



**“Valorisation of thistle-curdled CHEESES in MEDiterranean marginal areas”
Acronym VEGGIE-MED-CHEESES**

Deliverable title	D1.7 Report 2nd year
Deliverable Lead:	Università Politecnica delle Marche
Related Work Package:	WP1 Project coordination and overall management
Related Task:	Task 1.3 Communication with PRIMA-IS and the Funding Authorities
Author(s)	Lucia Aquilanti
Dissemination level	Public
Due Submission Date:	31.05.2021
Actual submission:	30.04.2021
Start date of project	01.05.2019
Duration	36 months (after project end extension: 48 months)
Abstract	The 2 nd year Report describes the main results and goals achieved during the second year of activity of the Veggie-Med-Cheeses Consortium

Versioning and Contribution History

Version	Date	Modified by	Modification reason
V1.0	15/04/2021	Lucia Aquilanti	First version
V2.0	30/04/2021	Lucia Aquilanti	Comments after peer review process

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Report 2nd year

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LIST OF PARTICIPANTS

Partner No.	Organisation	Participant (Permanent Staff)	Role
Coordinator (P1)	Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Italy (short name: D3A-UNIVPM)	Lucia Aquilanti	Project Coordinator (PC) and Principal Investigator (PI) of UNIVPM
Partner 2 (P2)	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca Alimenti e Nutrizione (short name: CREA-AN)	Pamela Manzi	PI of CREA-AN
Partner 3 (P3)	Departamento de Tecnología de Alimentos y Nutrición, Universidad Católica San Antonio De Murcia (short name: UCAM)	Luis Tejada Portero	PI of UCAM
Partner 4 (P4)	Department of Food Hygiene and Technology, Veterinary Research Institute, Hellenic Agricultural Organization, DEMETER (short name: DEMETER)	Akis Psomas	PI of DEMETER
Partner 5 (P5)	High Institute of Agronomy of Chott-Mariem, Sousse University (ISA-CM)	Bouthaina Al Mohandes Dridi	PI of ISA-CM

1. Explanation of the work carried out by the beneficiaries and Overview of the progress

WP1 Project coordination and overall management

OBJECTIVES

The objective of WP1 was to ensure optimal co-ordination and management of VEGGIE-MED-CHEESES, as well as collaboration among Partners, with the final aim of maximising progress of knowledge and innovation outputs.

TASKS AND STATUS

Task 1.1 Organization of general Project meetings. Partners involved: PARTY 1 **(IN PROGRESS)**

Task 1.2 Project coordination, management, supervision and quality control, Partners involved: All Partners **(IN PROGRESS)**

Task 1.3 Communication with PRIMA-IS and the Funding Authorities; Partners involved: All Partners **(IN PROGRESS)**

Task 1.4 Preparation of mid-term and final reports; Partners involved: All Partners **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

Deliverable number	Deliverable title	Due date of delivery	Effective date of delivery
D1.5	General Project meeting 3 report	31.10.2020 (month 18)	31.10.2020 (month 18)

D1.6	General Project meeting 4 report (Month 24)	30.04.2021 (month 24)	30.04.2021 (month 24)
D1.7	2st year (progress) report (Month 24)	30.04.2021 (month 24)	30.04.2021 (month 24)

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

- **Organization and scheduling of the General project meeting 3**, which has been held on-line (using TEAMS) on 29th October 2020 with the participation of all partners and the following agenda:

13 h 30 – 13 h 45 (Rome time) **Connection and Greetings**

13h 45– 14h 30 **Presentations of the main results achieved by Research partners involved in WP2, WP3, WP4**

14h 30 - 15h 00 **Planning of WP5 activities** (including packaging, delivery, analyses, etc.)

15h 00 – 15h 15 **Discussion and issues from the Partners**

15h 15 **Closure of the meeting**

All the details about the meeting are reported in the Deliverable 1.5.

- **Organization and scheduling of the General project meeting 4**, which has been held on-line (using TEAMS) on 18th March 2021 with the participation of all partners and the following agenda:

10 h 00 – 10 h 15 (Rome time) **Connection and greetings**

10h 15– 10h 30 **Communications from the Project Coordinator**

10h 30 - 11h 00 **Presentations of the main results achieved by Research partners (ISA-CM, UNIVPM) involved in WP2 and open discussion on WP2 issues**

11h 00 - 11h 30 **Presentations of the main results achieved by Research partners (ISA-CM, UNIVPM) involved in WP3 and open discussion on WP3 issues**

11h 30 - 11h 45 **Presentations of the main results achieved by Research partners involved in WP4 (UNIVPM, UCAM, CREA-AN, DEMETER) and open discussion on WP4 issues**

11h 45 - 12h 15 **Presentations of the main results achieved by Research partners (UNIVPM, DEMETER, CREA-AN, UCAM) involved in WP5 and open discussion on WP5 issues**

12h 15 - 12h 30 **Presentations of foreseen activities related to WP6 (UNIVPM)**

12h 30 – 13h 00 **WP7 products and deliverables**

13h 00 **Closure of the meeting**

All the details about the meeting are reported in the Deliverable 1.6.

- The Coordinator has reviewed the **periodic reports** prepared by the WP leaders to verify their consistency with the project tasks, in view of their assembling and transmitting them to PRIMA-IS and the funding agencies on behalf of the Consortium.
- The Coordinator has drafted the **minutes of general meetings 3 and 4** as well as the **2st year report**.

WP2 Characterization of spontaneously grown thistle populations

OBJECTIVES

The objective of WP2 was the characterization of Mediterranean spontaneous thistle populations ascribed to species and genera traditionally exploited in local cheese-making for the manufacture of thistle-curdled sheep's and goat's milk cheeses: *C. humilis*, *O. nervosum ssp. platylepis* and *O. tauricum*. To reach this goal both qualitative and quantitative morphological traits will be evaluated, and different ecotypes will be (eventually) identified (even using genetic markers).

TASKS AND STATUS

Task 2.1 Sampling and morphological characterization of spontaneously growing thistles; Partners involved: PARTY 1, PARTY 5 (**IN PROGRESS**)

Task 2.2 Lyophilization of sampled spontaneous thistles; Partners involved: PARTY 1, PARTY 5 (**COMPLETED**)

Task 2.3 Sampling, cataloguing, and storing of seeds; Partners involved: PARTY 1, PARTY 5 (**COMPLETED**)

Task 2.4 Elaboration of morphological data and potential identification of different ecotypes; Partners involved: PARTY 1, PARTY 5 (**IN PROGRESS**)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Morphological characterization is the first step in the description and classification of germplasm. Accordingly the genetic diversity in spontaneous thistles populations ascribed to *Cynara humilis*, *Onopordum platylepis* and *Onopordum tauricum*, occurring in different Mediterranean marginal areas (high altitude pastures, dry and arid lands, wasteland, etc.) have been morphologically characterized based on qualitative and quantitative traits, thus leading to the identification of different ecotypes. Samples and seeds from these populations have been collected, catalogued, and stored. Sampling has been performed within the natural areas of distribution of the thistle species under study, spanning from Spain in the West Mediterranean passing through North Africa and Italy.

The WP2 activities carried out in the reporting period are briefly summarized as follows:

- Samples from thistle populations spontaneously occurring in various Mediterranean marginal areas (PARTY 1: central Italy, South-eastern Spain; PARTY 5: North Tunisia), ascribed to the species *Cynara humilis*, *Onopordum tauricum* and *Onopordum platylepis* have been collected, classified and characterized through the evaluation of morphological (qualitative and quantitative) traits, according to the UPOV (International Union for the Protection of New Varieties of Plant) for artichoke. Sampling has been carried out using the simple random method and a

specimen of each population has been catalogued and stored with notes of collecting areas. Sampling was performed according to the following scheme:

- *Onopordum tauricum* has been sampled from two Italian sites (Cupi di Visso, Macerata; Colfiorito, Perugia)
- *Onopordum platylepis* has been sampled from one Tunisian site (Chott Meriem in the central-eastern regions of Tunisia)
- *Cynara humilis* has been sampled from one Spanish site (Alcaraz, Castiglia-La Mancia)

More details about sampling are reported in D2.2.

- Sampled thistles have been lyophilized (as capula from thistle flours) according to a standard procedure detailed in D2.1. The dried material has been crushed and the resulting powder has been kept in sealed bags at room temperature for storing.
- Seeds have been catalogued according to international protocols (ISTA, 2018), and further stored in rooms at controlled temperature (15°C) and relative humidity (15%) and in freezer cabinets at -20°C; all details about seeds cataloguing and storage are reported in D 2.2.
- Morphological data are being now elaborated using multiple statistical tools (PCA, PLS, PLS-DA, UPGMA cluster analysis, etc.) to identify eventual different thistle ecotypes (PARTY 1). For *Onopordum tauricum*, two ecotypes within the two sampled populations have been identified based on morphological traits; molecular tests based on the analysis of Simple Sequence Repeats (SSR) markers are currently in progress for the evaluation of the genetic variability between these two ecotypes.

WP3 Sustainable cultivation of thistles

OBJECTIVES

The objective of WP3 is the evaluation of the adaptability and agronomic productivity of *C. humilis*, *O. tauricum* and *Onopordum platylepis* in rainfed areas of the Mediterranean basin under zero/low inputs of fertilizers and use of non-chemical weed control methods. The final goal of this WP is to produce recommendations and guidelines for the sustainable cultivation, in the Mediterranean basin, of thistles exploitable by dairy industries for the manufacturing of high-quality and safe thistle-curdled cheeses.

TASKS AND STATUS

Task 3.1 Design of the field experiments; Partners involved: PARTY 1, PARTY 5 (**COMPLETED**)

Task 3.2 Germination tests; Partners involved: PARTY 1, PARTY 5 (**COMPLETED**)

Task 3.3 Soil characterization; Partners involved: PARTY 1, PARTY 5 (**COMPLETED**)

Task 3.4 Seeds multiplication and transplantation; Partners involved: PARTY 1, PARTY 5 (**IN PROGRESS**)

Task 3.5 Phenological development survey; Partners involved: PARTY 1, PARTY 5 (**IN PROGRESS**)

Task 3.6 Analysis of the above ground fresh and dry biomass; Partners involved: PARTY 1, PARTY 5 (**NOT STARTED**)

Task 3.7 Lyophilization of sampled cultivated thistles; Partners involved: PARTY 1, PARTY 5 (**NOT STARTED**)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Field experiments have been planned in 2 experimental sites, each modelling a Mediterranean scenario with different landscapes and climatic conditions, namely:

- a central Italian hill area next to the Adriatic coast, with a warm and rainy temperate climate (classified as Csa = hot-summer Mediterranean climate, according to Köppen and Geiger) at least three times as much precipitation in the

wettest month of winter as in the driest month of summer and driest month of summer receives less than 30 mm (1.2 in) (PARTY 1);

- an area in North Tunisia with an arid climate (classified as BSh = hot semi-arid climate, according to Köppen and Geiger), which is characterized by scarce rainfall and high temperatures during the whole year (PARTY 5). In both the two sites the following species and ecotypes have been compared: *Cynara humilis* L., *Onopordum tauricum* Willd (two ecotypes), *Onopordum platylepis* Murb., *Cynara cardunculus* L. This latter species has also been included, though its cultivation was not foreseen in the original Workplan (see for more details section 5).

The WP3 activities carried out in the reporting period are briefly summarized as follows:

- Selected thistle species/ecotypes have been compared in a randomized block experimental design with three replications. **Design of the experimental plan at the two selected sites is detailed in D3.1.**
- Seeds have been subjected to germination tests to identify the most suitable environmental conditions for their germination (temperature, light, pre-treatments, etc.).
- Influence of soil composition on thistles-based cropping system has been evaluated by measuring key soil parameters with standardised procedures already in use at the laboratories of the PARTY 1 and PARTY 5, being pH, organic matter content, electrical conductivity, total N, exchangeable K₂O, available P₂O₅, exchangeable Ca, Na and Mg.
- Regarding the sole Italian site, in March 2020 seeds have been planted in standard seed trays; four-week-old thistles with three-four true leaves have been transplanted (late April 2020) on previously prepared plots (see . Supplementation of low doses of organic fertilizer and relatively spaced irrigations (depending on rainfall). Tillage consisted of medium-depth ploughing (30 cm). Seed bed preparation has been conducted immediately before planting, by using a disk harrow. Weed are regularly eliminated by using non-chemical methods (hoeing).
- Regarding the Tunisian site, seed germination and young plants transplantation will be carried out on September 2020.
- At the Italian site, the monitoring of plants survival has been started in late April 2020 and it will be performed twice per growing season, as foreseen in Task 3.5. In the first monitoring at the beginning of the plant cycle, the number of plants that has re-grown will be recorded. In the second monitoring at the end of the season, the number of plants that has reached reproduction will be recorded. Plant survival will be then estimated as the ratio between the second and first monitoring. In parallel, twenty plants have been randomly selected within each plot and phenological growth stages are regularly recorded (every 3 days), according to the universal BBCH coding system for mono- and dicotyledonous plants (BBCH abbreviation stands for Biologische Bundesanstalt, Bundessortenamt and Chemical industry). Environmental parameters (rainfall and daily maximum and minimum air temperature) are regularly recorded at an agro-meteorological station located in of the two experimental field.

WP4 Characterization of thistle aqueous crude extracts (CEs)

OBJECTIVES

The objective of WP4 is the full characterization (chemical, microbiological, technological, and biochemical) of aqueous crude extracts (CEs) derived from both spontaneously grown and cultivated thistles (CE_{st} and CE_{ct}, respectively) from either whole plants or separate plant sections: stalks, leaves, capitula.

TASKS AND STATUS

Task 4.1 Chemical characterization of CEs; Partners involved: PARTY 1, PARTY 2, PARTY 5 **(IN PROGRESS)**

Task 4.2 Microbiological characterization of CEs; Partners involved: PARTY 1, PARTY 3, PARTY 4, PARTY 5 **(IN PROGRESS)**

Task 4.3 Evaluation of technological properties of CEs; Partners involved: PARTY 1, PARTY 3, PARTY 4 **(IN PROGRESS)**

Task 4.4 Purification of proteases from CEs; Partners involved: PARTY 1 **(IN PROGRESS)**

Task 4.5 Biochemical characterization of purified proteases; Partners involved: PARTY 1 **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

For this WP, during the reporting period UCAM (PARTY 3) completed the characterization of the crude extracts prepared by maceration of tubular flowers collected from wild *Onopordum tauricum*, *Onopordum platylepis*, and *Cynara humilis* (CE_st) by analysing the bioactive compounds occurring in CE_st. The biochemists working at UNIVPM-D3A (PARTY 1) went on with the biochemical characterization of the purified protease from *Onopordum tauricum*, according to the activities detailed in the Task 4.5.

WP5 Cheese-making trials and characterization of thistle-curdled and control cheeses

OBJECTIVES

The objective of WP5 is to carry out cheese-making trials allowing for the full characterization (physico-chemical, chemical, microbiological, textural and sensory) of local thistle-curdled cheeses as well as for the investigation of nutritionally valuable substances (e.g. minerals, vitamins, etc), health-beneficial (e.g. phenolic compounds, bioactive peptides with ANTI-ACE activity) and hazardous (biogenic amines) compounds

TASKS AND STATUS

Task 5.1 Cheese-making trials; Partners involved: PARTY 1, PARTY 3, PARTY 4 (**IN PROGRESS**)

Task 5.2 Physico-chemical and chemical analyses; Partners involved: PARTY 1, PARTY 3, PARTY 4 (**NOT STARTED**)

Task 5.3 Microbiological analyses; Partners involved: PARTY 1, PARTY 4 (**NOT STARTED**)

Task 5.4 Textural and sensory analyses; Partners involved: PARTY 2, PARTY 3, PARTY 4 (**IN PROGRESS**)

Task 5.5 Analysis of nutritionally valuable, health-beneficial and hazardous substances (Month 8-Month 14: cheeses made with CE_st; Month 22-Month 28: cheeses made with CE_ct); Partners involved: PARTY 1, PARTY 2, PARTY 3 (**NOT STARTED**)

Task 5.6 Statistical elaboration of data; Partners involved: PARTY 1 (**NOT STARTED**)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

During the reporting period, preliminary cheese-making activities were started at PARTY1 and PARTY4 for the optimization of the use of a commercially available thistle rennet for manufacturing of Caciofiore and Feta, respectively. These preliminary trials allowed to optimize the cheese-making process (T, t, quantity of vegetable rennet, etc.).

WP6 Evaluation of consumer needs, preferences and acceptance towards thistle-curdled and control

OBJECTIVES

The objective of WP6 is to evaluate the degree of liking of consumers towards experimental and control “second round” cheeses, as well as to provide analytical insights on the prospect value-chain potential of thistle-curdled traditional local cheeses in the Mediterranean

PLANNED TASKS AND STATUS

Task 6.1 Focus groups and consumer tests; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)

Task 6.2 Prospect value chain analysis and business model canvas analysis; Partners involved: PARTY 1, PARTY 3, PARTY 4 (NOT STARTED)

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

Not applicable

WP7 Multi-actor internal and external communication and technology transfer

OBJECTIVES

The objectives of WP7 are to ensure participation of stakeholders, as well as dissemination and exploitation of VEGGIE-MED-CHEESES outputs. A distinction can be made between objectives of internal and external communication.

Objectives of **internal communication** (within the Project), are:

- I. communicate project results within the Consortium so that all partners are updated timely on the knowledge generated by all WPs and tasks;
- II. coordinate the use of participatory approaches to utilise stakeholder and research knowledge and innovation to prioritise research activities within the project.

Objectives of **external communication** (to Stakeholders), are:

- I. use participatory approaches to utilise stakeholder and research knowledge to prioritise dissemination and training activities;
- II. communicate and disseminate project outputs to stakeholders and create a dairy farm-level observatory and knowledge exchange network;
- III. build capacity through technology transfer to ensure that the industry can effectively use the outputs from the project.
- IV. create a roadmap for future implementation and exploitation of project outputs at dairy farm, on regional and Mediterranean scales and for further research.

As a whole these actions aim to promote participation and effectively translate the Project outputs to meet the needs of the dairy industry and so that the results are disseminated in a way that can be promptly implemented and exploited by the following stakeholder groups: i) crop producers; ii) dairy farms, dairy industries, dairy operators, iii) retail and consumers and the general public; iv) Non Governative Organisations; v) scientists; vi) policy makers. All the partners will be involved in this WP.

TASKS AND STATUS

Task 7.1 Establishment of a stakeholder-platform (Month 1–Month 36); Partners involved: All partners (IN PROGRESS)

Task 7.2 Installation of a website and production of dissemination materials; Partners involved: All partners **(IN PROGRESS)**

Task 7.3 Documentation of scientific results; Partners involved: All partners **(IN PROGRESS)**

DELIVERABLES DELIVERED IN THE REPORTING PERIOD

None

BRIEF DESCRIPTION OF WORK DONE IN THE REPORTING PERIOD

- In the Reporting period, the Stakeholder platform was added with additional stakeholders (148 at the date of delivery of this deliverable)
- Stakeholders have periodically received updated information/news about the project and its progresses and main outcomes (4 additional newsletters have been sent, in the Reporting period)
- On 14.10.2020 Prof. Luis Tejada Portero (PI of UCAM, Spain) attended a divulgation on-line event ["26 Semana de la Torta del Casar"] organized by the Universidad del Queso" with a presentation titled: "Cynara cardunculus: science and technology in a millennial tradition; vegetable rennet in the preparation of cheeses and Torta del Casar". The Project objectives, Consortium partners, work plan and main results have been presented by Prof. Portero.
- Preliminary scientific results collected through the VEGGIE-MED-CHEESES research activities have been published in peer-reviewed Journals and presented at Internal Congresses, as detailed as follows:

CONFERENCE PROCEEDINGS

- M. Mozzon, N. Raffaelli. Potentialities of *Onopordum tauricum* (Willd.) as milk clotting agent. Proceedings of the 22nd International Conference on Dairy Science and Processing- Online event **12 November 2020** (ORAL Communication).

PUBLISHED PAPERS

- Full-length research paper published by the peer-reviewed journal Food Research International (ELSEVIER), 2022, 158, 111592 <https://doi.org/10.1016/j.foodres.2022.111592>
Title: *Potentialities of aqueous extract from cultivated Onopordum tauricum (Willd.) as milk clotting agent for cheesemaking*
Authors: Foligni R, Mannozi C., Gasparri M., Raffaelli N., Zamporlini F, Tejada L., Bande-De León C., Orsini R., Manzi P., Di Costanzo MG, Ritota M, Aquilanti L., Mozzon M.

PRESS RELEASES

- A press release dated 04/07/2021, titled: **La flor de cardo, ¿un nuevo 'superalimento'**?. [Thistle flower, a new 'superfood'?] <https://lasgastrocronicas.com/2021/07/04/la-flor-de-cardo-un-nuevo-superalimento/>

2. Update of the plan for exploitation and dissemination of result

No updates have been performed, yet

3. Update of the data management plan

No updates have been performed, yet

4. Follow-up of recommendations and comments from previous review(s)

Not applicable



The PRIMA programme is an Art. 185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation'



5. Deviations from original proposal in the Reporting period

A few deviations from the original proposal have been foreseen; they are described as follows:

UNFORESEEN USE OF IN-KIND CONTRIBUTION OF BOTANISTS FROM UNIVERSITY OF VALENCIA

Regarding the sampling of *Cynara humilis*, which grows spontaneously in Spain, a team of botanists from University of Valencia has given an in-kind contribution for the identification and sampling of flowers and seeds from this spontaneously growing thistle in July 2019..