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Serpa PDO cheese: towards identification of chemical markers involved in organoleptic attributes

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ABSTRACT
Serpa is a PDO cheese manufactured using raw ovine milk and extracts of *C. cardunculus* L. as rennet, without addition of starter cultures and followed by a minimum (but safe) ripening period. Both the processing technology and ingredients used result in a high microbial biodiversity that allows the development of a unique flavor. Variations in the manufacture process and distinct milk composition among producers result in a considerably heterogeneous cheese. The present study aimed at screening some groups of sensorial related compounds during two consecutive months of production, towards the identification of chemical markers involved in the specificity of Serpa cheese. The results suggested a high diversity and heterogenous chemical composition according to the producer and month. The free amino acids (FAAs) profile suggested the presence of almost all amino acids in the analyzed cheeses, being glutamic acid, alanine, leucine, valine and phenylalanine the most prevalent ones. Regarding the organic acid profile, lactic and acetic acids were the dominant groups. The volatile analysis suggested a high diversity and variability of volatile composition between cheeses, including several chemical groups, namely, ethyl esters, aldehydes and alcohols. The identification of sensorial chemical markers will be crucial to guide the selection and development of an autochthonous starter culture to improve Serpa's quality and safety.

1. INTRODUCTION
Serpa is a ripened cheese manufactured in a demarcated area in the South Alentejo region and considered one of the most famous Protected Designation of Origin (PDO) Portuguese cheeses. During the cheesemaking process, raw ovine milk and the *Cynara cardunculus* L. vegetable rennet are used, without addition of starter cultures and followed by a minimum (but safe) ripening period. Both the processing technology and ingredients used result in a cheese characterized by its high microbial biodiversity that allows the development of a characteristic strong and exquisite flavor coupled with a semi-soft and creamy texture [1].
Variations in the manufacturing process and distinct milk composition among producers influence the sensorial profile of raw milk cheeses, which may result in a final product considerably heterogeneous and, in some cases, with organoleptic defects [2]. The implementation of an autochthonous starter culture constitutes a possible way to improve the

cheesemaking process of PDO cheeses, minimizing their sensorial defects as well as the safety risks associated to the presence of some microbial groups in raw milk [2,3].

The screening of organoleptic related compounds is crucial for the identification of chemical markers involved in the specific properties of traditional cheeses and will guide the selection and development of an autochthonous starter culture [1]. Accordingly, this work aimed at assessing the volatile and a group of non-volatile compounds (FAAs and organic acids) present in Serpa cheese, from four distinct PDO producers in two consecutive months.

2. MATERIALS AND METHODS

2.1 Cheese samples

Serpa cheeses were obtained from four PDO certified industries (A, B, C and D), being manufactured according to the specifications described by Dos Santos *et al*. [4]. Cheeses were analyzed in duplicate for each producer from the core of ripened cheeses (30 days-old) in two consecutive months of production (February and March).

2.2 Free Amino acid profile

In order to separate, identify and quantify the FAAs, high performance liquid chromatography (HPLC) was used. The FAAs were extracted independently and in duplicate. Sample preparation was carried out using the Pico-Tag™ method [5]. In the HPLC analysis, the method and conditions described by Pripis-Nicolau *et al*. were followed [6].

2.3 Organic acid profile

The organic acids present in cheese samples were also separated and quantified by HPLC, where duplicate samples were extracted and analyzed independently. The sample preparation method and HPLC conditions for organic acid analysis described by Sousa *et al*. were followed, with slight modifications (temperature was maintained at 40 ºC) [7].

2.4 Volatile compounds screening

In order to evaluate the volatile content, headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS) was used. For sample preparation, 5 g of each cheese sample were macerated and homogenized. During pre-incubation, HS-SPME and GC-MS, conditions described by Oliveira *et al*. were followed [8].

3. RESULTS AND DISCUSSION

During the ripening period, several reactions occur in cheese resulting in a unique organoleptic profile. The screening of sensorial related compounds across different times of production such as, FAAs, organic acids and volatile compounds is important to identify chemical markers involved in Serpa cheese uniqueness. The FAAs composition of cheeses with 30 days during two consecutive months of production is shown in Table 1, suggesting the presence of almost all amino acids in the analyzed cheeses as well as a quantitative variation according to the PDO producer and month of production. However, Glu, Ala, Leu, Val and Phe were the most prevalent FAAs in all samples, with concentrations ranging between 13.10 and 83.48 mg/100g. The preliminary results may indicate that this group of FAAs is the more prevalent in Serpa cheese with 30 days of ripening and probably may have an important role in their specificity.

Table 1. Concentration of FAAs (mg/100g) in Serpa cheeses (n=4) with at least 30 days of ripening, from four PDO certified producers (A, B, C and D) in two consecutive months of production.

FAA	February				March			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Asp	8.64±1.41^{a}	5.92±1.29^{a}	8.64±4.82^{a}	7.82±3.14^{a}	5.35±2.52^{a}	7.65±2.43^{a}	11.18±3.02^{b}	9.32±2.88^{a}
Glu	64.43±9.49^{a}	43.87±10.74^{a}	47.82±27.13^{a}	51.30±6.56^{a}	54.07±10.98^{a}	22.65±6.39^{b}	53.78±6.84^{a}	55.67±12.40^{a}
Cys	0.10±0.10^{a}	0.05±0.01^{a}	0.046±0.04^{a}	0.05±0.04^{a}	0.93±1.42^{a}	0.18±0.11^{a}	0.14±0.11^{a}	0.19±0.13^{a}
Asn	0.61±0.2^{a}	0.36±0.20^{a}	0.76±0.53^{a}	0.32±0.04^{a}	4.51±4.75^{a}	0.55±0.13^{a}	0.31±0.07^{a}	0.41±0.41^{a}
Ser	0.47±0.09^{a}	1.02±0.58^{a}	2.16±1.37^{a}	1.81±1.21^{a}	5.83±6.40^{a}	0.60±0.20^{a}	1.50±0.24^{a}	0.48±0.16^{a}
His	0.35±0.49^{a}	1.71±0.35^{a}	4.85±4.75^{a}	3.78±3.64^{a}	4.70±4.58^{a}	4.23±1.53^{a}	4.54±0.80^{a}	7.77±1.07^{a}
Gln	1.55±0.26^{a}	1.26±0.36^{a}	1.74±1.06^{a}	2.21±1.08^{a}	1.82±1.30^{a}	2.06±0.67^{a}	3.80±0.43^{a}	3.44±1.89^{a}
Thr	4.51±0.56^{a}	4.05±1.08^{a}	7.08±4.15^{a}	4.38±1.37^{a}	5.57±0.80^{a}	2.56±0.56^{b}	12.23±2.22^{c}	4.34±1.50^{a,b}
Arg	3.23±0.48^{a}	6.42±4.93^{a}	9.92±8.45^{a}	10.49±9.25^{a}	6.23±8.76^{a}	1.78±0.82^{a}	1.16±0.38^{a}	3.25±1.66^{a}
Ala	13.10±1.07^{a}	14.19±8.38^{a}	14.44±8.54^{a}	20.88±3.33^{a}	21.76±1.84^{a}	14.28±4.05^{b}	13.85±2.57^{b}	14.84±2.16^{b}
Tyr	0.37±0.03^{a}	5.37±3.91^{a}	2.50±1.58^{a}	5.71±3.85^{a}	3.33±2.76^{a}	1.57±1.57^{a}	1.03±0.15^{a}	0.76±0.87^{a}
Val	26.38±5.3^{a}	34.83±13.25^{a}	46.29±25.64^{a}	46.55±9.89^{a}	42.88±6.81^{a}	65.00±23.50^{a}	55.63±8.93^{a}	82.44±19.24^{b}
Met	1.97±0.18^{a}	4.62±3.07^{a,b}	6.04±4.13^{a,b}	7.87±2.41^{b}	11.59±3.23^{a,d}	5.72±2.20^{a,c}	3.81±0.90^{c}	13.32±5.17^{d}
Trp	0.62±0.01^{a}	1.25±0.68^{a}	1.28±0.23^{a}	1.77±1.04^{a}	1.46±0.02^{a}	B.D.T.	B.D.T.	0.11±0.16^{a}
Phe	14.26±0.88^{a}	21.83±4.95^{a,b}	30.99±17.11^{b}	27.07±7.52^{a,b}	22.33±5.05^{a}	22.91±5.85^{a}	25.86±6.27^{a}	22.81±3.59^{a}
Ile	2.53±0.22^{a}	9.31±6.03^{a,b}	12.38±10.14^{a,b}	16.02±4.66^{b}	10.21±0.89^{a,b}	9.57±5.59^{a,b}	6.60±1.20^{a}	15.24±2.56^{b}
Leu	41.18±1.81^{a}	54.78±13.58^{a,b}	72.63±36.10^{b}	71.13±13.57^{b}	56.01±7.59^{a}	67.11±5.88^{a,b}	83.48±17.80^{b}	80.96±9.49^{b}

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

Regarding the organic acids profile, the results suggested significant differences according to the producer, with lactic acid as the most abundant, being followed by acetic acid (Table 2). The producers B and D also possess a high incidence of butyric and propionic acids comparatively to the other producers in both months. The presence of propionic and butyric acids is related with the intensity of bacterial fermentation during maturation period, typically increasing throughout this period. Acetic acid typically has higher concentrations in longer ripened cheeses. Although lactic acid is the most abundant in similar types of cheeses and maturation times [9] in producer B the content is much lower in both months, that showed the highest concentrations of propionic and butyric acids.

Table 2. Concentration of organic acids (mg/100g) in Serpa cheeses (n=4) with at least thirty days of ripening, from four PDO certified producers (A, B, C and D) in two consecutive months of production.

		Organic acids			
		Latic acid	Acetic acid	Propionic acid	Butyric acid
February	A	1881.26±60.35^{a}	170.66±8.71^{a}	5.87±2.05^{a}	4.37±4.38^{a}
	B	992.72±471.86^{b}	212.76±30.04^{a}	22.58±7.81^{b}	21.95±6.28^{b}
	C	1668.12±724.20^{a,b}	198.72±50.40^{a,b}	7.09±1.73^{a}	2.65±2.97^{a}
	D	1268.02±97.92^{b}	120.92±12.50^{b}	9.74±1.81^{a}	21.03±2.19^{b}
March	A	946.09±6.25^{a}	318.62±19.26^{a,b}	6.96±2.05^{a}	5.40±1.26^{a}
	B	645.03±160.41^{a}	359.44±82.31^{b}	77.01±16.83^{b}	41.82±29.40^{b}
	C	1700±348.81^{b}	238.27±41.03^{a}	5.87±1.07^{a}	B.D.T.
	D	1185.88±507.73^{a,b}	265.05±29.86^{a,b}	10.76±3.74^{a}	19.93±10.52^{a,b}

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

The target cheeses owned a great variability in volatile composition between producers and months of production. Despite of the diversity reported, the results suggested the presence of several chemical groups such as, aldehydes, aromatic compounds and ketones (data not shown). All samples analyzed possessed high levels of ethyl esters (e.g. butanoic, caprylic and caproic

acids), short-chain acids (e.g. acetic and butyric acid) and alcohols (e.g. 1-propanol and 1-octanol). The high variability and incidence of these groups was also found in Serra da Estrela cheese (also manufactured with raw ewe's milk and coagulated with *C. cardunculus* L.) [10].

4. CONCLUSIONS

Physicochemical and microbiological variations in milk composition as well as the differences in the manufacturing practices greatly influence the final sensorial characteristics of cheese. Accordingly, the screening over time of key compounds involved in the organoleptic attributes have been performed in order to evaluate Serpa PDO cheeses from distinct producers. The preliminary results included two consecutive months of production (February and March) but suggested a high chemical diversity and variation according to the industry and month of production. A higher incidence of some compounds in all cheeses was found: a group of FAAs (Glu, Ala, Leu, Val and Phe), lactic and acetic acids and, in the case of volatiles, short-chain fatty acids, alcohols and ethyl esters. It will be important to continue this screening over different times of production as well as throughout the ripening process to understand if this and other groups of compounds are involved in the specificity 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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