Parco Scientifico e Tecnologico della Sardegna

PortoConte Ricerche



PEPTIDOMICS OF YOUR SARDINIAN SNACK: ENRICHMENT AND CHARACTERIZATION OF PEPTIDES IN ARTISANAL CHEESE AND CRAFT BEER



The food peptidome describes the complete set of peptides generated in food from raw components during processing, ripening, and storage. Food peptidomics is a field of food proteomics aimed to investigate composition, interactions and properties of the peptides generated in a food matrix¹⁻². Adding to its interest for product characterization, food peptidomics can have reflections on human health, since many peptides possess bioactive properties impacting body functions or conditions, including antihypertensive, immunostimulating, antimicrobial, and opioid activities^{1,3}.

This study investigated the peptide profile of two products of Sardinia, the typical PDO cheese Fiore Sardo and the craft beer Zàgara from the Barley brewery, by combining FPLC and MS approaches.



Fiore Sardo is a traditional hard cheese 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 awarded Protected Designation of Origin status from the European Commission in 1996 (EC Regulation no.1263/96). The use of raw milk is one of its most relevant features. The original cheesemaking protocol is safeguarded by the **Consortium for the Protection of Fiore Sardo Cheese**. Currently, the consortium suspects that some cheese manufacturer are producing this cheese also using heated/pasteurized milk.

Here, we aimed to investigate the final peptide profile of ripened Fiore Sardo to evaluate possible distinctive traits between cheeses made from raw milk (R) and from heat-treated milk (H).





1. Methods

A total of **10 commercial Sardinian sheep milk cheese** samples were analyzed. Five were produced by the Consortium from raw whole sheep milk (**R**), while five, suspected to be produced using heated sheep milk (**H**), were bought at local markets. **All ten samples at 6 months of ripening** were subjected to peptide extraction, enrichment and analysis by mass spectrometry.



2.2 Principal component analysis (PCA) carried out using the NSAF values highlights the **within-group similarity of the two cheeses**. This result can be explained with the possibility that the reproducible use of raw milk for **R** cheeses leads to the generation of more similar peptide profiles during ripening. In addition, the more sparse clustering pattern shows a higher heterogeneity of **H**, that might be correlated with different heat-treatment processes applied to milk.



2.3 A total of **203 peptides displayed a significant differential abundance between the two cheeses**, with **124** higher in **R** cheeses (blue) and **79** higher in **H** cheeses (red).



2.4 Sequence analysis against the Biopep database assigned a bioactive potential to 138 peptides (both cheese preparations) of which 27 and 14 unique to **R** and **H** cheeses, respectively.



2.5 These bioactivities were mainly represented by immunomodulating peptides, followed by antibacterial, ACE-inhibitor, and antioxidative activities. According to NSAF values, a significant increase (T-test, p≤0.05) of antibacterial, antioxidative, binding, hemolytic and opioid peptides was observed in R cheese.







Zàgara is a craft Amber Ale produced in Sardinia by the Barley Brewery. In its recipe, organic honey from orange flowers (Zàgara) is used with barley and hops for brewing. Zàgara is described as follows: *"It presents itself orange in colour with a creamy head, tight and persistent. The nose experiences an explosion of orange and spices, whilst the palate enjoys the malt, orange and a finale of dry hops, which renders this beer both extremely quaffable and thirst quenching"*.

800

700

500

400

300

200

100

factor

undance

ab

spectral

malized

Our goal was to characterize the peptide profile of this craft beer in order to evaluate how the protein components originating from the brewing ingredients evolve along its fermentation and maturation.



2. Results

Peptidomic analysis led to the identification of 653 unique peptides, belonging to 220 proteins from barley (405 peptides from 136 proteins, 62%), yeast (245 peptides from 81 proteins, 37%) and honey (3 peptides from 3 proteins, 1%). No proteins or peptides from hops were identified.

Conclusion

The sample preparation and analysis methods applied to artisanal cheese and craft beer enabled to reach their profound peptidomic characterization. This study highlighted the ability of food peptidomics to describe and evaluate the peptide profile generated during a standard production process, as well as to highlight the possible changes that may be introduced in by variations in the production protocol or recipe. Fiore Sardo produced by the Consortium from raw whole sheep milk (R) showed a characteristic peptide profile that highlighted several differences with Fiore Sardo suspected to be produced using heated sheep milk (H). These differences are related to amino acid sequence, abundance and bioactive potential of peptides. In particular, R cheeses showed a higher abundance of several peptides and a higher bioactive peptide potential, especially concerning antibacterial, antioxidative, binding, hemolytic and opioid activities. Application of the peptidomic approach to a craft beer enabled to reach its profound characterization. Interesting data were obtained concerning the protein composition of the final product, and on the protein regions deriving from raw materials that can still be found in the final, ripened product. Insights on antimicrobial properties of several peptides were also obtained.



These results indicate that the peptidomic analysis of foods can offer useful hints for identifying how production process variables impact on the final product.

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