Innovative thistle-curdled cheeses from the Mediterranean area: nutritional evaluation of some relevant compounds

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SUPPLEMENTARY FILE

Cheesemaking trials

Two batches of experimental prototypes (Exp) and control cheeses (C) were produced according to the following procedures:

1. Cheeses traditionally produced with vegetable rennet: Caciofiore and Torta del Casar

- Caciofiore

Cheesemaking trials of *Caciofiore* cheese were performed in a dairy farm located in Pieve Torina (MC, Italy) in the Monti Sibillini National Park, according to an ancient local tradition involving the use of raw Sopravissana ewes' milk and thistle rennet.

Briefly, milk coagulants were added to raw milk previously filtered and pre-heated to 35-37 °C. After clotting (35-40 min), the curds were manually broken into rice-sized grains, transferred into plastic perforated moulds, and manually pressed to remove the whey. Moulded cheeses were held at 12–13 °C for 4 h, dry salted, and finally ripened for 60 days under controlled conditions (12–13 °C and 70% relative humidity).

Control Caciofiore (CF_C) cheeses were manufactured with commercial liquid *C. cardunculus* rennet (Dairen, Dairy and Food, Castel San Pietro Terme, BO, Italy) according to the manufacturers' instructions.

Experimental Caciofiore cheeses were produced with reconstituted aqueous extracts from flowers of spontaneous (coded as CF_ExpSP) and cultivated (coded as CF_ExpCT) *Onopordum tauricum* thistles, as previously described by Mozzon *et al.* (2020) and Foligni *et al*. (2022), respectively.

- Torta del Casar

Torta del Casar is a Protected Designation of Origin (PDO) cheese made from 100% ewes' milk from the Merino sheep, ripened for at least 60 days. The rind is semi-hard, with a uniform ochre colour without the addition of colouring agents. It may have small cracks on its surface. The paste has a soft to very soft consistency, is white to yellowish, and may have rounded eyes, typical of the ripening process distributed throughout the cut.

Torta del Casar cheesemaking was carried out in an industrial cheese factory located in Casar de Cáceres (Extremadura, Spain), strictly following its Product Specification (EC 2003). Briefly, milk coagulants were added to raw milk pre-heated at 30-32 °C and gently stirred. After clotting (about 60 min), the curds were manually broken using a cheese lyre into rice-sized grains, transferred into plastic perforated moulds and automatically pressed to remove the whey. The cheeses were salted in brine (16%), wrapped and soaked in an anti-mould bath, and ripened for 60 days under controlled conditions (9-11 °C and 90-92% relative humidity). The cheeses are turned every day from the beginning of the ripening period.

Control Torta del Casar cheeses (TC_C) were produced with commercial liquid *C. cardunculus* rennet (Dairen, Dairy and Food, Castel San Pietro Terme, BO, Italy).

Experimental Torta del Casar cheeses were produced with a reconstituted aqueous extract from flowers of spontaneous (coded as TC_ExpSP) and cultivated (coded as TC_ExpCT) *Cynara humilis* thistles, prepared as previously described by Bande-De León et al. (2023)

2. Cheeses traditionally produced with animal rennet: Queso de Murcia al vino and Feta

- Queso de Murcia al vino

Queso de Murcia al vino is made from 100% goat's milk and coagulated with animal rennet. Its main characteristic is that the rind is washed with red wine, according to its PDO Product Specification (EC 2002a).

In this work, Queso de Murcia al vino cheesemaking was carried out in an industrial cheese factory located in Caravaca de la Cruz (Murcia, Spain). For its production, mesophilic cultures and calcium chloride (512-532 g/L) were added to pasteurized milk pre-heated at 34 °C, and then milk coagulants were added and gently stirred. After clotting, the curds were manually broken using a cheese lyre into medium-sized grains, the paste was washed, and the temperature increased up to 38 ºC. The curd was then transferred into plastic perforated moulds and automatically pressed to remove the whey. The cheeses were salted in brine (18%), soaked in red wine bath for one day, and ripened for 30 days under controlled conditions (9-13 °C and 70-90% relative humidity).

Control Queso de Murcia al vino cheeses were manufactured with animal rennet (QM_C) according to the procedure described by the PDO Product Specification.

Experimental Queso de Murcia al vino cheeses were produced with a reconstituted aqueous extract from flowers of spontaneous (QM_ExpSP) and cultivated (QM_ExpCT) *Cynara humilis* thistles, elaborated as previously described by Bande-De León *et al.* (2023)

- Feta

Feta is a PDO (EC 2002b) soft cheese without rind with a pure white colour and slightly acid taste. It is made of sheep's milk or from its mixtures with goat's milk, of which the latter must not exceed 30% of the total cheese milk (Anyfantakis 1991).

For this research, Feta cheese was produced in the cheese dairy plant of Moni Velas located in Vela, Ioannina (Greece). In brief, pasteurized goat (30%) and ewes' milk (70%) were first inoculated with a commercial mesophilic starter culture, and then rennet was added. The clotting time was 40-45 min. When milk coagulation was completed, the curd was cut into 1-3 cm cubes, allowed to rest to remove whey. The curd was then gently transferred into moulds for draining. The next day, salting of the cheeses was performed (once) using clean, thick grained salt. The cheeses were placed in containers and transferred to the ripening room at 16 °C. Subsequently, the containers were moved to the cold room (3 °C) where they remained for 2 months from the production date until maturation was complete.

Control Feta was produced with animal/calf rennet, (CHR-Hansen, Horsholm, Denmark) (F_C) according to the procedure described by Feta PDO Product Specification.

Experimental Feta was produced with crude aqueous extract from flowers of spontaneous (F_ExpSP) and cultivated (F_ExpCT) *Cynara humilis* thistles, as previously described by Bande-De León *et al.* (2023).

After ripening time, each cheese was shipped under vacuum to CREA laboratories to perform analytical determinations and related nutritional assessment.

References

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