# Free amino acid and organic acid profile of Serpa PDO cheeses from distinct dairy industries

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## Rationale


Serpa PDO cheese

- Protected Designation of Origin
- Raw ovine milk
- No starter cultures
- *Cynara cardunculus* L. as coagulant
- Minimum ripening period of 30 days
- Long-lasting cultural heriterage


High microbial biodiversity
- strong and exquisite flavor
- semi-soft and creamy texture

→ Although its unique organoleptic profile, several parameters might impact Serpa final attributes. For instance:

- Coupled with the safety risks of raw milk

+ Autochthonous starter culture → Promote a consistent quality, reducing variability and minimizing safety risks of final product

- Resulting in a high heterogeneity


Feeding systems


Climatic conditions


Milking conditions


Manufacturing methods

## Aims

Screening of flavor and texture related compounds in Serpa PDO cheese:
- Free Amino Acids (FAAs)
- Organic acids

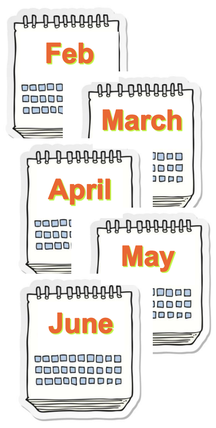
→ Towards the identification of chemical markers involved in the specificity of Serpa cheese → Guide the selection and development of an autochthonous starter culture

## Materials and methods

Cheeses from 4 PDO certified dairies

A B C D


core of 30-day old cheeses



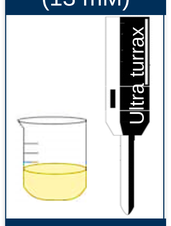
Screening during 5 consecutive months

Organic acids profile


Serpa PDO cheese

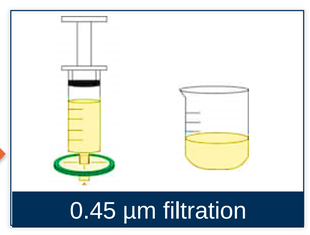
Free amino acids profile

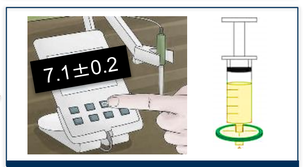
Sulfuric acid (13 mM)



Perchloric acid (0.6 M)


Centrifugation and filtration


0.45 µm filtration



pH adjustment, incubation and filtration

Sousa *et al*., 2015


HPLC analysis

Pripis-Nicolau *et al*., 2001

## Results and discussion

- Lactic acid is the most abundant organic acid (ranging from 645 to 2650 mg/100 g of cheese), followed by acetic acid.
- There are significant differences between the PDO certified dairies.
- The cheeses from dairy B possess a higher incidence of butyric and propionic acids as well as a lower content of lactic acid comparatively to the other producers in all months of production.

Table 1. Concentration of main organic acids (mg/100 g) in Serpa cheeses (n=4)

| Organic acid | February | | | | March | | | | April | | | | May | | | | June |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | C |
| Lactic | 1881.3±60.4$^{a}$ | 992.7±471.9$^{b}$ | 1668.1±724.2$^{a,b}$ | 1268.0±97.9$^{b}$ | 946.1±6.3$^{a}$ | 645.0±160.4$^{a}$ | 1700.0±348.8$^{b}$ | 1185.9±507.7$^{a,b}$ | 1977.7±250.4$^{a}$ | 807.8±375.0$^{b}$ | 2548.4±375.0$^{a}$ | 827.2±71.4$^{b}$ | 2164.3±442.7$^{a}$ | 832.4±102.4$^{b}$ | 1807.5±442.0$^{a,c}$ | 1475.1±182.2$^{c}$ | 2649.8±252.7 |
| Acetic | 170.7±8.7$^{a}$ | 212.8±30.0$^{a}$ | 198.7±50.4$^{a,b}$ | 120.9±12.5$^{b}$ | 318.6±19.3$^{a,b}$ | 359.4±82.3$^{b}$ | 238.3±41.0$^{a}$ | 265.1±29.9$^{a,b}$ | 268.4±58.9$^{a}$ | 161.1±30.9$^{b}$ | 335.3±45.0$^{a}$ | 134.5±45.7$^{b}$ | 226.5±63.5$^{a}$ | 178.16±65.1$^{a}$ | 171.6±28.1$^{a}$ | 184.5±78.5$^{a}$ | 294.2±40.3 |
| Propionic | 5.9±2.1$^{a}$ | 22.6±7.8$^{b}$ | 7.1±1.7$^{a}$ | 9.7±1.8$^{a}$ | 7.0±2.1$^{a}$ | 77.0±16.8$^{b}$ | 5.9±1.1$^{a}$ | 0.8±3.7$^{a}$ | 11.9±3.8$^{a}$ | 30.9±12.8$^{a}$ | 11.6±3.9$^{a}$ | 22.6±2.9$^{a}$ | 17.4±5.6$^{a}$ | 21.3±3.5$^{a}$ | 15.3±4.8$^{a}$ | 14.1±6.8$^{a}$ | 23.8±3.8 |
| Butyric | 4.4±4.4$^{a}$ | 22.0±6.3$^{b}$ | 2.7±3.0$^{a}$ | 21.0±2.2$^{b}$ | 5.4±1.3$^{a}$ | 41.8±29.4$^{b}$ | B.D.T. | 19.9±10.5$^{a,b}$ | 3.9±1.0$^{a}$ | 31.0±6.4$^{b}$ | 3.5±1.1$^{a}$ | 18.0±7.3$^{c}$ | 3.1±0.4$^{a}$ | 14.1±4.1$^{b}$ | 4.3±2.2$^{a,b}$ | 8.3±2.8$^{b}$ | 2.8±0.8 |

Means in the same line and corresponding to the same month of production with different superscript letters differ significantly ($p<0.05$).
*B.D.L. Below the detection limit.

Table 2. Concentration of main FAAs (mg/100 g) in Serpa cheeses (n=4)

| FAA | February | | | | March | | | | April | | | | May | | | | June |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | C |
| Glu | 64.4±9.5$^{a}$ | 43.9±10.7$^{a}$ | 47.8±27.1$^{a}$ | 51.3±6.6$^{a}$ | 54.1±10.9$^{a}$ | 22.7±6.4$^{b}$ | 53.8±6.4$^{a}$ | 55.7±12.4$^{a}$ | 79.2±6.4$^{a}$ | 42.8±9.4$^{b}$ | 92.0±10.9$^{a}$ | 88.1±10.4$^{a}$ | 97.5±16.5$^{a}$ | 47.2±9.9$^{b}$ | 66.0±1.5$^{c}$ | 109.8±18.5$^{a}$ | 52.5±3.3 |
| Ala | 13.1±1.1$^{a}$ | 14.2±8.4$^{a}$ | 14.4±8.5$^{a}$ | 20.9±3.3$^{a}$ | 21.8±1.8$^{a}$ | 14.3±4.1$^{b}$ | 13.9±2.6$^{b}$ | 14.8±2.2$^{b}$ | 18.8±1.4$^{a}$ | 17.7±5.8$^{a}$ | 31.6±2.8$^{b}$ | 36.8±5.6$^{b}$ | 24.4±5.7a | 16.1±2.2a | 18.7±0.7a | 32.3±8.5a | 13.1±0.9 |
| Val | 26.4±5.3$^{a}$ | 34.8±13.3$^{a}$ | 46.3±25.6$^{a}$ | 46.6±9.9$^{a}$ | 42.9±6.8$^{a}$ | 65.0±23.5$^{a}$ | 55.6±8.9$^{a}$ | 82.4±19.2$^{b}$ | 38.6±3.4$^{a}$ | 61.5±9.1$^{a}$ | 105.4±15.2$^{b}$ | 88.9±18.7$^{b}$ | 50.7±6.7$^{a,b}$ | 39.2±4.0$^{a}$ | 57.9±2.7$^{b}$ | 88.9±11.9$^{c}$ | 46.9±1.2 |
| Phe | 14.3±0.9$^{a}$ | 21.8±5.0$^{a,b}$ | 31.0±17.1$^{b}$ | 27.1±7.5$^{a,b}$ | 22.3±5.1$^{a}$ | 22.9±5.9$^{a}$ | 25.9±6.3$^{a}$ | 22.8±3.6$^{a}$ | 26.9±2.2$^{a}$ | 38.6±9.4$^{a}$ | 74.3±7.4$^{b}$ | 58.8±12.9$^{b}$ | 45.5±7.8$^{a}$ | 27.7±6.0$^{b}$ | 58.3±5.9$^{a}$ | 57.7±11.5$^{a}$ | 29.6±1.3 |
| Leu | 41.3±1.8$^{a}$ | 54.8±13.6$^{a,b}$ | 72.6±36.1$^{b}$ | 71.1±13.6$^{b}$ | 56.0±7.6$^{a}$ | 67.1±5.9$^{a,b}$ | 83.5±17.8$^{b}$ | 81.0±9.5$^{b}$ | 65.2±7.6$^{a}$ | 111.9±24.5$^{b}$ | 128.6±12.4$^{b}$ | 107.6±24.1$^{b}$ | 90.6±11.5$^{a}$ | 93.6±10.4$^{a}$ | 108.7±3.2$^{a}$ | 144.5±17.7$^{b}$ | 89.9±2.5 |

Means in the same line and corresponding to the same month of production with different superscript letters differ significantly ($p<0.05$).

- Glu, Ala, Leu, Val and Phe were the most prevalent FAAs with concentrations ranging between 13 and 145 mg/100 g of cheese, although almost all FAAs are present in all analyzed cheeses.
- There are quantitative variations according to the PDO producer and month of production.

## Conclusions

- The results suggest a high chemical diversity and variation according to the PDO dairy and month of production as well as a higher incidence of a group of FAAs (Glu, Ala, Leu, Val and Phe) and lactic and acetic acids in all samples analyzed, which may indicate that this chemical group may have an important role in Serpa specificity.
- This screening over different times of production with distinct dairies, coupled with the monitorization throughout the ripening process will be important to understand if this and other groups of compounds are involved in the authenticity of Serpa cheese.
- The identification of sensorial markers will be crucial to guide the selection and development of an autochthonous starter culture to improve Serpa's quality and safety.

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