Deliverable title	**D5.6 Report with statistical elaboration of data overall collected in WP5 and identified biomarkers for demonstration of quality/authenticity of Mediterranean thistle-curdled cheese**
Deliverable Lead:	CREA-AN
Related Work Package:	**WP5 Cheese-making trials and characterization of thistle-curdled and control cheeses**
Related Task:	Task 5.6 Statistical elaboration of data
Author(s)	Antonio Raffo
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Abstract	Data collected onto the first- and second-round experimental and control cheeses are statistically elaborated using the most appropriate tools: Analysis of variance (ANOVA); Principal Component Analysis (PCA); Partial Least Squares regression (PLS); Unweighted pair group method with arithmetic mean (UPGMA); cluster analysis. The impact of the coagulants onto the cheese traits is evaluated. Biomarkers for demonstration of quality and/or authenticity of Mediterranean thistle-curdled cheeses are searched.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v 1.0	25/05/2022	Pamela Manzi	First version
v 2.0	01/06/2022	Antonio Raffo	Comments after peer reviewing process
v 3.0	21/02/2023	Antonio Raffo	Comments after peer reviewing process
v 4.0	27/04/2023	Antonio Raffo	Final version

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Acronyms

CF: Caciofiore
F: Feta
QMV: Queso de Murcia al Vino
TDC: Torta del Casar
CH: Cynara humilis
OT: Onopordum tauricum
OP: Onopordum platylepis
St: spontaneous flowers
Ct: cultivated flowers
AR: Animal rennet
VR: Vegetable rennet, commercial

1. Caciofiore cheese

The results of the analyses on the Caciofiore cheese samples are reported in deliverables 5.2 (Round 1) and 5.4 (Round 2). At each Round, cheesemaking trials were repeated on two different days with two different batches of milk (B1 & B2). The data overall collected were elaborated in order to investigate the effect of the coagulant (control: commercial vegetable coagulant extracted from *Cynara cardunculus* and experimental: extract from spontaneous *Onopordum tauricum* flowers in Round 1 and extract from cultivated *Onopordum tauricum* flowers in Round 2).

1.1. Statistical Elaboration of data

Several statistical approaches have been adopted for the elaboration of the results:

- One-way ANOVA and Tukey – Kramer's Honest Significant Diffenrece (HSD) to investigate differences among samples;
- Two-way ANOVA to investigate the effect of the Batch, Rennet, and Batch x Rennet factors;
- Principal Component Analysis (PCA)
- Kruskall wallis test on alpha and beta diversity indices.

The results of the statistical analyses have been presented in D5.2 and 5.4 with the data collected from the analyses performed on the cheese samples.

1.2. Main outcomes

- Commercial vegetable rennet from *Cynara cardunculus* (C) *vs Onopordum tauricum* (OT)

Statistically significant differences have been found between control and experimental cheeses in both rounds of cheesemaking, mainly ascribable to the Batch factor. The experimental rennet (extracts from spontaneous and cultivated flowers of *Onopordum tauricum*) was to found to consistently affect the Soluble Nitrogen -Total Nitrogen ratio (SN/TN) and Total Titratable Acidity (TTA) in both R1- and R2-cheese samples. In detail, the experimental cheeses showed lower soluble nitrogen values than the control cheeses. The extract from *O. tauricum* of wild flowers had a greater proteolytic effect on alpha and beta casein compared to the extract obtained from cultivated flowers. On the other hand, the extract of *O. tauricum* from cultivated flowers hydrolysed gamma casein more strongly than its wild counterpart, being its effect on this fraction even greater than that of the control coagulant. Commercial rennet, from *Cynara cardunculus*, showed more proteolysis effect on beta casein compared to the two extracts of *O. tauricum* from both wild and cultivated flowers. No significant differences were found for the ratio between hydrophobic and hydrophilic peptides (Ho/Hi) between control and experimental cheeses in both rounds.
Concerning the study of the microbial dynamics during maturation, very few differences were found in the viable counts between control and experimental cheeses in both production rounds, suggesting that the type of rennet did not affect neither the development of bacteria nor fungi. This outcome was also confirmed by the results from the metataxonomic analysis. No significant differences were found in the species composition (bacterial and fungal biota) of 60 days-ripened control and experimental cheeses.
The texture of R1 cheeses was found to not be affected by the rennet type, except for adhesiveness; by contrast, in R2 cheeses all texture parameters were significantly affected by the rennet type. ANOVA analysis also showed a significant effect of the type pf rennet on all colour attributes. The PCA applied on VOCs data suggested a clear differentiation between cheese samples produced in the two separate rounds (in this figure also cheese samples obtained from the second round, with cultivated thistle extract, to give a more comprehensive picture of the influence of the rennet type). In the first round a marked effect of the cheesemaking replicate (batch) on the volatile profile was observed, while in the second round this factor had a limited impact on volatile formation. The rennet type did not affect the total phenolic, cholesterol, vitamin E, and Vitamin A contents, as well as the minerals under investigation. The peptides extracted from experimental cheeses in Round 2 showed a greater radical scavenging activity (RSA).

- Commercial vegetable rennet from Cynara cardunculus (C) *vs Onopordum platylepis* (OP)

Significant differences were found for the values of a_w, salt content, Non Proteic Nitrogen (NPN) and Water Soluble Nitrogen (WSN) among cheeses manufactured with C, OP_st and OP_ct.

Concerning the microbiological analysis, the type of rennet was found to strongly affect ($p < 0.001$) the viable counts of presumptive lactococci, presumptive termophilic lactococci, Enterobacteriaceae, coliforms, and *Escherichia coli*. On the same groups, even an effect of the batch and batch x rennet factors was revealed by two-way ANOVA.

2. Feta cheese

The results of the analyses conducted on Feta cheese samples are shown in D5.2 and 5.4.

2.1. Statistical Elaboration of data

- One-way ANOVA and Tukey – Kramer's Honest Significant Difference (HSD) to investigate differences among samples;
- Two-way ANOVA to investigate the effect of the Batch, Rennet, and Batch x Rennet factors;
- Kruskall wallis test on alpha and beta diversity indices.

2.2. Main outcomes

In both rounds, significant differences were revealed for free amino acids content by one-way ANOVA between control and experimental cheeses (see D5.2 and D5.4). No significant differences were found on the casein (CN) fractions between control and experimental on both rounds. Round 2 experimental cheeses (F_CHct) showed a higher Ho/Hi ratio compared to control cheeses manufactured with animal and commercial vegetable rennet. The texture of thistle-curdled cheeses appeared to have an increased hardness, fracturability, springiness and chewiness. Moreover, the rennet type was found to affect the formation of most Feta VOCs (24 compounds). By contrast, as for colour parameters, only h° was found to be affected by the rennet type, while no significant differences were found for L*, a*, b*, and C* parameters. In both rounds, control feta cheese manufactured with animal rennet showed higher values for total phenolic content. No significant differences were found for vitamin A, vitamin E and cholesterol among the samples. In Round 1 feta samples, higher levels of Ca, P, Mg and Zn were found in experimental cheeses compared to control cheeses. Significant differences were found in the antioxidant and antihypertensive activity of peptides extracted from control and experimental samples. From a microbiological point of view, no significant differences were found between control and experimental samples from all cheese-making trials.

3. Queso de Murcia al Vino cheese

Round 1 of cheesemaking trials were conducted by using extract from spontaneous *Onopordum platylepis* flowers, in comparison with the commercial vegetable rennet and animal rennet. Since the experimental extract was found to be not suitable for the manufacture of Queso de Murcia al Vino cheese (too long coagulation time), cheesemaking trials were repeated with extracts from spontaneous and cultivated *Cynara humilis* flowers. The results of the analyses are presented in D5.2 and D5.4.

3.1. Statistical Elaboration of data

- One-way ANOVA and Tukey – Kramer's Honest Significant Difference (HSD) to investigate differences among samples;
- Two-way ANOVA to investigate the effect of the Batch, Rennet, and Batch x Rennet factors;
- Principal Component Analysis (PCA);
- Kruskall wallis test on alpha and beta diversity indices.

3.2. Main outcomes

Considering the round of cheesemaking with extracts from spontaneous flowers, many differences were revealed by one-way ANOVA among the samples (see D5.2); by contrast, R2 cheeses were characterized by similar physico-chemical parameters. Many differences were shown in the free amino acids content as revealed by one-way ANOVA. The casein fractions of Round 1 cheeses showed differences in the β-CN and Residual α_s-CN among samples, whereas no differences were found in Round 2 samples. A great variability was observed among R1 samples for the Ho/Hi ratio as revealed by one-way anova, while R2 samples were characterized by similar results (no significant difference). Two-way anova applied to the results of the microbiological analysis revealed an effect ($p < 0.001$) of the rennet type on most of the viable counts of the microbial groups under investigation. Despite the batch factor was also found to affect most of the microbial groups, a greater effect of the rennet compared to the batch was observed in the viable counts of total mesophilic aerobes, presumptive lactobacilli, presumptive lactococci, and presumptive termophilic lactococci. Differences in the hardness, cohesiveness, springiness and adhesiveness were seen in the texture of R1 cheeses; no significant differences were observed among R2 samples. Results of 2-way ANOVA analysis highlighted a significant effect of the rennet type on most of quantified VOCs in Queso de Murcia cheeses; short-chain fatty acids and ethyl esters were found with lower level in cheeses manufactured with animal rennet. Round 1 cheeses showed significant differences for most of the attributes analyzed by sensory analysis; no significant differences were seen in the external attributes of R2 samples; as revealed by one-way anova, significant differences were observed in the color of the paste, the "red wine" odor, texture attributes, bitterness and persistency. According to the results of the *t test* analysis no differences ($P>0.05$) could be detected in cholesterol, vitamin E and vitamin A between control and experimental Queso de Murcia al Vino respectively manufactured with animal rennet and extract from spontaneous flowers of *Cynara humilis*, while Queso de Murcia made with crude aqueous extract of cultivated *Cynara humilis* showed the lowest contents of these two fat soluble vitamins. Concerning minerals, significant differences ($P<0.05$) were detected in Na, K and Mg: Control cheeses showed the highest contents for all these three elements compared to experimental cheeses manufactured with extract from spontaneous flowers of *C. humilis*. As for the comparison between control and experimental (from cultivated *C. humilis* flowers), significant differences ($P<0.05$) were detected in all elements except for calcium and sodium. Queso de Murcia al vino made with crude aqueous extract of cultivated *Cynara humilis* showed the highest contents for P and Zn but the lowest for K and Mg. Cheese from R1 resulted significantly different for the antioxidant activity of bioactive peptides, whereas no significant differences were found in the antihypertensive activity. Conversely, no significant differences were observed in the antioxidant activity of R2 cheeses, whereas the samples showed significant differences in the antihypertensive activity.

4. Torta del Casar cheese

The results of the analyses on the Torta del Casar cheese samples are reported in deliverables 5.2 (Round 1) and 5.4 (Round 2). At each Round, cheesemaking trials were repeated on two different days with two different batches of milk (B1 & B2). The data overall collected were elaborated in order to investigate the effect of the coagulant (control: commercial vegetable coagulant extracted from *Cynara cardunculus*, experimental: extract from spontaneous *Cynara humilis* flowers in Round 1 and extract from cultivated *Cynara humilis* flowers in Round 2). Cheeses manufactured by the company with the same batches of milk and extracts from wild flowers of *Cynara cardunculus* self-produced by the company were also subjected to the analyses.

4.1. Statistical Elaboration of data

- One-way ANOVA and Tukey – Kramer's Honest Significant Difference (HSD) to investigate differences among samples;
- Two-way ANOVA to investigate the effect of the Batch, Rennet, and Batch x Rennet factors;
- Principal Component Analysis (PCA);
- Kruskall wallis test on alpha and beta diversity indices.

4.2. Main outcomes

Significant differences in the chemical characterization were found among the cheese samples from both rounds of cheesemaking as revealed by one-way ANOVA. In detail, among R1 cheeses, control (commercial vegetable rennet) and experimental (extract from spontaneous *C. humilis* flowers) showed lower pH values compared to the cheeses manufactured with the extract from *Cynara cardunculus* commonly used by the company. Significant differences were also found in the salt, ash, and total fat content. Torta del Casar cheeses manufactured with the extract from *Cynara cardunculus* prepared by the company showed higher values of non proteic and water soluble nitrogen. As for R2 cheeses, significant differences were found in pH, moisture, total fat, total protein, non proteic and water soluble nitrogen results, as revealed by one-way anova. The type of rennet seemed to affect most of the free amino acids contents in R1 cheeses (ala, arg, asn, asp, gln, gly, his, ile, leu, met, phe, pro, ser, trn,trp, tyr); significant differences were found in ala, arg, asn, gly, ser and val contents in R2 cheeses. As for the casein hydrolysis profile, no significant differences were found among both R1 and R2 cheese samples, except for α-CN in R2 samples (lower in experimental cheeses). Concerning the peptide profile, significant differences were found in R1 samples; in detail, cheeses manufactured with the extract prepared by the company from *Cynara cardunculus* flowers showed higher hydrophilic peptides and a lower Ho/Hi ratio. No differences were found among R2 cheeses. In R1 samples, the rennet type was found to affect the viable counts of all the microbial groups under investigation , except for coliforms. Significant differences were also found in R2 samples, with the rennet type highly affecting the viable counts of presumptive termophilic cocci, presudomonadaceae, enterobacteriaceae, coliforms and molts, although an effect of the batch was also revealed for most of the aforementioned microbial groups. Significant differences were found in the texture parameter of both R1 and R2 cheeses, except for adhesiveness in R1 and cohesiveness in R2. A PCA analysis described clearly the effect of the rennet type on the volatile profile. Briefly, the extract from *Cynara cardunculus* prepared by the company promoted the formation of branched chain fatty acids, which are important odorants of Torta del Casar cheese. On the other hand, the use of the *C. humilis* based rennet was associated with an enhanced level of butanoic acid and related esters. Finally, the Dairen rennet cheese was associated with higher levels of some ethyl esters and 2-methylpropanoic acid. Significant differences were observed in most of the cheese attributes evaluated by sensory analysis in both R1 and R2 cheeses, as well as in the colour parameters instrumentally measured. According to the results of the ANOVA analysis no significant differences ($P>0.05$) could be detected in vitamin E, vitamin A, and cholesterol in both R1 and R2 cheese samples. Concerning the analysis of minerals, only K and Mg contents showed significant differences in R1 samples, and Mg content in R2 samples. In both rounds cheeses, significant differences were found in the antioxidant activity as revealed by one-way ANOVA, whereas similar results were observed for the ACE inhibitory activity.

5. Identification of biomarkers

Identification of biomarkers was based on the analysis of peptide sequences resulting from hydrolysis by the enzymes of the different thistle species under investigation.

In detail, casein hydrolysates were produced by preparing a 1% (w/v) solution of bovine casein in distilled water and adjusting the pH to 6.2 with NaOH. Citrate buffer and sodium azide were not used in the preparation of the hydrolysates to avoid interference in the subsequent peptide activity assays. The hydrolysis reaction was carried out in falcon tubes with a volume of 9 mL of substrate solution immersed in a water bath at 50°C with stirring. Once this temperature was reached inside the tube, 360 µL of the reconstituted lyophilised extract from thistle flowers were added at a concentration of 0.6 mg protein/mL. The reaction was stopped after 16 hours, and the pH was adjusted to 4.6 with HCl. Subsequently, the hydrolysate was centrifuged at 4000 g for 20 minutes, the supernatant was collected and filtered through 0.45 µm nylon filter using kitasate and vacuum pump. Finally, the filtered hydrolysate was pH adjusted to 7 with NaOH and distributed into falcon tubes for storage at -20°C until use.

The peptides present in the hydrolysates were sequenced by LC-MS/MS analysis. Moreover, to verify the bioactivity of the peptide sequences identified in the different casein hydrolysates, these sequences were searched in the BIOPEP database. As results, 2 types of biopeptides were detected: those in their bioactive form in the casein hydrolysates; and potential ones, which contain bioactive sequences within their primary structure.

Regarding the peptides found in their bioactive form, in the *C. cardunculus* casein hydrolysate we have identified 15 peptides (table 1), 4 of them (marked in red) being only detected in this hydrolysate, and the rest 11 ones also being identified in *C. humilis* or *O. platylepis* hydrolysates.

Regarding the *C. humilis* casein hydrolysate, we have identified 20 peptides (table 2), 9 of them (marked in red) being only detected in this hydrolysate, being the rest 11 ones also being also identified in *C. cardunculus* or *O. platylepis* hydrolysates. This hydrolysate from C. *humilis* has yielded the higher number of different bioactive sequences.

Regarding the *O. platylepis* casein hydrolysate, we have identified 5 peptides (table 3) that were also identified in the previously mentioned hydrolysates.

The quantification of potential bioactive peptides in bovine casein total hydrolysates was performed using C. *cardunculus*, C. *humilis* and O. *platylepis* water-soluble extract, considering their peptide spectral matches (PSM) in liquid chromatography–mass spectrometry and taking into account the presence of demonstrated bioactive sequences in their primary structure. The identified peptides and their respective quantification for each sample were extracted. The quantification values were normalized according to the total PSM of all peptides in the samples. In this way, quantification of the same peptide between samples was comparable.

The results of the peptides identified that have potential bioactivity because of containing bioactive sequences within their primary structures show that O. *platylepis* casein hydrolysates showed great potential as a source of this type of precursor (Figure 1). Exerting for example, potential antidiabetic, antifungal, inmunostimulating and immunomodulating, antithrombotic, and inhibitory insuline secretion activities. On the other hand,

Moreover, according to the heat map (figure 2), where the colour blue indicates a lower quantity of potential bioactive peptides, and the colour red indicates a higher presence of these precursors, it can be observed that *O. platylepis* casein hydrolysates yielded a greater amount of potential bioactive peptides such as stimulating, antiamnestic, antidiabetic, antithrombotic, opioid agonist, etc. than *C. cardunculus* or *C. humilis* ones. On the other hand, C. *cardunculus* showed a greater amount of potential bioactive peptides with osteoanabolic, anti-apoptotic, lipoxygenase inhibitor, anti-inflammatory, antioxidative, hypolipidemic, haemolytic, etc. than the rest of the species. Hydrolysates from C. *humilis* showed higher amounts of peptides with bioactivity more related to opioid antagonist, alpha-glucosidase inhibitor, angiotensin-converting enzyme (ACE) inhibitor, hypotensive, anticancer, antiviral, antibacterial, and dipeptidyl peptidase IV inhibitor.

Table 1. Peptide sequences identified in bovine casein hydrolysates from ***Cynara cardunculus*** proteases that were registered in the bioactive peptide database (BIOPEP).

Sequence	ID	Name	Chemical mass	IC50 (μM)	Activity
AYFYPELF	8377	ACE inhibitor from alphas1-CN (143-150)	1049.1715	–	ACE inhibitor
GVSKVKEAMAPKHKEMPFPKYPVEPFTESQ	9853	Peptide stimulating mucin secretion	3417.9414	–	stimulating
HQPHQPLPPTVMFPPQ	10186	Zinc binding peptide	1851.1311	–	binding
INNQFLPYPY	9231	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	1268.4124	40.08	dipeptidyl peptidase IV inhibitor
KTVYQHQKAMKPWIQPKTKVIPYVRYL	8255	f(181-207) of bovine alpha s2-casein	3345.0034	–	antibacterial
	8273	Cr1			
LLYQEPVLGPVRGPFPIIV	8174	peptide derived from bovine b-casein (1-28)	2107.5295	–	immunomodulating
RPKHPIKHQGLPQEVLNENLLRF	8171	Isracidin - peptide derived from alphaS1-casein (1-23)	2764.1833	–	immunomodulating
	3035			–	antibacterial
	9530			–	antiviral
SDIPNPIGSENSEK	8335	f(195-208) of alpha S1 casein	1486.5331	–	antibacterial
SQSKVLPVPQKAVPYPQ	9907	Antioxidative peptide	1866.1592	–	antioxidative
VEELKPTPEGDLEIL	10170	Zinc binding peptide	1681.8731	–	binding
VYQHQKAMKPWIQPKTKVIPY	3966	–	258.4082	–	haemolytic
VYQHQKAMKPWIQPKTKVIPYVRY	3965	–	3002.5708	–	haemolytic
	3033	CaMPDE inhibitor		–	CaMPDE inhibitor
	3435	f(183-206) of bovine alpha s2-casein		–	antibacterial

VYQHQKAMKPWIQPKTKVIPYVRYL	3034	CaMPDE inhibitor	3115.728	–	CaMPDE inhibitor
	5469	(fr.183-207 of bovine alpha s2-casein)		–	antibacterial
	3964	–		–	haemolytic
WMHQPHQPLPPTVM	10184	Zinc binding peptide	1699.0052	–	binding
YQKFPQY	9254	ACE inhibitor	973.079	20.08	ACE inhibitor

Table 2. Peptide sequences identified in bovine casein hydrolysates from ***Cynara humilis*** proteases that were registered in the bioactive peptide database (BIOPEP).

Sequence	**ID**	**Name**	**Chemical mass**	**IC50 (μM)**	**Activity**
ALPQYLKTVYQHQK	9904	Antioxidative peptide	1716.9716	–	antioxidative
AMKPWIQPKTKVIPYVRYL	3030	CaMPDE inhibitor	2331.8558	–	CaMPDE inhibitor
AYFYPELF	8377	ACE inhibitor from alphas1-CN (143-150)	1049.1715	–	ACE inhibitor
FSDKIAK	8266	Antibacterial peptide	807.9315	–	antibacterial
	8181	ACE inhibitor		113.6	ACE inhibitor
GVSKVKEAMAPKHKEMPFPKYPVEPFTESQ	9853	Peptide stimulating mucin secretion	3417.9414	–	stimulating
HIQKEDVPSER	9559	antioxidative	1337.4346	–	antioxidative
HKEMPFPKYPVEPF	10188	Zinc binding peptide	1746.0322	–	binding
HKEMPFPKYPVEPFTESQ	9896	Antioxidative peptide	2191.4555	–	antioxidative
INNQFLPYPY	9231	dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	1268.4124	40.08	dipeptidyl peptidase IV inhibitor

KTVYQHQKAMKPWIQPKTKVIPYVRYL	8273	Cr1	3345.0034	–	antibacterial
	8255	f(181-207) of bovine alpha s2-casein		–	antibacterial
LLYQEPVLGPVRGPFPIIV	8174	peptide derived from bovine b-casein (1-28)	2107.5295	–	immunomodulating
NAVPITPTL	10253	Antioxidative peptide	925.0774	–	antioxidative
	10250	Osteoanabolic peptide		–	osteoanabolic
RPKHPIKHQ	7483	ACE inhibitor (fr. of as1-casein, 1-9)	1140.3379	13	ACE inhibitor
RPKHPIKHQGLPQEVLNENLLRF	3035	isracidin	2764.1833	–	antibacterial
	8171	Isracidin - peptide derived from alphaS1-casein (1-23)		–	immunomodulating
	9530	Isracidin - antiviral		–	antiviral
SQSKVLPVPQKAVPYPQ	9907	Antioxidative peptide	1866.1592	–	antioxidative
VKEAMAPK	7796	Lipoxygenase inhibitor	873.0703	–	lipoxygenase inhibitor
	9561	Antioxidative peptide		–	antioxidative
VYQHQKAMKPWIQPKTKVIPY	3966	–	2584.0820	–	haemolytic
VYQHQKAMKPWIQPKTKVIPYVRY	3965	–	3002.5708	–	haemolytic
	3033	CaMPDE inhibitor		–	CaMPDE inhibitor
	3435	f(183-206) of bovine alpha s2-casein		–	antibacterial
VYQHQKAMKPWIQPKTKVIPYVRYL	5469	(fr.183-207 of bovine alpha s2-casein)	3115.7280	–	antibacterial
	3034	CaMPDE inhibitor		–	CaMPDE inhibitor
	3964	–		–	haemolytic

YQKFPQY	9254	ACE inhibitor	973.0790	20.08	ACE inhibitor

Table 3. Peptide sequences identified in bovine casein hydrolysates from ***O. platylepis*** proteases that were registered in the bioactive peptide database (BIOPEP).

Sequence	**ID**	**Name**	**Chemical mass**	**IC50 (μM)**	**Activity**
LLYQEPVLGPVRGPFPIIV	8174	peptide derived from bovine b-casein (1-28)	2107.5295	–	immunomodulating
RPKHPIKHQGLPQEVLNENLLRF	9530	Isracidin - antiviral	2764.1833	–	antiviral
	8171	Isracidin - peptide derived from alphaS1-casein (1-23)		–	immunomodulating
	3035	isracidin		–	antibacterial
SQSKVLPVPQKAVPYPQ	9907	Antioxidative peptide	1866.1592	–	antioxidative
VYQHQKAMKPWIQPKTKVIPYVRYL	5469	(fr.183-207) of bovine alpha s2-casein	3115.7280	–	antibacterial
	3034	CaMPDE inhibitor		–	CaMPDE inhibitor
	3964	–		–	haemolytic
YQKFPQY	9254	ACE inhibitor	973.0790	20.08	ACE inhibitor

Figure 1. Standardized quantification of peptide precursors (×103) in bovine casein hydrolysates samples of C. *cardunculus* C. *humilis* and O. *platylepis.*

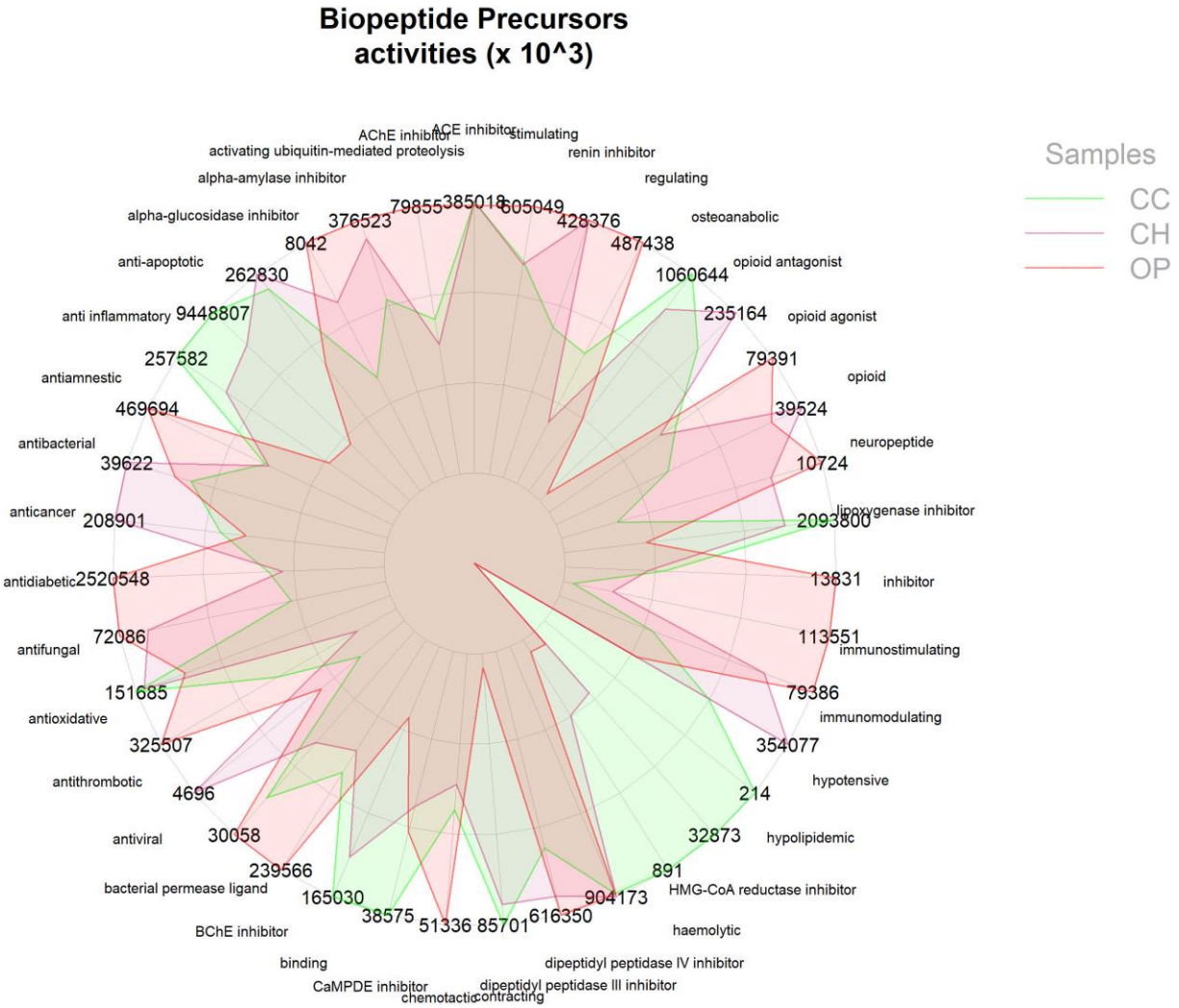

Figure 2. Heatmap and dendrogram of bioactivities of the different replicates evaluated. Quantification of bioactivity is regarding the mean. The grouping relationship between the groups of activities is defined.