella beninensis</i>                    |        |
| 0.01%  | 0.00%  | 0.00%  | 0.00%  | 0.02%    | 0.05%  | <i>Weissella uncultured</i>                    |        |
| 0.00%  | 0.01%  | 0.01%  | 0.02%  | 0.02%    | 0.05%  | <i>Lactococcus</i> sp. <i>INBio_4514B</i>      |        |
| 0.02%  | 0.02%  | 0.02%  | 0.03%  | 0.00%    | 0.00%  | <i>Acetobacter uncultured</i>                  |        |
| 0.02%  | 0.01%  | 0.02%  | 0.02%  | 0.00%    | 0.00%  | <i>Acetobacter unidentified</i>                |        |
| 0.04%  | 0.02%  | 0.03%  | 0.02%  | 0.00%    | 0.02%  | <i>Gluconacetobacter</i>                       |        |
| 0.02%  | 0.01%  | 0.02%  | 0.01%  | 0.02%    | 0.03%  | <i>Gluconobacter morbifer</i>                  |        |
| 0.20%  | 0.10%  | 0.14%  | 0.12%  | 0.03%    | 0.04%  | <i>Gluconacetobacter</i> sp. <i>AXB(X)</i>     |        |
| 0.08%  | 0.02%  | 0.06%  | 0.06%  | 0.71%    | 0.16%  | <i>Buttiauxella agrestis</i>                   |        |
| 0.00%  | 0.00%  | 0.00%  | 0.00%  | 0.24%    | 0.00%  | <i>Escherichia coli</i>                        |        |
| 0.02%  | 0.01%  | 0.01%  | 0.01%  | 0.22%    | 0.02%  | <i>Hafnia-Obesumbacterium</i>                  |        |
| 0.02%  | 0.01%  | 0.01%  | 0.01%  | 0.20%    | 0.03%  | <i>Kluyvera ascorbata</i>                      |        |
| 0.41%  | 0.06%  | 0.09%  | 0.11%  | 0.59%    | 0.23%  | <i>Acinetobacter uncultured</i>                |        |

Taxonomic identification and filtering with 0.01% of relative abundance.

**Table S2. Fungal taxonomic identification.**

|                 | Core                            |                                |                                 |                          |                     | Others                         |                        |                             |                                |               |              |                       |              |                                     |                           |                             |                          |         |
|-----------------|---------------------------------|--------------------------------|---------------------------------|--------------------------|---------------------|--------------------------------|------------------------|-----------------------------|--------------------------------|---------------|--------------|-----------------------|--------------|-------------------------------------|---------------------------|-----------------------------|--------------------------|---------|
|                 | <i>Kazachstaniana gamospora</i> | <i>Kluyveromyces marxianus</i> | <i>Saccharomyces cerevisiae</i> | <i>Hanseniaspora sp.</i> | o_Saccharomycetales | <i>Aureobasidium pullulans</i> | <i>Penicillium sp.</i> | <i>Botrytis caroliniana</i> | <i>Torulaspota delbrueckii</i> | o_Hypocreales | p_Ascomycota | <i>Rectipilus sp.</i> | o_Agaricales | <i>Erythrobasidium hasegawianum</i> | <i>Malassezia globosa</i> | <i>Malassezia restricta</i> | <i>Naganishia albida</i> | k_Fungi |
| <b>Metzal</b>   | 40.76%                          | 47.67%                         | 8.96%                           | 0.72%                    | 1.01%               | 0.01%                          | 0.05%                  | 0.00%                       | 0.01%                          | 0.01%         | 0.00%        | 0.32%                 | 0.39%        | 0.00%                               | 0.08%                     | 0.01%                       | 0.00%                    | 0.00%   |
| <b>Aguamiel</b> | 14.22%                          | 61.04%                         | 22.63%                          | 0.46%                    | 0.14%               | 0.09%                          | 0.48%                  | 0.10%                       | 0.01%                          | 0.05%         | 0.06%        | 0.11%                 | 0.05%        | 0.05%                               | 0.02%                     | 0.40%                       | 0.07%                    | 0.03%   |
| <b>T0</b>       | 2.52%                           | 0.02%                          | 97.30%                          | 0.09%                    | 0.06%               | 0.00%                          | 0.00%                  | 0.00%                       | 0.00%                          | 0.00%         | 0.00%        | 0.00%                 | 0.00%        | 0.00%                               | 0.01%                     | 0.00%                       | 0.00%                    | 0.00%   |
| <b>T3</b>       | 1.97%                           | 0.05%                          | 97.65%                          | 0.19%                    | 0.12%               | 0.00%                          | 0.00%                  | 0.00%                       | 0.02%                          | 0.00%         | 0.00%        | 0.00%                 | 0.00%        | 0.00%                               | 0.00%                     | 0.00%                       | 0.00%                    | 0.00%   |
| <b>T6</b>       | 1.23%                           | 0.02%                          | 98.63%                          | 0.07%                    | 0.04%               | 0.00%                          | 0.00%                  | 0.00%                       | 0.00%                          | 0.00%         | 0.00%        | 0.00%                 | 0.00%        | 0.00%                               | 0.00%                     | 0.00%                       | 0.00%                    | 0.00%   |
| <b>Pulque</b>   | 4.37%                           | 0.01%                          | 95.26%                          | 0.08%                    | 0.24%               | 0.00%                          | 0.00%                  | 0.00%                       | 0.03%                          | 0.00%         | 0.00%        | 0.00%                 | 0.01%        | 0.00%                               | 0.00%                     | 0.00%                       | 0.00%                    | 0.00%   |

Taxonomic identification and filtering with 0.01% of relative abundance.

**Table S3. Bacteria taxonomic identification per sample and genus.**

|                | <i>Zymomonas</i> | <i>Weissella</i> | <i>Acetobacter</i> | <i>Lactococcus</i> | <i>Leuconostoc</i> | <i>Obesumbacterium</i> | <i>Gluconobacter</i> | <i>Lactobacillus</i> | Others |
|----------------|------------------|------------------|--------------------|--------------------|--------------------|------------------------|----------------------|----------------------|--------|
| <b>MetzaF1</b> | 79.5%            | 13.8             | 0.2                | 0.6                | 2.8                | 0.01                   | 0.1                  | 0.01                 | 3.1    |
| <b>MetzaF2</b> | 81.5             | 0.01             | 1.3                | 9.5                | 2.0                | 0.1                    | 0.01                 | 0.1                  | 5.5    |
| <b>MetzaF3</b> | 7.8              | 56.9             | 0.2                | 4.5                | 10.7               | 0.6                    | 0.01                 | 0.01                 | 19.1   |
| <b>AMF1</b>    | 63.2             | 22.1             | 0.3                | 8.1                | 2.4                | 0.1                    | 0.01                 | 0.01                 | 3.8    |
| <b>AMF2</b>    | 83.7             | 0.0              | 0.6                | 14.2               | 0.6                | 0.01                   | 0.01                 | 0.1                  | 0.8    |
| <b>AMF3</b>    | 7.0              | 18.5             | 1.5                | 46.6               | 14.5               | 4.9                    | 0.01                 | 0.01                 | 7.0    |
| <b>T0F1</b>    | 51.8             | 1.1              | 6.3                | 6.8                | 3.9                | 0.1                    | 0.1                  | 0.01                 | 30.0   |
| <b>T0F2</b>    | 85.8             | 0.2              | 1.1                | 4.2                | 1.1                | 0.01                   | 0.01                 | 0.01                 | 7.5    |
| <b>T0F3</b>    | 51.9             | 2.1              | 16.5               | 7.4                | 5.0                | 0.3                    | 0.1                  | 0.1                  | 16.7   |
| <b>T3F1</b>    | 59.4             | 1.2              | 5.1                | 7.5                | 5.0                | 0.1                    | 0.1                  | 0.1                  | 21.6   |
| <b>T3F2</b>    | 80.5             | 0.3              | 1.8                | 4.7                | 1.5                | 0.01                   | 0.01                 | 0.1                  | 11.1   |
| <b>T3F3</b>    | 55.8             | 1.8              | 18.6               | 3.0                | 3.8                | 0.1                    | 0.2                  | 0.01                 | 16.7   |
| <b>T6F1</b>    | 67.5             | 0.3              | 3.7                | 3.8                | 2.9                | 0.1                    | 0.1                  | 0.01                 | 21.6   |
| <b>T6F2</b>    | 86.6             | 0.1              | 1.2                | 2.5                | 1.2                | 0.01                   | 0.01                 | 0.01                 | 8.4    |
| <b>T6F3</b>    | 53.6             | 0.7              | 12.8               | 3.3                | 3.9                | 0.1                    | 0.1                  | 0.01                 | 25.5   |
| <b>PQF1</b>    | 51.8             | 1.3              | 6.2                | 7.3                | 2.5                | 0.1                    | 0.1                  | 0.1                  | 30.5   |
| <b>PQF2</b>    | 43.7             | 2.3              | 4.1                | 6.9                | 3.7                | 0.2                    | 0.0                  | 0.1                  | 39.0   |
| <b>PQF3</b>    | 46.5             | 4.9              | 15.8               | 3.9                | 3.0                | 0.1                    | 0.2                  | 0.1                  | 25.5   |



Table S4. Percentage of core per sample and per genus.

| OTU ID                            | Metzal        | Aguamiel      | T0            | T3            | T6            | Pulque        | % of total core per genus |
|-----------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------------------|
| <b>BACTERIAL</b>                  |               |               |               |               |               |               |                           |
| <i>Zymomonas</i>                  | 56.29%        | 51.27%        | 63.18%        | 65.24%        | 69.25%        | 47.35%        | <b>58.76%</b>             |
| <i>Lactococcus</i>                | 4.86%         | 22.98%        | 6.14%         | 5.05%         | 3.19%         | 6.03%         | <b>8.04%</b>              |
| <i>Weissella</i>                  | 23.56%        | 13.54%        | 1.12%         | 1.10%         | 0.35%         | 2.83%         | <b>7.08%</b>              |
| <i>Acetobacter</i>                | 0.54%         | 0.77%         | 7.97%         | 8.50%         | 5.91%         | 8.71%         | <b>5.40%</b>              |
| <i>Leuconostoc</i>                | 5.16%         | 5.83%         | 3.30%         | 3.42%         | 2.69%         | 3.08%         | <b>3.91%</b>              |
| <i>Hafnia-Obesumbacterium</i>     | 0.24%         | 1.67%         | 0.11%         | 0.09%         | 0.05%         | 0.14%         | <b>0.38%</b>              |
| <i>Gluconobacter</i>              | 0.05%         | 0.01%         | 0.07%         | 0.10%         | 0.05%         | 0.09%         | <b>0.06%</b>              |
| <i>Lactobacillus</i>              | 0.05%         | 0.03%         | 0.04%         | 0.05%         | 0.02%         | 0.10%         | <b>0.05%</b>              |
| <b>% of total core per sample</b> | <b>90.75%</b> | <b>96.10%</b> | <b>81.93%</b> | <b>83.55%</b> | <b>81.51%</b> | <b>68.33%</b> | <b>83.70%</b>             |
| Others                            | 9.24%         | 3.89%         | 18.06%        | 16.44%        | 18.50%        | 31.68%        | 16.30%                    |
| <b>FUNGI</b>                      |               |               |               |               |               |               |                           |
| <i>Saccharomyces</i>              | 8.96%         | 22.63%        | 97.30%        | 97.65%        | 98.63%        | 95.26%        | <b>70.07%</b>             |
| <i>Kluyveromyces</i>              | 47.67%        | 61.04%        | 0.02%         | 0.05%         | 0.02%         | 0.01%         | <b>18.13%</b>             |
| <i>Kazachstania</i>               | 40.76%        | 14.22%        | 2.52%         | 1.97%         | 1.23%         | 4.37%         | <b>10.84%</b>             |
| <i>Hanseniaspora</i>              | 0.72%         | 0.46%         | 0.09%         | 0.19%         | 0.07%         | 0.08%         | <b>0.27%</b>              |
| O_Saccharomycetales               | 1.01%         | 0.14%         | 0.06%         | 0.12%         | 0.04%         | 0.24%         | <b>0.27%</b>              |
| <b>% of total core per sample</b> | <b>99.12%</b> | <b>98.49%</b> | <b>99.99%</b> | <b>99.98%</b> | <b>99.99%</b> | <b>99.96%</b> | <b>99.58%</b>             |
| Others                            | 0.88%         | 1.52%         | 0.01%         | 0.03%         | 0.01%         | 0.05%         | 0.42%                     |

