



Newsletter #3

Quarterly newsletter on project status and last results

February 2022



UNIVERSITÀ
DEGLI STUDI
DI MILANO



INSTITUT DE
L'ELEVAGE **idele**



INRAE



CSIC



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PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

Milk quality along the dairy chain for a safe and sustainable milk



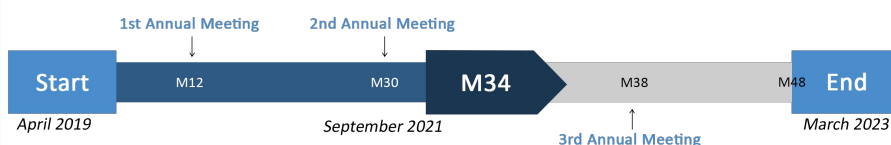
MilkQua project in a few words & status

MilkQua in a few words:

Plant extracts to prevent mastitis in Tunisia.

MilkQua's strategic aim is to enhance global food security and dairy food quality by reducing antimicrobial use on Tunisian farms. Our main objective is to improve the quality and sustainability of the Tunisian dairy sector by addressing a high priority species-specific disease, mastitis, which is of great importance to the Tunisian agricultural sector economy. MilkQua aims to reduce the use of antibiotics related to mastitis by valorising Tunisian plant extracts. The project brings together 10 partners in Tunisia, France, Italy, Spain and Portugal.

Project timeline:



[More information on MilkQua](#)

News from our last Scientific meeting:

On the 17th December 2021, all the partners of MilkQua met for a remote Scientific Meeting dedicated to the presentation of the results of WP5 (System biology assessment of the effects of extracts after in vivo and in vitro studies) led by Fabrizio Cecilian, UMIL. This was followed by a discussion among partners. Other internal meetings will be held to deepen and share on the analysis of results.

Newsletter #3: Focus on WP3 and WP6

WP3: *In vitro* evaluation of bioactive molecules and extracts

WP3 objectives are:

The overall objectives of this WP are to select the most promising plants regarding their biological potential and provide information regarding their phytochemical composition and mechanism of anti-inflammatory and antimicrobial activity. The other objectives are:

1. Standardization of plant sampling procedures (T3.1)
2. Optimization of extraction protocols; Obtention of bioactive extracts; Phytochemical characterization of bioactive extracts and sampling (T3.2)
3. To evaluate the *in vitro* effects of plants and essential oils on ruminal fermentation parameters and methane production when included in the diet of ruminants (T3.3)
4. To elucidate the anti-inflammatory, antioxidant and antimicrobial activity of essential oils and bioactive extracts, and their detailed mechanism of action (T3.4)
5. To assess the potential toxicity of plant materials, thus ensuring that only safe materials and doses are used (T3.5)

WPL: UPFF. Partners involved: CSIC, LPAM



Renato and David working in the laboratory

Activities performed so far: interview of David Pereira, UPFF, leader of WP3

What is the role of UPFF in the project

At UPFF (University of Porto, Faculty of Pharmacy), the team involved is from the Laboratory of Pharmacognosy. This means that we specialize in natural products, both from a chemical and biological point of view. Our role in MilkQua is the biological assessment of the natural products obtained by our colleagues in Tunisia, reason for which we coordinate WP3.

What are the main actions you already implemented ?

Quite a few ! We initially evaluated all essential oils sent by our colleagues and conducted some preliminary toxicological assessment. As one would expect, we need to know which samples are the safest and which concentration ranges can be used. From here, we evaluated all samples for their anti-inflammatory activity using a gene reporter assay in macrophages. This allowed us to identify the most active samples, which we subsequently selected for more in-depth mechanistic studies using *in vitro* models.

What are further actions?

Yes, we are happy with our results! The actions referred above allowed us to select 4 out of 10 samples for more detailed studies. These 4 samples have been characterized for their anti-inflammatory effect and we now know their impact upon an array of parameters, from genetic expression to cytokines and proteins. Using the phytochemical information received from our partners in Tunisia, we were also able to test the most abundant molecules in each essential oil, which allowed us to identify, in each sample, the molecules responsible for the activity of the essential oil.

What is your personal vision of the project ?

My vision is that the collaborative framework used in MilkQua is the future (present?) of a significant part of research projects. Indeed, an increasingly high number of topics and scientific fields can only be tackled when a multidisciplinary team consisting of several partners is assembled. We have clear objectives with societal impact and our expectations is that they will be achieved. MilkQua has generated a significant amount information, most of which can have real impact in animal science and the practices of local producers. As so, our ambitions are to be able to conduct impactful research in this field, even after MilkQua, by further exploiting the team and skills that MilkQua brought together.

What interest do you see in collaborating with other partners in the project/WP?

WP3 benefits from the contribution of several partners, each of which adds value and know-how to our activities. For example, LPAM was involved in the sampling, extraction and phytochemical analysis of all samples. They collected the plants and conduct GC analysis to identify the qualitative and quantitative profile. These samples were then analyzed by UP for their anti-inflammatory properties and also by CSIC, who conducted several studies on the effects of these materials on ruminal fermentation parameters and methane production. In all cases, the chemical knowledge generated by LPAM has been

used to plan the work and interpret results.

This integrated approach is pivotal to the success of the WP and also the project, as it allows each partner to contribute with their know-how and expertise, and thus add to the impact and reach of the project. As we speak, we are finishing the first manuscript that resulted from this work, and which also benefited from the participation of partners from other WPs.

WP6: Milk and dairy product processing studies

WP6 objectives are:

We will evaluate the interest and the benefit of adding essential oils on the quality and stability of milk and its derived processed products. Two innovative encapsulation strategies of EOs will be compared. Analysis of milk obtained from the *in vivo* trials (WP4) and subsequent derived processed dairy products (flavour, bioactivity, composition) will be performed in connexion with WP4 and WP7 activity of EOs and bioactive extracts, and their detailed mechanism of action. The other objectives are:

1. Analysis of milks and processed products (T6.1)
2. Comparative mechanisms of two encapsulation strategies (T6.2)
3. Supplemented milk properties assessment (T6.3)

WPL: INRAE. Partners involved: Idele, LPAM

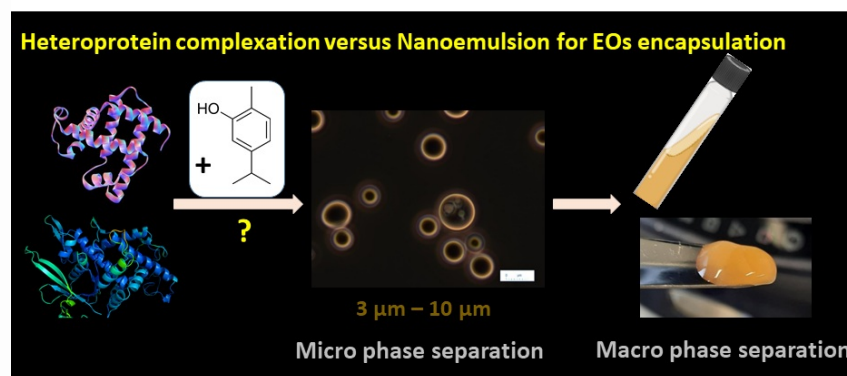


Illustration of heteroprotein complexation versus Nanoemulsion for EO's encapsulation

Activities performed so far: interview of Saïd Bouhallab, INRAE, leader of WP6

What is LPAM? What is the role of LPAM in the project?

I am Saïd Bouhallab, Director of Research at INRAE. INRAE is strongly involved in sustainability issues and the reduction of antibiotic use, which is one of the objectives of the MilkQua project. The role of INRAE is to lead the work of WP6, for which I am responsible, on the impact of the addition of essential oils in milk both *in vivo* (animal feed) and *in vitro* (direct addition of essential oils in milk). INRAE is also involved in animal experiments in the form of collaboration through the experimental installation on the UMR Pegase in Rennes.

What interest do you see in collaborating with other partners in the project/WP?

Milkqua is an interdisciplinary project involving expertise and actors from plant chemistry, fundamental biochemistry, microbiology, animal health, milk production and processing, and even surveys on farmers' practices. I let you imagine the great richness of our exchanges during the monthly meetings.'

What are the main actions you already implemented?

INRAE is the leader of WP6, but also collaborates with partners in WP4 and WP7 on the *in vivo* treatment of cows by the application of essential oils. We have already tried to see what properties the essential oils have on the quality of the milk, then we will characterize the potential difference between control milk and milk from animals treated with the selected essential oils and we have progressed on the development of encapsulation technology to protect the essential oils to enhance their bioactivity. This work is done in close collaboration with LPAM..

What results did you achieve, what are the further actions to implement?

For the moment, we have begun organoleptic tests on the controlled milks and those treated with essential oils, and we haven't found any difference in sensory properties. In addition, at the level of encapsulation, we will set up two technologies: first, the protection of essential oils by nano-emulsion (work carried out by LPAM) that shows encouraging results and we made the proof of concept on the capacity of the assemblies of dairy proteins to encapsulate hydrophobic molecules. In the near future, we will move on to studies of essential oil molecules.

What is your vision of the project?

As a protein biochemist, I was not used to work in this type of interdisciplinary project, which I am discovering is really essential to solve the major challenges facing public research. PRIMA projects allow us to have a very global vision of the problems of many sectors. It is also in line with the values that I hold and that we promote within the framework of the "Milk, vector of development" network initiated by INRAE - CIRAD a few years ago to strengthen North/South cooperation and collaboration.

Latest News & Events



WP2 resumed its activities in Tunisia

A team of IDELE carried out a mission in Tunisia in December 2021 within the framework of WP2 of MilkQua. WP2 objectives are to set up a program destined to enhance milk quality, change the practices used to reduce the mammary's infections and reduce the use of antibiotics in the Tunisian dairy farms, using a training program, and information and communication technics.

After a break of 18 months because of the Covid - 19 crisis, the present mission aim was to resume the program with :

1. Meetings at Tunis with the Office of Livestock and Pasture, Tunisian Veterinary School, General Direction of the Veterinary Services in order to identify the determinants, the brakes and the motivations of the decision making of the breeders to change the technics they use to enhance the milk quality and reduce the use of antibiotics. The purpose of those meetings was also to finalize messages on good practices of medicines in breedings that will be send on a regular basis as SMS to the breeders in order to make them aware of this.
2. Organisation of training program of 3 days in Bizerte for the OEP engineers and a veterinary about the mammary's infections and the good practices of antibiotics use. The team also met the Natilait's representatives at Utique (Dairy company) in order to inform them of the project and be able to find targeted breedings to involve them in the surveys and the tests of the SMS campaign
3. Visit of a dairy farm at Ariana.

After the conclusion of all these works, we will jointly with our Tunisian colleagues, propose the best approaches to advice breeders and field workers as well as national actions for the control of mastitis, and the absence of inhibitors in milk.



Vote to support MilkQua!

ITA'nnov contest rewards the best innovations in the field of agriculture and Agri-food. MilkQua applied to the ITA'nnov contest that is now open.

A public price will be given to the project that gathered the higher number of "likes" and the price will be attributed during the Paris International Agricultural Fair, "Salon de l'Agriculture" in March 2022! A wonderful video has been created to present MilkQua: [discover it and support MilkQua!](#)



MilkQua video is ready

The official video of MilkQua is ready and available on [MilkQua website](#) and social media. This first video realized at M30 of the project is a state of the work already done so far and the results that came up yet. Partners are interviewed in their premises, labs and workspaces and explain their objectives and tasks within the project framework. A good occasion to better know the MilkQua project and its aims.

Tunisia-Japan Symposium

LPAM will participate in the [Tunisia-Japan Symposium](#) from 11th to 13th March of 2022. This event that had



been delayed from 2020 will gather experts, students and academics from both countries to discuss new perspectives on materials and nanomaterials and their applications in diverse fields such as energy, biotechnology, water, biomedicine

Dr. Mariem Ben Jemaa et Hanen Falleh from LPAM will speak at the Symposium on the following subjects:

- Simplex-Centroid mixture design to define an anti-escherichia coli formula (Dr. Mariem Ben Jemaa)
- Encapsulation of orange and sesame oils into nanoemulsion: physical, chemical and stability characterizations (Hanen Falleh)

Latest publications :

- ANDRES, S. et al., *Administración de l-carnitina durante el periodo de cebo en corderos con restricción alimentaria durante la lactancia*, XIX Jornadas sobre Producción Animal, AIDA, p57.
- Andrés, S., Abdennebi-Najar, L., Giráldez, F.J., *Dietary administration of oregano essential oil to newborn dairy calves improves feed efficiency and weight gain during the suckling period*, EAAP - 72nd Annual Meeting of the European Federation of Animal Science
- Cecilian, F., Audano, M., Addis, M-F., Lecchi, C., Ghaffari, M-H., Albertini, M., Tangorra, F., Piccinini, R., Caruso, D., Mitro, N., Bronzo, V., *The untargeted lipidomic profile of quarter milk from dairy cows with subclinical intramammary infection by non-aureus staphylococci*, Journal of Dairy Science, 16/06/2021
- Falleh, H., Benjemâa, M., Zohra Rahali, F., Hamrouni, I., Daaloul, M., Ksouri, R., Pereira, D., and Abdennebi-Najar, L., *Investigation of selected essential oils antibacterial potentials*. 4 th International Congress on Biochemistry & Microbiology Applied Technologies - BMAT-2021, May 28-30 th , 2021. Hammamet, Tunisia
- Martín, A., Abdennebi-Najar, L., Ksouri, R., Mateos, I., Ranilla, M.J., López, S., Giráldez, F.J., Andrés, S., *The effect of natural essential oils and synthetic essential oils on ruminal fermentation*, EAAP - 72nd Annual Meeting of the European Federation of Animal Science

Deliverables already submitted

- D1.1 Management committee constitution
- D1.2_MilkQua Mid term Reporting to PRIMA-IS
- D3.1 Sampling/extraction protocol and list of plants
- D3.2 Bioactive extracts for subsequent biological assessment
- D3.3 Phytochemical profile of selected samples
- D3.4. Effects of essential oils on *in vitro* ruminal fermentation parameters and methane production
- D3.5_Report anti-inflammatory, antimicrobial and immunomodulatory properties
- D3.6 Safety report on selected samples
- D4.1 Protocols and data collection
- D6.2 Mechanisms of EOs encapsulation and selection of the most stable and efficient one
- D6.3_MilkQua Antimicrobial activity of encapsulated EOs
- D7.2 Questionnaire establishment on milk consumption
- D7.3 Validation of the questionnaire
- D8.1 Communication and dissemination materials
- D8.2 Plans for dissemination and exploitation of the results
- D8.3 Completed and planned communication activities

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Don't forget to forward this email to your friends and contacts who could be interested in our project and its achievements.

MilkQua Project
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This project is part of the PRIMA programme supported by the European Union



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