Parco Scientifico e Tecnologico della Sardegna

PortoConte Ricerche

# DIFFERENTIAL PEPTIDOMIC PROFILES BETWEEN FIORE SARDO CHEESE OBTAINED FROM RAW AND PASTEURIZED MILK

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Consorzio per la Tutela del Formaggio FIORE SARDO DOP

REGIONE AUTONOMA DELLA SARDEGNA

## 1. INTRODUCTION

Fiore Sardo is a traditional hard cheese exclusively produced in Sardinia (Italy) from raw whole ewe's milk. It is one of the oldest known Mediterranean cheeses, dating back to Bronze Age, and it was awarded the Protected Designation of Origin (PDO) status from the European Commission in 1996 (EC Regulation no.1263/96). The Consortium for the Protection of Fiore Sardo Cheese safeguards the original cheesemaking protocol, which contemplates the use of raw milk as one of its most essential features.

Aim of this study was to optimize a method to evaluate the peptide profile of ripened Fiore Sardo and to investigate possible differences between cheese made from raw milk (R) and from pasteurized milk (P).

## 2. MATERIALS & METHODS

CHEESEMAKING
original protocol of Fiore Sardo;
same batch of ewes' milk, same dairyman



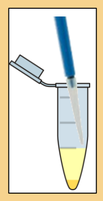
RIPENING
6 months

MINCING
FREEZE-DRYING

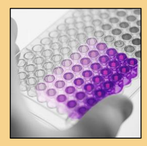
MECHANICAL HOMOGENIZATION
bead beating
$Na_2HPO_4$ 50 mM

CENTRIFUGATION
3,500 x *g*, 30 min, 4°C
SUPERNATANT COLLECTION
water soluble extract (WSE)

FILTRATION
Whatman paper No.2,
0.45 µm PVDF filters



CASEIN PRECIPITATION
HCl 0.1 N, pH 4.6

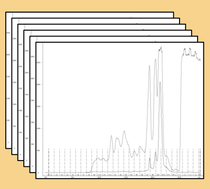


QUANTITATION
BCA protein assay

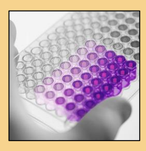
Cys
RED CAM

FILTRATION
Ultrafree MC
12,000 x *g*, 4 min

SIZE EXCLUSION CHROMATOGRAPHY
FPLC system, AKTA explorer
Superdex Peptide 10/300 GL
30% ACN, 0.1% TFA



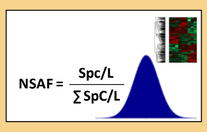
FRACTION COLLECTION
DRYING OUT
resuspension in HCOOH 0.2%



QUANTITATION
BCA protein assay

Q EXACTIVE LC-MS/MS
UltiMate 3000 RSLCnano LC system
485 min gradient
2 run replicates, 4 µg

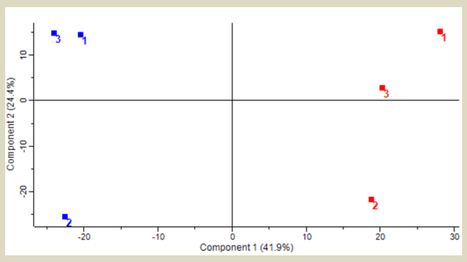

PEPTIDE IDENTIFICATION
search engine: Sequest-HT
peptide validation: Percolator
FDR < 1 % based on peptide q-value
database: UniProtKB
(*O. aries*, *B. taurus*, *C. hircus*, Dec 2014)
id parameters: no enzyme, 4-50 amino acids length



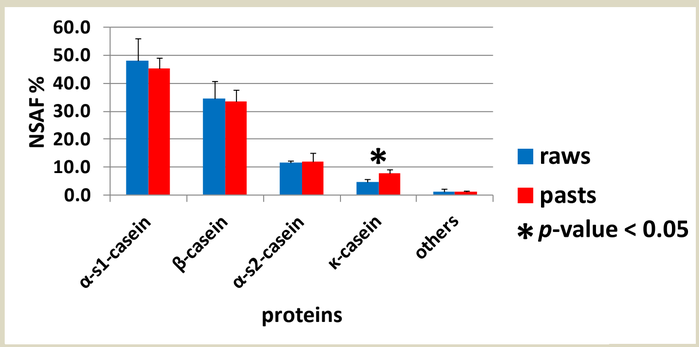

DATA ANALYSIS
label free quantification
*via* normalized spectral abundance factor (NSAF)[1]

## 3. RESULTS

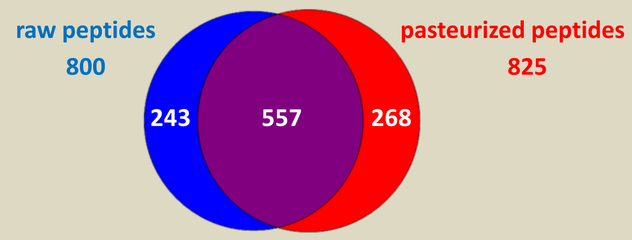
**3.1 Principal component analysis (PCA)** performed using **NSAF** values highlights a clear separation between **R** and **P** samples.



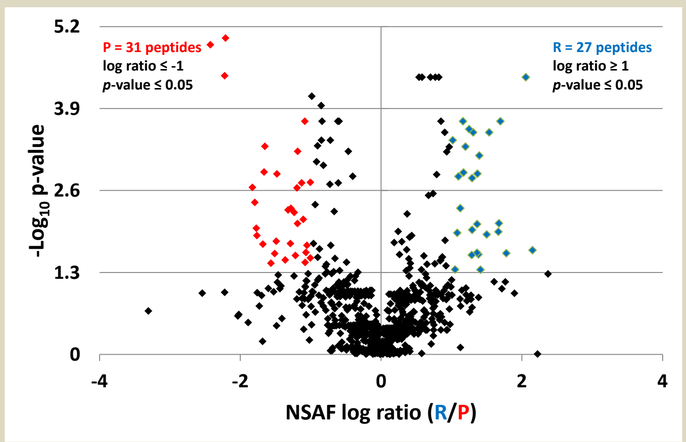

**3.2** All peptides were mainly derived from the hydrolysis of **α-s1-casein** and **β-casein**, followed by **α-s2-casein** and **κ-casein**. **P** samples showed a statistically significant increase of peptides derived from **κ-casein** (t-test *p*-value < 0.05).




**3.3** A total of **1068 peptides** from the two groups were identified.

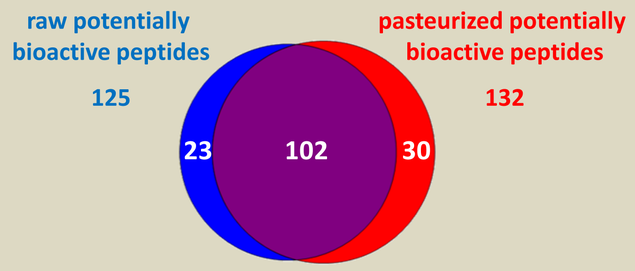



**3.4** A total of **1068 peptides** from the two groups were identified. **58 peptides** displayed a significant differential abundance between the two cheeses, with **27 higher** in **R** samples and **31 higher** in **P** samples. T-test was performed on logarithmized NSAF.

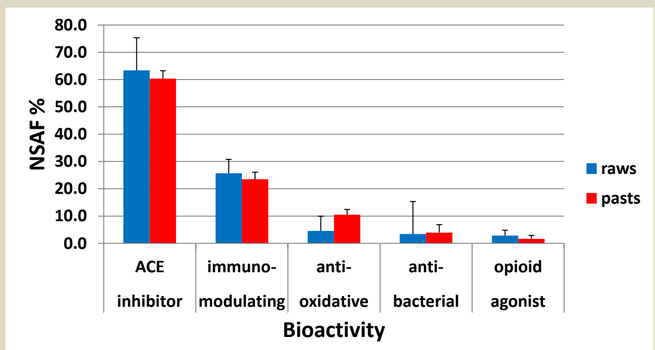



**3.5** Bioactivity assignment was performed by sequence analysis against the **BIOPEP database**[2].

Peptides with identical sequence, or containing sequence with biological activities, were considered potentially bioactive (**156 total peptides**, **125** in **R** and **132** in **P** cheeses).




**3.6** Bioactivity was mainly represented by **ACE-inhibitor** peptides, followed by **immunomodulating**, **antioxidative**, **antibacterial** and **opioid agonist** activities. According to NSAF values, no significant increase (t-test, *p*-value ≤ 0.05) was observed in **R** cheese.




## 4. CONCLUSIONS

An extensive peptidomic characterization of Fiore Sardo cheese was achieved. Qualitative and quantitative differences in peptide profiles were observed between raw and pasteurized samples. The presence of bioactive peptides was also revealed in both cases, but their abundance showed only a mild increase in raw cheese .

The experimental protocol enabled to distinguish cheese produced with raw or pasteurized milk based on peptide profiles. Furthermore, potentially bioactive peptides were characterized. This provides important opportunities for valorization of Fiore Sardo cheese.

## 5. FUTURE PERSPECTIVES

**proteolytic enzyme prediction**

**ACE-inhibition and antibacterial assays**

## 6. REFERENCES


[1]Zybailov B, Mosley AL, Sardiu ME, Coleman MK, Florens L, Washburn MP (2006). *Journal of Proteome Research*, **5**(9): 2339–2347.

[2]Minkiewicz PD, Jerzy D, Iwaniak A, Dziuba M, Darewicz M. (2008). *Journal of AOAC International*, **91**(4): 965-980.