

Biodiversity matters

Marlene Barros, of Universidade Católica Portuguesa, introduces iCheese, which is at the forefront of efforts towards a vegetable coagulant compliant with food quality and safety guidelines

Traditionally in the production of cheese from ewes' and goats' milk in several Mediterranean countries, cardoon flowers, rich in enzymes, are used. There is European regulation regarding enzyme use in food. It is necessary, however, to develop and implement procedures to achieve a vegetable coagulant from cardoon flowers compliant with food quality and safety guidelines.

Vegetable coagulants and cardoon: a Mediterranean resource

Aspartic proteinases (APs) are enzymes that cleave peptide bonds and are involved in different biological mechanisms from viral replication to protein digestion. Their capacity to fragment milk proteins has made them ideal coagulants for the cheese industry. The global market for coagulating enzymes is interesting from the economic standpoint and, so far, has been dominated by enzymes of microbial origin.

The diversity of APs present in different organisms is currently acknowledged; however, the diversity of aspartic proteinases expressed in the cardoon flower is a gift from Nature, turned into a scientific challenge.

Cynara cardunculus L. (cardoon) is an important Mediterranean multipurpose and versatile crop with a wide spectrum of potential applications. Cardoon's biodiversity throughout the Mediterranean basin presents an invaluable genetic heritage, which is translated in great biochemical diversity with a wide potential for applications from food to the health industry. Cardoon is also an extremely resilient natural



Mediterranean resource, with a high impact on the decrease of the carbon footprint.

A multigene family encoding APs in *C. cardunculus* L. is known and six cardosins have been isolated, purified, and biochemically characterised. This is one of the highest numbers of APs purified from a single organism. Several Mediterranean civilisations have discovered this gift of Nature, and the dried cardoon flowers are used as a vegetable coagulant for the production of traditional cheeses along the Mediterranean basin, a cultural heritage worth preserving as an integral part of the Mediterranean diet.

Tradition and innovation - iCheese challenges

In Portugal, the protected designation of origin (PDO) for cheese from ewes' and goats' milk

determines that cheese is produced using cardoon flower extracts, to guarantee the specific characteristics of each traditional cheese. It is consensual among the scientific and producer communities that cardoon vegetable coagulants are fundamental for the identity of traditional cheeses and that cardoon flowers are a unique source of coagulating enzymes. Therefore, the valorisation and preservation of these endogenous resources depend on the establishment of procedures ensuring vegetable coagulants with a sustainable and reproducible quality to ensure the quality of the final products – the traditional cheeses.

Additionally, to fulfil the European regulation (EC No. 1332/2008) regarding enzyme use, and enable the utilisation of cardoon flowers as a natural source

of enzymes compliant with the EU regulation, it is necessary to develop and implement procedures to achieve a vegetable coagulant compliant with food quality and safety guidelines.

The Centre for Interdisciplinary Research in Health (CIIS) of Universidade Católica Portuguesa (UCP), alongside its partners, has the scientific and technological knowhow on cardoon, cardosins, and their applications, and has followed the regulatory issues. For these reasons, it was considered that the existing knowhow should be transferred for the empowerment of the stakeholders involved in this sector.

Therefore, a national operational group, iCheese, was set up under the Programa de Desenvolvimento Rural with a total investment of €430,121.47 from national and European funds. This group is co-ordinated by UCP and has as scientific partners:

- Universidade de Évora (Instituto de Ciências Agrárias e Ambientais Mediterrânicas);
- Instituto Nacional de Investigação Agrária e Veterinária (INIAV);
- Centro de Biotecnologia Agrícola e Agroalimentar do Alentejo (CEBAL); and
- Institutos Politécnicos de Viseu, Castelo Branco and Beja.

ANCOSE – Associação Nacional de Criadores de Ovinos Serra da Estrela and CATAA – Associação Centro de Apoio Tecnológico Agroalimentar are partners for the knowledge transfer, and Confraria do Queijo Serra da Estrela and ANIL-Associação Nacional dos Industriais de Laticínios are privileged partners to disseminate project results to the producers. Enterprises managing PDO, SATIVA and Beira Tradição, are also important in the implementation of the knowledge transfer. iCheese also includes knowledge transfer to other Mediterranean countries, and the Centre National de Recherche en Biotechnologie from Algeria and the company Klöckner-Pentaplast España, S.A.U. are also partners in this project.

Empowering the traditional cheese sector

To guarantee the sustainable and safe supply of coagulants, iCheese capitalises on Nature's gift to choose the best cardoon genotypes for the production of each variety of PDO cheese in Portugal.

iCheese will innovate at the product level by:

- 1) Developing formulations of the vegetable coagulant obtained from dried cardoon flowers of well-characterised ecotypes established in

the experimental fields. These new standardised formulations will be applied to the production of each particular PDO region (Serra da Estrela, Beira Baixa, Nisa, Évora, Azeitão, Serpa) and will be designated by MixEcoCyn 1-6; and

- 2) Developing a new formulation of powdered dried cardoon flowers with coagulating activity adequate for different milks and destined to new cheese design (InovEcoCyn), creating opportunities for innovation in the dairy sector. This goal will also enable the empowerment of the SMEs to gain new markets at the national and international levels, especially for consumers with restrictions regarding the use of animal coagulants. The InovEcoCyn coagulant is extremely interesting for producers who currently use other types of coagulants such as chymosin and are interested in adding value to their product. It is known that cardoon flowers add new textures and flavours to the cheese, and each producer will be able to imprint in their cheese-specific characteristics. Different producers will be able to use distinct cardoon genotypes and use the different coagulant properties for the production of 'designer' cheese.

Innovation at the process level will be achieved by:

- 1) Transfer of knowhow on the establishment of different cardoon genotype cultures to ensure sustainability and biodiversity maintenance of this crop;
- 2) Evaluating and establishing the strategies for flower cutting and processing guaranteeing the hygiene guidelines and legislation; and
- 3) Establishing the guidelines for the processing and packaging of the coagulant compliant with food safety and quality norms, contributing to the establishment of new national and international markets for the natural vegetable coagulants.

These innovations in products and processes will mainly create value in the production chain in the primary sector where a new business may develop through the creation of cardoon cultures for flower production. Additionally, in the transformation sector, the processing of flowers and other plant components such as stems, leaves, and seeds is an important opportunity for new health and biotechnology applications of cardoon.



Marlene Barros

Knowledge transfer and dissemination

The main goal of the project will be achieved with the creation of the iCheese web platform, which will function as a window into this project, reinforcing the knowledge transfer to the producers. Additionally, researchers and the general public may monitor project results and progress through this platform, which will be designed to include scientific, technical, and also non-specialist information.

To be able to reach as many producers as possible, a series of itinerant demonstration and dissemination actions in every PDO region of cardoon coagulated cheese will be organised.

iCheese is an example of the concentration of national scientific and technology network institutions which have congregated knowledge generated on cardoon, aspartic proteinases, and their applications since 1980, as well as their wide dissemination in the traditional cheese sector. This project promotes the preservation of the cultural heritage associated with the cardoon coagulated traditional cheese industry, simultaneously promoting the creation of new markets through innovation in the processes and products involved.



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