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

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Research Article

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Abstract

Since ancient times, thistles have been used as clotting agents in the production of traditional cheeses, particularly in the Mediterranean area. In recent years, their use in cheesemaking has increased to satisfy the growing requests from vegetarian consumers. In this research paper, four different cheeses, typical of the Mediterranean area, were evaluated from a nutritional point of view: *Caciofiore* (from Italy) and *Torta del Casar* (from Spain), both typically produced using vegetable rennet, and *Queso de Murcia al vino* (from Spain) and *Feta* (from Greece), traditionally produced using animal rennet. All the cheeses were manufactured according to their traditional cheesemaking procedures and used as controls. Experimental cheeses were produced using aqueous extracts obtained from flowers of either spontaneous or cultivated thistles indigenous to the Mediterranean area (respectively *Onopordum tauricum* for Caciofiore, and *Cynara humilis* for Torta del Casar, Queso de Murcia al vino, and Feta). All cheeses were characterized for fat-soluble and mineral compounds to assess their nutritional adequacy according to the recommended daily intake of each evaluated nutrient. All the cheeses were found to be a good source of vitamin A, calcium and phosphorus, with an optimal Ca/P molar ratio, except for Feta. By consuming the recommended serving (50 g) of the studied cheeses, the salt and cholesterol intake is, on average, 16.4% and 15.9%, respectively of recommended intake. The use of aqueous thistle extracts in cheesemaking appears to have no effect on the nutritional quality of the studied cheeses.

Plant-based replacements for dairy products have been gaining popularity worldwide, especially in North America, Europe and East Asia, driven by concerns about health and the environmental impact of dairy production (Schiano *et al.*, 2020; Carlsson Kanyama *et al.*, 2021; Ramsing *et al.*, 2023). Additionally, lactose intolerance is also a key factor affecting the dietary choices of some consumers. Sales of these products are constantly growing, however there is ongoing debate regarding their environmental impact and relative health benefits (OECD and Food Agriculture Organization of the United Nations, 2023). Health, religious, and dietary factors, along with changes in eating habits, have led to an increase in the demand for "vegan cheeses" or vegetarian-friendly alternatives to cheeses made with animal rennet, resulting in a growing interest in alternative sources of milk coagulants for cheesemaking. These sources include enzymes of microbial origins or plant extracts (Fresno *et al.*, 2023). As a result, vegetable rennet is becoming increasingly popular for coagulating milk in cheese production. In particular, thistle-curdled cheese is a type of cheese made using thistle-derived rennet instead of traditional animal-derived rennet. The process involves the use of vegetable extracts from thistle for milk coagulation, resulting in curds that are then processed into cheese.

Vegetable coagulants are obtained through the maceration in water of different parts or organs of the plant (leaves, flowers, seeds, rhizomes, etc: (Bande De León *et al.*, 2023). Many plant-derived extracts have been evaluated as milk coagulants in cheese production (Zhang *et al.*, 2023). Their clotting activity is attributed to specific proteases present in the plant extract, with specificity for cleaving the peptide bonds of caseins. Aspartic, serine or cysteine proteases, able to hydrolyse k-casein, have been reported in cardoon flowers such as *Cynara scolymus, Cynara cardunculus* or *Onopordum tauricum* (Nicosia *et al.*, 2022). The market for thistle-curdled cheese is growing, especially in regions like the Mediterranean. This growth is driven by increasing consumer demand for plant-based and sustainable food products (Aschemann-Witzel *et al,* 2021). Thistle-curdled cheeses can support local agriculture, particularly in Mediterranean areas where thistles spontaneously grow and are also cultivated. The use of thistles in cheesemaking also promotes biodiversity and encourages the cultivation of local plants, thereby supporting local ecosystems and economies.

For centuries, crude aqueous extracts from *C. cardunculus* flowers have been used as milk coagulants in the production of many sheep and goat milk cheeses, particularly in the Iberian Peninsula. Most of these Spanish and Portuguese cheeses are protected designation of origin (PDO) products (e.g., *Queijo Serra da Estrela, Torta del Casar, Queso de La Serena, Queso de Flor de Guía*, etc: Almeida and Simões, 2018). In Italy, some of these cheeses are included in the national list of *"traditional agri-food products"*, such as different local versions of Caciofiore (Rampanti *et al.*, 2023b). The use of extracts from *C. cardunculus* flowers in cheesemaking is associated with increased creaminess, softer textures, different taste attributes and a slightly piquant flavour (Tejada *et al.*, 2007). However, there are some limitations to their application at industrial scale due to a lack of standardisation (Almeida and Simões, 2018). The use of emerging sensory methodologies (ESM) could be helpful for a better sensory evaluation of these products (Ribeiro *et al.*, 2024).

The use of *C. cardunculus* was previously reported in the cheesemaking of *Queso de Murcia al vino* (Tejada *et al.*, 2008), a Spanish cheese produced with goat's milk, in comparison to the control cheese made with animal rennet. The type of coagulant affected pH, titratable acidity, ash, and calcium contents, whereas esterified fatty acids did not significantly change. Many other studies have been reported in the literature exploring the potential use of vegetable rennet from other thistles, such as *Cynara scolymus, Cynara humilis* and *Onopordum tauricum* (Almeida and Simões, 2018; Bravo Bolívar *et al.*, 2023; Rampanti *et al.*, 2023a, 2023b), which are common in the Mediterranean area, growing in countries such as Italy, West Africa and the Iberian Peninsula. However, nutritional evaluation of cheeses produced with vegetable rennet is a topic rarely discussed in the literature.

Milk and dairy products are known for their unique combination of micronutrients and associated health benefits (Gaucheron, 2011). Dairy products are considered rich sources of calcium, which is present in a highly bioavailable form (Paggio *et al.*, 2023). Additionally, they are good sources of other minerals and vitamins (Manzi *et al.*, 2013), including phosphorus, magnesium, zinc, selenium, and vitamins (Manzi *et al.*, 2021; Zhou *et al.*, 2022). However, dairy products are also associated with a high content of cholesterol and saturated fatty acids (Rashidinejad *et al.*, 2017). Earlier literature associated dairy products with low-density lipoprotein cholesterol levels, a marker for cardiovascular disease risk (Hooper *et al.*, 2020). Howver, more recent studies indicate that dairy foods, especially cheese, benefit from a matrix effect, making them neutral or even beneficial in terms of risk. The term '*food matrix*' refers to the overall structure of a food, including the arrangement of nutrients and components within it, and how these elements interact with one another (Thorning *et al.*, 2017). In cheese various components within the dairy matrix interact with its overall structure, resulting in notable health advantages. For example, the presence of proteins, fats, minerals, and vitamins in cheese doesn't just deliver individual benefits, their interaction within the cheese matrix enhances nutrient absorption and utilization by the body. (Feeney and McKinley, 2020; Feeney *et al.*, 2021).

This work is part of the PRIMA Project "Valorisation of thistle-curdled cheeses in the mediterranean marginal areas" (https://veggiemedcheeses.com/), aimed at improving and valorising typical products of the Mediterranean area, with particular attention to the production of cheeses made with vegetable coagulants (aqueous extracts from wild and cultivated thistles with sustainable agronomic practices), in order to obtain new cheeses based on vegetable rennet. This PRIMA Project involved replacing animal rennet with vegetable rennet (both spontaneous and cultivated) in cheeses typically made with animal rennet (Queso di Murcia al vino and Feta). Moreover, for cheeses normally produced with vegetable rennet (Caciofiore and Torta del Casar), the Project aimed to substitute the commercial rennet with a local vegetable rennet, whether cultivated or spontaneous in Mediterranean areas. For these reasons, *Onopordum tauricum* and *Cynara humilis*, generally used only for very small-scale local cheesemaking, were previously evaluated for their clotting activity (Foligni *et al.*, 2022; Bande De León *et al.*, 2023) and used to produce traditional Mediterranean cheeses: *Caciofiore* from Italy, *Torta del Casar* and *Queso de Murcia al vino* from Spain, and *Feta* from Greece. Caciofiore and Torta del Casar cheeses are already curdled with vegetable rennet, namely *Cynara cardunculus*, which undoubtedly represents the most exploited species of cardoon in cheesemaking. However, to increase the quality and sustainability of their cheesemaking process, aqueous extract from other species of spontaneous and sustainably cultivated thistles, namely *Onopordum tauricum* and *Cynara humilis*, were exploited to produce *Caciofiore* and *Torta del Casar*, respectively. Aqueous extracts from spontaneous and cultivated *Cynara humilis* were also used as clotting agents in the production of *Queso de Murcia al vino* and *Feta* cheeses, currently produced with animal rennet.

In this research paper, nutritional evaluation of these Mediterranean cheeses (*Caciofiore, Torta del Casar, Queso de Murcia al vino*, and *Feta*), made with aqueous extract from typical thistles of the Mediterranean area (*Onopordum tauricum* and *Cynara humilis*), was addressed to assess the daily intake of recommended minerals, fat-soluble vitamins (A and E) and cholesterol, provided by one serving of each type of cheese. This aspect is generally neglected in the literature despite the considerable number of scientific works concerning the production and characterization of thistle-curdled cheeses. To the best of authors' knowledge, this is the first paper addressing the nutritional assessment of such cheeses.

Materials and methods

Cheesemaking

The differnet cheeses produced are described here, and details of the cheesemaking procedures are given in the Supplementary Materials.

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

Control Caciofiore (CF_C) cheeses were manufactured with commercial liquid *C. cardunculus* rennet. 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. Control Torta del Casar (TC_C) were produced with commercial liquid *C. cardunculus* rennet according to PDO procedure

(EC, 2003). 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)

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

Control Queso de Murcia al vino was manufactured with animal rennet (QM_C) according to the procedure described by the PDO Product Specification (EC, 2002a). 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). Control Feta was produced with animal/calf rennet (F_C) according to the procedure described by Feta PDO Product Specification (EC, 2002b). 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.

Analytical determinations

Compounds of the unsaponifiable fraction (retinol isomers and β carotene, α tocopherol, and cholesterol) were determined according to Panfili *et al.* (1994) by normal phase high performance liquid chromatography. Detailed experimental conditions and equipment were described in the work of Paggio *et al.* (2023). Vitamin A was calculated from the determination of retinol isomers (13-cis and trans retinol) and β-carotene if any, considering the different contributions to vitamin A of all compounds showing the biological activity of retinol (Weiser and Somorjai, 1992). For vitamin E, the amount of α-tocopherol was considered. Total solids content was detected according to ISO (2004). Minerals (Ca, P, Na, K, Mg, and Zn) were determined according to AOAC (2002) method by atomic absorption spectroscopy. Further details and experimental conditions are described in the work of Paggio *et al.* (2023).

Estimated dietary intake

The estimated dietary intake (EDI) for adults was considered as the percentage contribution of the intake of the compounf of interest through each cheese consumption to the daily reference intakes set by the authority. It was calculated using the following equation:

$$\mathrm{EDI}(\%) = C \times IR \times 100/\text{Reference intake}$$

where C represents the concentration of the studied nutrient in cheese (g/100 g of cheese), and IR is the ingestion rate (g/day) of cheese, equivalent to a serving of 50 g/person (SINU, 2014). Reference Intakes for adults are set by Regulation EU (2011). No reference intake or recommended value is set in Regulation EU (2011) for cholesterol, therefore, the suggested dietary target for cholesterol (<300 mg/day) set by SINU (2024) was considered. Moreover, SINU (2014) suggested three servings of 50 g weekly for cheese with > 25% fat.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed with the mean and standard deviation. One-way analysis of variance (ANOVA) test, coupled with the Tukey's post hoc test, was performed to evaluate significant differences ($P < 0.05$) among the mean values. Statistical analysis was performed using XL-STAT Base 18.06 (Addinsoft 1995–2017).

Results and discussion

In Table 1, the contents of cholesterol (mg/100 g dry weight), fat-soluble vitamins (μg/100 g dry weight) and minerals (mg/100 g dry weight) of the studied cheeses are reported. For each type of cheese under study, control and experimental samples showed similar cholesterol contents, except for Torta del Casar where TC_ExpSP shows a statistically lower value compared to TC_C and TC_ExpCT. Though, as López (2012) indicates, the kind of coagulant had no effect on fat or protein cheeses, it is possible that the clot network created with wild *C. humilis* may retain more cholesterol than the other two coagulants. Among all cheeses, Caciofiore had, on average, the lowest cholesterol content, while Queso de Murcia al vino showed the highest ($P < 0.01$).

There is limited data in the literature regarding the cholesterol content of these cheeses, except for Feta. (Andrikopoulos *et al.*, 2003). According to the authors, cholesterol levels in different Greek Feta cheeses ranged from 129 to 180 mg/100 g dry weight. These data agree with the results of this work, where cholesterol ranged from 165.4 to 191.9 mg/100 g dry weight for Feta cheese (Table 1), with no significant differences among control and experimental cheeses. Fat-soluble vitamins (E and A) showed no significant variation between control and experimental cheeses for Caciofiore and Feta. In contrast, some significant differences were observed for the Spanish samples Queso de Murcia al vino and Torta del Casar. These differences are probably not due to the type of coagulant (vegetable or animal rennet). Moreover, Queso de Murcia al vino and Torta del Casar are made with goat or ewe milk, hence only retinol isomers contribute to their vitamin A content. In these kinds of milk, carotene is absent because it is totally converted into vitamin A (Park and Haenlein, 2013; Raynal-Ljutovac *et al.*, 2008; Verruck *et al.*, 2019).

In the literature, there is very scarce data on fat-soluble vitamins for the cheeses studied in this work. Kondyli *et al.* (2016) reported a decrease in retinol and alpha tocopherol during the ripening of Feta cheese, attributed to the sensitivity of these vitamins to light, oxygen and temperature. At 60 days of ripening time (standard Feta marketing period), vitamin A and E levels were 1.22 and 6.75 μg/g of edible weight, respectively. However, compared to our data, the vitamin A levels reported by Kondyli *et al.* (2016) were markedly lower. As it is well known, variations in fat-soluble vitamins in dairy products are attributed to animal feed, breed and cheesemaking procedures (Lucas *et al.*, 2006).

Regarding minerals (Table 1), Caciofiore showed the highest calcium and phosphorus levels, while Feta showed the lowest ($P < 0.01$). Contents of calcium and phosphorus in Queso de Murcia al Vino and Torta del Casar were lower than those reported by Moreno-Rojas *et al.* (2010). No significant differences in the calcium levels were observed in Queso de Murcia al vino between control (QM_C) and experimental cheeses produced with aqueous extract from flowers of spontaneous (QM_ExpSP) and cultivated (QM_ExpCT) *Cynara humilis*. However, in a previous work by Tejada *et al.* (2008), the authors observed an effect of the type of

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Table 1. Content of cholesterol, vitamins E and A and minerals in control and experimental cheeses

		Cholesterol		*Vit E*		*Vit A*		*Ca*		*P*		*Na*		*K*		*Mg*		*Zn*	
Cheeses		*mg/100 g dw*		*µg/100 g dw*				*mg/100 g dw*											
CF_C	*mean*	123.9	*a*	1974.4	*a*	600.0	*a*	1243.4	*b*	909.1	*a*	1453.2	*a*	232.3	*a*	78.7	*a*	4.2	*a,b*
	sd	9.9		741.2		71.9		187.2		141.2		195.1		57.4		9.0		1.1	
CF_ExpCT	*mean*	130.6	*a*	2538.1	*a*	618.7	*a*	1357.6	*a.b*	893.9	*a*	1524.9	*a*	160.6	*b*	75.5	*a.b*	3.5	*b*
	sd	15.2		390.0		68.6		47.5		39.8		103.6		9.4		0.8		0.1	
CF_ExpSP	*mean*	122.7	*a*	1890.6	*a*	586.0	*a*	1423.1	*a*	1006.5	*a*	1513.0	*a*	209.6	*a.b*	70.4	*b*	4.8	*a*
	sd	8.8		646.7		53.7		116.9		111.9		391.3		46.6		7.9		0.8	
TC_C	*mean*	161.9	*a*	1185.4	*b*	560.7	*b*	894.2	*a*	621.4	*a*	1122.1	*a*	268.7	*a*	64.7	*a*	3.2	*a*
	sd	10.8		349.9		30.4		136.6		164.3		184.4		12.2		5.5		1.0	
TC_ExpCT	*mean*	167.0	*a*	843.3	*c*	567.8	*b*	661.1	*b*	562.8	*a*	1259.0	*a*	277.7	*a*	53.6	*b*	3.2	*a*
	sd	7.3		74.7		37.8		14.8		44.8		46.1		17.6		1.6		0.2	
TC_ExpSP	*mean*	149.9	*b*	1684.5	*a*	688.7	*a*	941.2	*a*	729.9	*a*	1210.4	*a*	265.3	*a*	66.7	*a*	4.0	*a*
	sd	3.2		75.2		21.9		55.5		22.4		175.2		8.1		2.4		0.1	
QM_C	*mean*	192.0	*a*	1033.2	*a*	414.2	*a*	998.6	*a*	655.1	*b*	679.0	*a*	207.1	*a*	58.8	*a*	4.3	*b*
	sd	4.8		40.7		12.8		60.7		6.5		40.0		3.0		1.0		0.1	
QM_ExpCT	*mean*	187.5	*a*	800.5	*b*	393.5	*b*	1000.6	*a*	701.7	*a*	644.3	*a*	174.4	*b*	56.8	*b*	4.8	*a*
	sd	1.5		176.8		13.5		28.6		13.0		26.3		19.6		1.0		0.4	
QM_ExpSP	*mean*	190.4	*a*	1068.9	*a*	412.9	*a*	938.7	*a*	651.7	*b*	571.6	*b*	189.8	*a.b*	56.8	*b*	4.3	*b*
	sd	5.7		41.1		11.3		34.7		15.4		67.8		4.5		1.7		0.1	
F_C	*mean*	165.4	*a*	1340.0	*a*	621.7	*a*	338.2	*b*	393.2	*b*	1951.9	*a*	94.6	*a*	20.2	*b*	1.9	*b*
	sd	13.1		139.2		141.4		98.6		50.4		331.8		41.4		4.8		0.2	
F_ExpCT	*mean*	170.8	*a*	1484.3	*a*	585.0	*a*	425.9	*b*	526.0	*a*	1800.9	*a*	114.3	*a*	14.4	*b*	2.0	*b*
	sd	3.7		9.5		18.6		9.7		4.7		13.8		1.1		0.1		0.0	
F_ExpSP	*mean*	191.9	*a*	1449.7	*a*	707.5	*a*	633.0	*a*	584.9	*a*	2469.5	*a*	66.4	*a*	29.2	*a*	2.7	*a*
	sd	30.0		365.0		58.3		100.3		22.6		576.1		15.9		8.1		0.4	

CF: Caciofiore, TC: Torta del Casar, QM: Queso de Murcia al vino, F: Feta.
C: control, ExpCT: cultivated thistle extract, ExpST: spontaneous thistle extract. For details see Material and method section
Data are mean ± standard deviation of three independent measurement
In column, for each cheese, different letters mean significant differences ($P < 0.05$).

coagulant (*Cynara Cardunculus vs.* animal rennet) on the calcium levels in Queso de Murcia al vino, but not on phosphorus levels. To the contrary, we observed a significantly higher phosphorus content in Queso de Murcia al vino made with aqueous extract from flowers of cultivated *Cynara humilis* compared to the control cheese and cheeses made with aqueous extract from flowers of wild *Cynara humilis*.

Commonly, sodium shows significant variation among different types of cheeses (McSweeney, 2007), as the cheesemaking process markedly affects its content. In this work, on average, the highest sodium levels were observed in Feta cheeses, while Queso de Murcia al vino showed the lowest contents ($P < 0.01$). Gatzias *et al.* (2020) were able to discriminate Feta samples produced in various regions of northern Greece based on specific physicochemical parameters, including sodium content. According to the authors, cheeses from the Ioannina region, the same where the Feta cheeses of this work were produced, showed the highest sodium contents (3.28% of salt in edible weight). Instead, slightly lower sodium levels (ranging from 964 to 1800 mg/100 g dry weight) in Feta produced from the milk of ewes and goats grazing in the Greek mountainous region were reported by Maggira *et al.* (2023). High sodium contents were reported by Moatsou *et al.* (2004) when evaluating Feta produced with artisanal liquid rennet: at 60 days of ripening, the salt content was 3.40 and 3.73% (2.8 and 3.1 g of sodium per100g dry weight) in control (commercial animal rennet) and experimental cheeses, respectively.

Concerning the sodium content of Queso de Murcia al vino, we found values of the same order of magnitude as those previously reported by Moreno-Rojas *et al.* (2010). The same authors (Moreno-Rojas *et al.*, 2010) also reported the sodium content of Torta del Casar as 1007 mg/100 g dry weight. This value is in accordance with our own data (1122 mg/100 g dry weight). As for Caciofiore, no significant differences in sodium content were observed between control and experimental samples. Our results herein are consistent with those reported by Rampanti *et al.* (2023b) in a previous study on Caciofiore cheese. In more detail, the authors observed sodium values ranging from 1066 to 1256 mg/100 g of dry weight in control cheese manufactured with a commercial thistle rennet and from 1096 to 1341 mg/100 g of dry weight in experimental cheeses manufactured with *O. tauricum* extracts.

The aqueous extracts of both *Cynara humilis* and *Onopordum tauricum* have been previously characterised for their high potassium levels (Bande De León *et al.*, 2023; Foligni *et al.*, 2022). Specifically, values ranging from 5358 to 7578 mg/100 g dry weight were found in the extracts obtained from the flowers of these thistle species (Bande De León *et al.*, 2023; Foligni *et al.*, 2022). However, the experimental cheeses did not result in high potassium content due to the low amount of coagulant used compared to the processed milk and a likely loss in whey. Moreover, Foligni *et al.* (2022) observed that the extracts obtained from flowers of cultivated *O. tauricum* were characterized by a higher content of macro elements compared to the same extract obtained from wild plants. However, this difference is not noticeable in the resulting Caciofiore cheeses (Table 1). Cheesemaking certainly affects the retention of minerals in the curd. In previous research (Rampanti *et al.*, 2023b), comparing curdled Caciofiore prototypes with wild or cultivated *Onopordum. tauricum*, only a few rennet-dependent differences emerged, such as a lower SN/TN% ratio, a lower titratable acidity and a darker colour compared to control cheeses.

The magnesium content exhibited significant variability among the four cheeses, with levels ranging from 14.4 to 78.7 mg/100 g dry weight (Table 1), with Feta displaying the lowest contents ($P < 0.05$). The magnesium contents in Queso de Murcia al Vino and Torta del Casar were quite similar to those reported by Moreno-Rojas *et al.* (2010). Zinc levels ranged from 1.9 to 4.8 mg/100 g dry weight (Table 1). On average, Caciofiore and Queso de Murcia al vino showed the highest contents, while Feta showed the lowest ($P < 0.05$). The zinc contents observed for Queso de Murcia al vino and Feta were higher than those reported in the literature (Dai *et al.*, 2023; Moreno-Rojas *et al.*, 2010), while similar zinc contents were reported by Moreno-Rojas *et al.* (2010) for Torta del Casar.

Table 2. Molar ratio Ca/P in control and experimental cheeses

Cheeses		Ca/P
		Molar ratio
Caciofiore	CF_C	1.04
	CF_ExpCT	1.15
	CF_ExpSP	1.08
Torta del Casar	TC_C	1.17
	TC_ExpCT	0.90
	TC_ExpSP	0.98
Queso de Murcia al Vino	QM_C	1.16
	QM_ExpCT	1.08
	QM_ExpSP	1.09
Feta	F_C	0.64
	F_ExpCT	0.81
	F_ExpSP	0.61

CF: Caciofiore, TC: Torta del Casar, QM: Queso de Murcia al vino, F: Feta.
C: control, ExpCT: cultivated thistle extract, ExpST: spontaneous thistle extract. For details see Material and method section.

It is well established that an adequate calcium intake is essential for maintaining good bone health, with the optimal dietary Ca/P molar ratio being approximately 1.0 (Kemi *et al.*, 2010). During growth and adulthood, consuming a combination of calcium and phosphorus in a ratio similar to that found in dairy products can have a positive impact on bone health (Bonjour, 2011). In childhood, an adequate Ca/P ratio (ranging from 0.9 to 1.7) is required (SCF – Scientific Committee on Food, 1993), while in adults, this ratio can tolerate variations depending on the diet. According to our results (Table 2), all cheeses showed a good Ca/P molar ratio, with Caciofiore, Queso de Murcia al vino, and Torta del Casar showing, on average, the highest value ($P > 0.05$). Hence, consumption of all these cheeses may positively impact bone health, potentially reducing the risk of osteoporosis and other bone-related issues (Givens, 2017).

Cheeses contain many essential nutrients, including proteins, vitamins, and minerals such as calcium, phosphorus, magnesium, potassium, and zinc. However, evidence suggests that dairy products offer specific health benefits that cannot be solely attributed to individual nutrients (Peters, 2017). For instance, consuming full-fat dairy products has been shown to help maintain a healthy body weight, reduce the risk of cardiovascular diseases and type 2 diabetes, and improve bone health (Givens, 2017; Thorning *et al.*, 2017). These positive effects are not simply due to the presence of certain nutrients in dairy products. Instead, it is believed that the structure of dairy products causes interactions within the

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dairy matrix, resulting in various metabolic reactions with beneficial outcomes (Feeney and McKinley, 2020; Feeney *et al.*, 2021). Dairy products contain cholesterol and saturated fats, which may adversely affect human cholesterol concentrations. However, it is worth noting that the role of dietary cholesterol in cardiovascular risk is controversial and may depend on individual predisposition to synthesize versus absorb cholesterol (Visioli and Strata, 2014).

A healthy adult should include cheese as a part of a balanced diet. However, it is important to note that the SINU (2014) guidelines advise limiting cheese consumption to no more than three servings (50 g) of cheese per week. Table 3 shows the nutritional evaluation of the cheeses studied. According to the authors' knowledge, this is the first study where cheeses produced with rennet coming from aqueous extracts of spontaneous and cultivated thistle flowers are compared with their relative control cheeses. Data given in Table 3, refer to one serving of 50 g. Caciofiore and Torta del Casar account for 13.7% and 14.1%, respectively, of the suggested dietary target for cholesterol (<300 mg/day), while Feta and Queso de Murcia al vino have the highest values, at 15.7% and 20.1%, respectively. From these results, a portion of cheese contributes on average, about 15.9% of cholesterol. Replacing animal rennet with vegetable rennet or replacing commercially vegetable rennet with different types of vegetable rennet, does not seem to affect the nutritional assessment of cholesterol in cheese.

The daily percentage contributions to the reference intakes of fat-soluble vitamins for the studied cheeses for one serving (50 g) is reported in Table 3. Vitamin E (α-tocopherol) is a scavenger of peroxyl radicals and is a component of the antioxidant defence system, specifically protecting polyunsaturated fatty acids found in membrane phospholipids and plasma lipoproteins. Dietary sources of α-tocopherol typically include whole grain cereals, nuts, seeds, egg yolks, certain fatty seafood, vegetable oils, and fat spreads derived from vegetable oils (EFSA Panel on Dietetic Products Nutrition and Allergies, 2015b). It has been shown that cheeses do not contain a significant amount of vitamin E (Raynal-Ljutovac *et al.*, 2008) and, as expected, the contribution of the studied cheeses to the standard reference intake for vitamin E is limited, ranging from 2.6% (Queso de Murcia al vino) to 5.8% (Caciofiore).

Dairy products, including cheeses, are recognized as a good source of vitamin A. According to Regulation EU (2011), a food item is considered a source of vitamins and minerals if it contains more than "*15% of the nutrient reference values per portion*". In all the studied cheeses (Table 3), a serving (50 g) provides more than 15% contribution to the daily reference intake of vitamin A. Caciofiore has the highest value, with an average of 24.5%, while Queso de Murcia al vino has the lowest one, with an average of 16.2%. Referring to the daily % contribution of fat-soluble vitamins A and E, neither Caciofiore nor Feta show differences between the control cheeses and the experimental cheeses. In the case of the Spanish cheeses, however, both in Queso de Murcia al vino and in Torta del Casar, the experimental cheeses with cultivated thistle show the lowest percentage contribution of both vitamins.

The daily percentage contributions to the reference intakes of minerals in control and experimental cheeses are also shown in Table 3. A well-known advantage of cheese consumption is related to its high calcium concentration, which is crucial for maintaining strong bones and teeth. In addition, calcium in cheese is known to positively affect blood pressure and, when combined with a low-energy diet, can even contribute to weight loss. Moreover, the presence of bioactive peptides (caseinphosphopeptides) and the absence of chelating agents promote calcium absorption from cheese (Walther *et al.*, 2008). A serving of cheese provides a significant nutritional intake of calcium. Among the cheeses under study, the calcium intake was highest in Caciofiore, followed by Queso de Murcia, Torta del Casar, and Feta. Notably, Feta cheese showed a particularly low calcium content. This is likely attributed to calcium migration into the preservation brine and the high sodium content of Feta, which can affect the calcium distribution by replacing casein calcium (Panteli *et al.*, 2015).

Phosphorus is the second most abundant mineral in the human body, following calcium. It plays a crucial role in bones and teeth formation, being a fundamental constituent of their mineral fraction. Additionally, phosphorus is a component of enzymes, proteins, phospholipids, nucleic acids, and nucleotides. Moreover, it allows the proper functioning of certain vitamins. In the blood, phosphorus acts as an important buffer system for regulating pH levels (Bird and Eskin, 2021). One serving (50 g) of the studied cheeses guarantees, on average, 29.5% of the recommended daily intake of this element (Table 3). As already observed for calcium, Caciofiore showed the highest phosphorus nutritional intake, while Feta had the lowest.

Sodium is the primary cation within extracellular fluids. It helps to regulate fluid and electrolyte balance, blood pressure and cellular homeostasis. However, an excessive intake of sodium is a cause of raised blood pressure and increased risk of cardiovascular disease. According to the World Health Organization (2023), the global average sodium intake is 4310 mg/day, more than twice the recommended value of < 2000 mg/day of sodium for adults. Reducing sodium intake can prevent numerous cardiovascular events and deaths at a minimal cost. Consequently, WHO recommends several sodium-related policy actions (e.g., mass media campaigns, lowering sodium content in food products, implementing front-of-pack labeling to help consumers) that should be promptly undertaken to prevent cardiovascular disease and its associated costs. In cheese, salt is necessary to reduce spoilage and prevent the growth of pathogens, but high sodium intake through the diet has been reported to favor calcium excretion, which may lead to osteoporosis (Guinee and Fox, 2017). Consuming a serving (50 g) of the cheeses under study allows a daily sodium intake ranging from 8.5% (Queso the Murcia al vino) to 23.1% (Feta).

Dairy products are not primary sources of magnesium (EFSA Panel on Dietetic Products Nutrition and Allergies, 2015a). In the studied cheeses, magnesium intake per serving (50 g) averaged 4.3 %, with Feta showing the lowest contribution (1.5%) to the daily intake of this mineral and Caciofiore the highest (6.5 %). Regarding the daily percentage contribution to potassium intake with a serving (50 g) of cheese, the cheeses under investigation (on average 2.8 %) confirm what was previously reported (EFSA Panel on Dietetic Products Nutrition and Allergies, 2016). Cheeses are not a significant source of this element.

Zinc is a mineral that plays a significant role in several crucial biological processes in the human body. The daily dietary requirement for zinc depends on phytate intake and body weight (EFSA Panel on Dietetic Products Nutrition and Allergies, 2014). According to Regulation EU (2011), the adult's daily reference intake for zinc is 10 mg/day. However, it is important to highlight that this value has some limitations, as it does not account for differences in diet composition, especially the inhibitory effect of dietary fibre, phytate, or polyphenols on zinc bioavailability and absorption. While vegetables contain high amounts of phytate, therefore providing less available zinc, animal foods like dairy products do not contain phytate (Foster and Samman, 2015). Zinc content in animal food products is usually higher compared to vegetables. Consuming dairy products can significantly contribute to

Table 3. Daily % contribution of a portion (50 g) to the suggested dietary target for cholesterol[#] and to the reference intake[§] of vitamins E and A as well as minerals in control and experimental cheeses

Cheeses		Cholesterol		Vit E		Vit A		Ca		P		Na		K		Mg		Zn	
CF_C	*mean*	13.2	*a*	5.3	*a*	24.0	*a*	49.8	*b*	41.6	*b*	19.7	*a*	3.7	*a*	6.7	*a*	13.5	*a,b*
	sd	1.1		2.0		2.9		7.5		6.5		2.6		0.9		0.8		3.7	
CF_ExpCT	*mean*	14.2	*a*	6.9	*a*	25.3	*a*	55.5	*a,b*	41.7	*a,b*	21.1	*a*	2.6	*b*	6.6	*a*	11.4	*b*
	sd	1.7		1.1		2.8		1.9		1.9		1.4		0.2		0.1		0.3	
CF_ExpSP	*mean*	13.5	*a*	5.2	*a*	24.2	*a*	58.7	*a*	47.4	*a*	21.1	*a*	3.5	*ab*	6.2	*a*	15.8	*a*
	sd	1.0		1.8		2.2		4.8		5.3		5.5		0.8		0.7		2.5	
TC_C	*mean*	14.4	*a*	2.7	*b*	18.7	*b*	30.0	*a,b*	24.3	*a,b*	12.5	*b*	3.6	*b*	4.7	*a*	8.7	*b*
	sd	0.8		1.1		1.5		10.9		8.6		0.8		0.3		0.9		3.4	
TC_ExpCT	*mean*	13.1	*b*	1.7	*c*	16.7	*c*	19.5	*b*	19.0	*b*	12.6	*b*	3.3	*c*	3.4	*b*	7.5	*b*
	sd	0.4		0.2		0.9		0.2		1.1		0.2		0.1		0.0		0.3	
TC_ExpSP	*mean*	15.0	*a*	4.2	*a*	25.8	*a*	35.2	*a*	31.2	*a*	15.4	*a*	4.0	*a*	5.3	*a*	12.0	*a*
	sd	0.3		0.2		0.6		1.8		0.7		2.3		0.2		0.2		0.2	
QM_C	*mean*	19.9	*a*	2.7	*a*	16.1	*a,b*	38.7	*a*	29.0	*b*	8.9	*a*	3.2	*a*	4.9	*a*	13.2	*b*
	sd	0.5		0.1		0.5		2.4		0.2		0.5		0.0		0.1		0.2	
QM_ExpCT	*mean*	20.0	*a*	2.1	*b*	15.7	*b*	40.1	*a*	32.1	*a*	8.8	*a,b*	2.8	*b*	4.8	*a*	15.3	*a*
	sd	0.8		0.4		0.6		2.9		1.9		0.7		0.2		0.2		2.0	
QM_ExpSP	*mean*	20.5	*a*	2.9	*a*	16.6	*a*	37.8	*a*	30.0	*b*	7.8	*b*	3.1	*a*	4.9	*a*	14.0	*a,b*
	sd	0.7		0.1		0.6		1.1		0.6		0.9		0.1		0.1		0.3	
F_C	*mean*	15.0	*a*	3.1	*a*	20.8	*a*	11.3	*b*	15.2	*b*	22.2	*a*	1.3	*a*	1.4	b	5.1	*c*
	sd	1.1		0.6		2.4		2.8		0.9		1.4		0.6		0.3		0.2	
F_ExpCT	*mean*	16.2	*a*	3.5	*a*	20.8	*a*	15.2	*a,b*	21.4	*a*	21.7	*a*	1.6	*a*	1.1	b	5.8	*b*
	sd	0.3		0.0		0.7		0.4		0.2		0.2		0.0		0.0		0.1	
F_ExpSP	*mean*	15.9	*a*	3.0	*a*	21.6	*a*	19.2	*a*	20.5	*a*	25.3	*a*	0.8	*a*	1.9	a	6.6	*a*
	sd	3.7		1.0		0.6		1.4		1.1		3.8		0.1		0.4		0.4	

CF: Caciofiore, TC: Torta del Casar, QM: Queso de Murcia al vino, F: Feta.
C: control, ExpCT: cultivated thistle extract, ExpST: spontaneous thistle extract. For details see Material and method section
Data are mean ± standard deviation of three independent measurement
In column, for each cheese, different letters mean significant differences ($P < 0.05$)
Calculated according to the suggested dietary target for cholesterol (SINU, 2014);
§Calculated according to the reference values of European Food Safety Authority (2011).

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meeting the recommended daily intake of zinc (Manzi *et al.*, 2021). According to the FoodEx2 food classification system (European Food Safety Authority, 2011), "*milk and dairy products group*" contributes to zinc intake in adults (18 to <65 years), specifically for 12.1–23.9 % and 13.2 – 26.5 % for males and females, respectively (EFSA Panel on Dietetic Products Nutrition and Allergies, 2014). On average, a serving (50 g) of the studied cheese provides 10.7% of the daily reference intakes of zinc. In particular, a serving of Queso the Murcia and Caciofiore provides the highest daily zinc intake (14.1 % and 13.6 %, respectively), whereas a serving of Feta provides the lowest (5.8 %).

When evaluating the nutritional content of minerals in thistle-curdled cheeses, the existing data is insufficient to draw clear and definitive conclusions regarding the impact of the specific types of thistles used. Nevertheless, the results suggest that the nutritional assessment is only marginally affected by the cheesemaking process involving different varieties of thistles.

In conclusion, the studied cheeses were good sources of essential nutrients such as calcium, vitamin A, phosphorus and zinc, with an optimal Ca/P molar ratio that makes calcium and phosphorus easily absorbable by the body, although with Feta cheese being an exception to this rule. It is noteworthy that the contribution of the studied cheeses to the standard reference intakes for vitamin E, Zn, Mg, and K is limited, but this is a common characteristic among all types of cheese. To maintain a healthy intake of salt and cholesterol, it is recommended to limit cheese consumption to no more than three servings (50 g each) per week. The use of aqueous thistle extracts in the cheesemaking process, whether for cheeses traditionally produced with animal rennet or those made with commercial vegetable rennet, seems to have minimal impact on nutritional quality and does not negatively impact the health benefits typically derived by consumption of these types of cheese. These findings hold significant implications for the food industry, especially as consumer interest in cheeses made with vegetable rennet are constantly growing. By supporting the production of these plant-based rennet, the research could help manufacturers meet this increasing demand, while also preserving and promoting traditional cheese-making practices in the Mediterranean region. Additionally, the results may be of significant interest to consumers and health professionals, particularly those focused on the nutritional aspects of dairy products. As more consumers are looking for healthier options, this knowledge can guide informed choices and contribute to broader discussions on sustainable and health-conscious food production. Overall, this study highlights the potential for innovation in cheesemaking without compromising nutritional quality.

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