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Cladodes of *Opuntia ficus-indica* (L.) as source of bioactive compounds in dairy products

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ABSTRACT

Recently, the interest on improving livestock products' nutraceutical profiles by sustainable feeding systems has increased. In this context, the overall quality and, in particular, the nutraceutical profile of dairy products obtained by 16 lactating Cinisara cows fed integrated in dry season with *Opuntia ficus-indica* cladodes, were investigated. Two homogeneous groups of cows (milk yield: 6.3 ± 1.5 kg; body weight: 213 ± 55 kg) were in succession fed with 2 different diets (CON: pasture and wheat bran; OFI: pasture, wheat bran and cladodes), according to a 2×2 Latin square design. The bulk milk was used to make Caciotta cheeses, analyzed at 0, 15 and 30 storage days. Milk and cheeses samples were analyzed for chemical, physical and microbiological traits. On the final products, the nutraceutical and sensorial profiles, together with the antioxidant's capacity were also determined. On milk, only the urea content in individual samples was reduced in OFI. Considering the cheeses, the integration with cladodes did not influence the starter cultures development acted with 2 strains of *S. thermophilus*, but caused a higher content of polyphenols and a consequent greater antioxidant capacity, together with a change in the fatty acids profile. In particular, the caprylic, capric, lauric, myristic, and palmitic fatty acids were higher, as well as the petroselinic, vaccenic, rumenic, and α -linolenic fatty acids. Differently, the oleic and the γ -linolenic fatty acids were lower. The cheeses of OFI showed better overall acceptability, and a higher yellow color, odor intensities, and butter flavor. The multivariate analysis well distinguished the cheeses belonging to the 2 groups. Further investigations should be conducted to formulate well balanced diets including cladodes for Cinisara lactating cows, but also to determine the content

of other important bioactive compounds in fresh and in treated cladodes, as well as their effects on animals' welfare and their productions.

Keywords: Cinisara cow, cladodes, Caciotta, fatty acid, polyphenols, antioxidant capacity

INTRODUCTION

In recent years, the interest in the benefits for human health by consumption of functional foods as bioactive sources of antioxidants, vitamins, minerals, fiber, omega-3 fatty acids, antioxidants, and phenolic compounds has increased. This category includes vegetables (such as fruits, herbs, oil seeds, and vegetables), but also other foods or food products such as dairy products, fortified eggs, and seafood, presenting different functional components transferred by dietary intake in a closed relationship with the animal's physiology, which can modify levels and profiles of nutraceutical compounds in the final products (Arshad et al., 2021). In this context, many researchers have focused their attention on the use of natural resources (Alenisan et al., 2017; Salami et al., 2019; Benchaar, 2020) but also of industrial products and by-products in livestock feed to improve, in a sustainable and circular way, the nutraceutical profile of foods (Gaglio et al., 2021a; Santa et al., 2021; Todaro et al., 2021; Kolif & Olafadehan, 2022), taking also into account the possible transfer of environmental contaminants from feed to foodstuffs (Di Bella et al., 2020; Amutova et al., 2021; Giosuè et al., 2022).

From this perspective, *Opuntia ficus-indica* could be considered a strategic integrative resource in livestock rearing in the marginal Mediterranean, thanks to the cladodes' proprieties (Valentini et al., 2018; Gama et al., 2022) and the plant's adaptability, growing in arid, rocky and steep places (Vastolo et al., 2020; Prisa et al., 2021). In the subtropical Mediterranean climate, the growth of pastures begins in autumn at the time of the first rainfall that follows the dry summer period, with a slowdown in winter and intense spring growth when

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most of the plants bloom; finally, during the dry summer period the growth is interrupted and almost all of the pabular essences withers. In the presence of optimal soil conditions and water availability, the production of ensiled fodder represents an alternative to compensate for the summer deterioration of the pastures. However, in marginal areas it is difficult or impossible to produce silage; in these areas, the use of cladodes of *O. ficus-indica* represents a sustainable alternative (Nyamushamba et al., 2016; Oduor et al., 2022) for the administration of fresh forage in the dry period, when this plant presents high green matter production per unit area and a low NDF content requiring, therefore, an addition of a fiber source (Wanderley et al., 2012). The cladodes of *O. ficus-indica* change composition with the season, plant age, cladode order (position), cultivar, fertilization and harvest management, planting density, and environmental factors (Dubeux et al., 2021); in general, they present high moisture and energy contents, thanks to the high content of non-fibrous carbohydrates (Santos et al., 2017; Siqueira et al., 2017; Ferreira et al., 2022), and are rich in pectin, mucilage, minerals, malic acid, vitamins, carotenoids, polyphenols (tannins and flavonoids below the level considered harmful to the health of animals) and other antioxidants (Valentini et al., 2018; De Santiago et al., 2018; Rocchetti et al., 2018). However, cladodes are low in protein, and therefore nitrogen compounds should be included in the diet to increase the protein content (Mashoe, 2007; Felix et al., 2016).

Diverse compounds contained in cladodes can determine in different ways positive effects on animals' productive results. As known, the presence of compounds as mucilage, pectin, and phenols in animal feeds can improve the fatty acids profile in ruminant milk, modulating the microbiota in the rumen and/or the transit rate (de Araujo et al., 2021; Netto et al., 2022). Valentini et al. (2018) found that the fermentation of *Opuntia* pectin in ruminants can determine an improvement in the rumen environment in increased acetate production, influencing positively the fat synthesis in milk. Moreover, Gama et al. (2022) found that lactating cows fed with the integration of cladodes can increase the $\omega 6/\omega 3$ fatty acids ratio in milk, with a transfer of 18:2 $\omega 6$. Another study showed how the use of cladodes of *Opuntia* in a diet enriched with sorghum oil could reduce the biohydrogenation of polyunsaturated fatty acids in the rumen, increasing the 18:1 trans-11 and the C18:2 CLA cis-9, trans-11 in milk (Gama et al., 2021). Moreover, the use of cladodes in substitution of some kind of hay, such as *Cynodon* spp., or of floured corn, could decrease stearic acid (de Oliveira Moraes et al., 2019). Porto et al. (2020) observed also the maintenance of an optimal acetate:propionate ratio and

the increase in the production of microbial proteins in sheep fed with growing proportions of cladodes. Indeed, the high presence of rapidly fermentable carbohydrates and the consequent great levels of volatile fatty acids determined an increase in the height and surface of the papillae in the ruminal epithelium, in an attempt to absorb the larger quantities of volatile fatty acids produced. Another study conducted on dairy cows showed an increase in daily production with a linear reduction of urea nitrogen in milk and urine thanks to the replacement of corn silage with increasing percentages of *O. ficus-indica* cladodes (de Oliveira Moraes et al., 2019). Kamble et al. (2017) showed that cladodes can replace hay to 60%, with minimum effects on cow and sheep production. Moreover, their use as forage integration seems to improve the taste and color of dairy products (Shetty et al., 2012).

In Italy, the *O. ficus-indica* is mainly found in Sicily and Calabria, but it is also found in some mild-temperature areas of central and southern Italy (Sardinia, Apulia, Basilicata) (Prisa, 2021). In these regions, as well as in the arid areas of Africa and Brasil, the use of cladodes as feed integration for some autochthonous cattle and sheep breeds is recurrent in the dry period (Lins et al., 2016; Moraes et al., 2019; Saraiva et al., 2020; Nyambali et al., 2023). One of these breeds is the dual-purpose Cinisara cow, which is very adapted to be grazed in constrained environments, such as the marginal areas of north-western Sicily. The whole and raw milk is used to make, according to the traditional methods, both "Caciocavallo Palermitano" (a typical stretched-curd cheese with a firm paste and a parallelepiped shape) and "Caciotta" (fresh cheese with a spherical shape and a weight varying from 0.5 to 2 kg), while the meat is used for fresh consumption and recently also for the production of bresaola and salami (Gaglio et al., 2016; Alabiso et al., 2020).

The aim of the present study was to evaluate the effect of integrating *O. ficus-indica* cladodes in the diet of lactating Cinisara cows during the dry period (summer) on milk yield, and different traits of cheeses. In the specific, the microbiological and sensorial profiles together with those of specific bioactive compounds, as fatty acids and polyphenols, and the antioxidants capacity of final products, were investigated.

MATERIALS AND METHODS

Animals, experimental design and diets

The experiment was carried out for 6 weeks (June–July 2021) involving 16 Cinisara cows raised in a farm located at 540 m above sea level (a.s.l.) in Cinisi (Italy

- latitude 38° 07' 39" N – 13° 07' 42" E), in the typical production area of this breed.

The selected animals (body weight: 213 ± 55 kg - calving in autumn, winter, and spring) were divided into 2 homogeneous groups considering the lactation stage (154 ± 93 d) and the morning milk yield (6.3 ± 1.5 kg). The 2 groups were in succession fed with 2 different diets, according to a 2×2 Latin square design, with each phase composed of 2 weeks of adaptation to the diets and a week for data and sample collection (sampling week). Specifically, the cows were reared adopting the traditional semi-extensive system based on feeding in natural pastures integrated into the barn with the 2 following supplements: 4 kg/day of commercial wheat bran (CON) or 4 kg/day of commercial wheat bran plus 15 kg/d of fresh *Opuntia ficus-indica* cladodes (OFI) of 1-year and 2-year-old. The integration was divided into equal parts and given twice a day.

The experiment was conducted in accordance with the Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and received the approval of the local Bioethics Committee (protocol number: UNPA-CLE-Prot. 84097 DATA).

Feed sampling and analysis

In each sampling week (on d 3 and 5), the integrations (wheat bran and cladodes of *O. ficus-indica*) were sampled, and the cows were observed during the grazing, recording and sampling their selection of pabular resources, which were recomposed by manual plucking of plant parts following the method described by Di Grigoli et al. (2019). The feed administered into the stable was totally consumed by the animals, while the pasture ingestion was not evaluated. The samples, placed in sterile vacuum containers, were refrigerated at 8°C for the transfer to laboratory where they were processed as following: pasture (being dry) and wheat bran were homogenized and stored at -20°C; the cladodes were freeze-dried and then homogenized and stored at -20°C.

All feeds were analyzed for dry matter (DM - method 967.03), crude protein (CP, $N \times 6.25$ - method 988.05), ether extract (EE - method 920.29), and ash (method 942.05) contents, following the recommendations of the AOAC (2012). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined in accordance with Van Soest et al. (1991). Non-fiber carbohydrate (NFC) content was calculated as $(100 - [CP + EE + ash + NDFom])$.

Moreover, fatty acids (FA) from all feeds were extracted according to the method developed by O'Fallon et al. (2017), with C23:0 as the internal standard (Sigma-Aldrich, Milano, Italy). An autosampler in-

jected each sample (1 μ L) into an HP 6890 gas chromatography system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA, USA). The separation and identification of each FA were performed as described by Di Grigoli et al. (2022).

In all feed, the total content of phenolic compounds and antioxidant capacity (as Trolox equivalent antioxidant capacity - TEAC assay) were determined. The extraction was performed following the procedure described by López-Andrés et al. (2013).

On the extract, the total concentration of polyphenols was measured using the Folin-Ciocalteu colorimetric method, as described by López-Andrés et al. (2014). The absorbance of the samples was read at 725 nm using an HACH DR/4000U spectrophotometer (HACH, Loveland, CO, USA) against a blank containing all of the reagents except the sample extract. Gallic acid aqueous solutions of different concentrations (0–1 mg/ml) were used for the calibration curve ($R^2 = 0.99$). The results were expressed as grams of gallic acid equivalent (GAE) per kilogram of sample DM.

The antioxidant activity on extracts was determined by the TEAC assay, as described by Re et al. (1999). The absorbance of the samples was read at 734 nm using an HACH DR/4000U spectro-photometer (HACH, Loveland, CO, USA). The antioxidant capacity of samples was calculated by relating the percentage of inhibition to that measured under the same conditions (after 6 min and at 734 nm) exerted by solutions of known concentrations (ranging from 0 to 2.5 mM) of Trolox in PBS obtained from a stock solution of 2.5 mM in PBS; these results were used to perform a calibration curve ($R^2 = 0.99$). The results were expressed as mmol of Trolox equivalent (TE) per 100 g of sample DM.

The diet formulation, gross chemical composition and the fatty acids profile of the feed are reported in Table 1.

Milk sampling and analysis

For each experimental phase, during the sampling week on d 2,4 and 6, the cows were milked once a day (at 9:00 a.m.) and individual milk yield was recorded and sampled. The second milking (at 5:00 p.m.) was substituted by the calves' suckling. Moreover, on d 3 and 5 of the same week, bulk milk from the morning milking of each group were sampled and used to make Caciotta cheeses.

Individual and bulk milk samples were analyzed for fat, protein, casein, lactose, urea and somatic cells count by infrared method (CombiFoss 6000; Foss Electric A/S, Hillerød, Denmark); pH was measured with a 70+DHS pH-meter (XS Instruments, Carpi, Modena

Table 1. Diet formulation and feed composition (means $\pm$ SD)

Diet (fresh weight)	Bran	Cladodes	Pasture
Control (CON) (kg/animal per day)	4	0	ND
Experimental (OFI) (kg/animal per day)	4	15	ND
Chemical composition	Means value $\pm$ SD		
Dry Matter (DM), %	89.20 $\pm$ 0.80	11.88 $\pm$ 0.49	90.67 $\pm$ 0.20
Crude protein, % DM	18.60 $\pm$ 0.49	6.76 $\pm$ 0.18	5.88 $\pm$ 0.97
Ether extract, % DM	5.58 $\pm$ 0.62	1.90 $\pm$ 0.02	1.67 $\pm$ 0.25
Ash, % DM	5.46 $\pm$ 0.45	21.16 $\pm$ 0.35	8.95 $\pm$ 2.07
NDF, % DM	36.68 $\pm$ 1.15	34.52 $\pm$ 1.99	70.33 $\pm$ 0.83
ADF, % DM	16.01 $\pm$ 1.84	25.71 $\pm$ 2.79	64.53 $\pm$ 0.47
ADL, % DM	3.25 $\pm$ 0.28	3.07 $\pm$ 0.34	10.68 $\pm$ 1.27
NFC, % DM	34.18 $\pm$ 1.20	41.33 $\pm$ 1.54	16.24 $\pm$ 0.44
Total FA, % DM	5.50 $\pm$ 0.68	1.74 $\pm$ 0.13	1.48 $\pm$ 0.03
C12:0% FA	1.82 $\pm$ 0.59	3.83 $\pm$ 0.19	0.79 $\pm$ 0.17
C14:0% FA	0.18 $\pm$ 0.05	2.70 $\pm$ 0.28	2.17 $\pm$ 0.05
C15:1 cis % FA	0.13 $\pm$ 0.02	1.31 $\pm$ 0.03	0.00 $\pm$ 0.00
C16:0% FA	16.15 $\pm$ 0.41	21.65 $\pm$ 0.19	27.32 $\pm$ 0.87
C16:1% FA	0.20 $\pm$ 0.01	0.00 $\pm$ 0.00	0.60 $\pm$ 0.02
C17:0% FA	0.22 $\pm$ 0.00	0.76 $\pm$ 0.07	0.65 $\pm$ 0.05
C18:0% FA	1.56 $\pm$ 0.51	5.20 $\pm$ 0.14	5.92 $\pm$ 1.18
C18:1 cis ω 9 OA % FA	19.35 $\pm$ 0.94	10.75 $\pm$ 1.76	14.35 $\pm$ 0.10
C18:2 cis ω 6 LA % FA	50.90 $\pm$ 0.14	27.00 $\pm$ 0.63	28.19 $\pm$ 5.50
C18:3 cis ω 3 ALA % FA	4.66 $\pm$ 0.84	19.12 $\pm$ 3.89	6.29 $\pm$ 2.40
C20% FA	—	0.76 $\pm$ 0.10	—
Polyphenols, g GAE/kg DM	4.13 $\pm$ 0.84	16.74 $\pm$ 1.73	7.13 $\pm$ 0.55
Teac, mmol trolox/kg DM	46.83 $\pm$ 3.87	106.06 $\pm$ 6.39	69.08 $\pm$ 3.08

The results indicate mean values of three measurements performed on each sample. NFC: non-fiber carbohydrates = 100 – (CP + ether extract + ash + NDFom). OA = oleic acid. LA = linoleic acid. ALA = α -linolenic acid. DE = delphinidin equivalent. GAE = gallic acid equivalent. TEAC = trolox equivalent antioxidant capacity. TBARS = thiobarbituric acid–reactive substances. MDA = malonylaldehyde.

- Italy); titratable acidity was assessed by the Soxhlet-Henkel method ($^{\circ}\text{SH}/50\text{ mL}$).

Individual and bulk milk samples were evaluated for the aptitude to clotting by measuring clotting time (r, min), curd-firming time (k20, min) and curd firmness (a30, mm) with a Formagraph instrument (Foss Electric).

Natural Milk Starter Culture Development

The Natural Milk Starter Cultures (NMSC) was developed with 2 strains of *Streptococcus thermophilus* (WVS18 and WVS271) previously evaluated for their aptitudes in cheese production (Scatassa et al., 2015). These strains were first sub-cultured in Medium 17 (M17) broth (Biotec, Grosseto, Italy), washed and re-suspended in Ringer's solution (Sigma-Aldrich, Milan, Italy) as reported by Gaglio et al. (2021b). The washed cells of *S. thermophilus* were inoculated (1% vol/vol) into bovine whole-fat UHT milk (Granarolo, Bologna, Italy) and incubated at 30°C for 24 h. NMSC containing the multi-strain culture were then used for cheese production.

Cheese making and sampling

On d 3 and 5 of the 2 experimental weeks, a total of 30 kg of bulk milk of each animals' group (CON and OFI) was heated to 37°C, inoculated with the NMSC to a final cell densities of 10^7 cfu/mL and processed as described by Bonanno et al. (2013) to produce Caciotta cheese.

In each of 4 cheesemaking, 3 forms for each animals' group were obtaining.

At the end, a total of 24 cheeses (12 for each animals group) were made and weighted after 24 h (yield at 24 h). After weighing, the cheeses were placed in saturated brine (NaCl) at 5°C for 24h.

For each cheesemaking and for each animal group, 24 h after salting (d 0) one of the 3 wheels was sampled while the other 2 wheels were vacuum packed and stored in a refrigerator at 10°C to be sampled respectively at 15 and 30 d.

Microbiological investigation

Plate counts. Milk samples (1 mL) were directly serially diluted (Health Canada, 2015), while 10 g of curd and cheese samples were first homogenized in 90 mL of sodium citrate [2% (wt/vol)] solution by a stomacher (Solís et al., 2009) and then serially diluted. Cell

Table 2. Microorganisms and growth conditions

Microorganisms	Media	Incubation conditions	Company
TMM	Skim Milk Agar	30°C for 72 h	Microbiol Diagnostici, Uta, Italy
<i>Streptococcus thermophilus</i>	Medium 17 Agar	44°C for 48 h	Biotec, Grosseto, Italy
<i>Pseudomonas</i> spp.	<i>Pseudomonas</i> Agar Base	25°C for 48 h	Condalab, Madrid, Spain
Enterobacteriaceae	Violet Red Bile Glucose Agar	37°C for 24 h	Condalab, Madrid, Spain
<i>Listeria monocytogenes</i>	<i>Listeria</i> Selective Agar Base	37°C for 24 h	Oxoid, Hampshire, UK
<i>Escherichia coli</i>	Chromogenic Medium Agar	37°C for 24 h	Condalab, Madrid, Spain
<i>Salmonella</i> spp.	Hektoen Enteric Agar	37°C for 24 h	Microbiol Diagnostici, Uta, Italy
CPS	Baird Parker Agar	37°C for 48 h	Oxoid, Hampshire, UK

Abbreviations: TMM, total mesophilic microorganisms; CPS, coagulase positive staphylococci.

suspensions were plated on selective agar media to enumerate the main microbial groups associated with dairy productions as reported in Table 2. All plate counts were performed using the spread plate method, except those for the LAB and members of Enterobacteriaceae family, which were plated by pour plate (Tinebra et al., 2022). Microbiological analyses were carried out in duplicate at each sampling time.

Persistence of Starter Cultures. The dominance of *S. thermophilus*, inoculated as starter cultures, over indigenous milk lactic acid bacteria (LAB) was performed by randomly amplified polymorphic DNA (RAPD)-PCR analysis as reported by Guarcello et al. (2016). Briefly, the DNA extracted from the colonies of presumptive LAB (Gram-positive and catalase-negative) isolated from final cheese were used as template for PCR. The monitoring of the added strains was performed by comparison between RAPD profiles obtained from pure cultures isolated from control and experimental cheese and those of *S. thermophilus* WVS18 and WVS271.

Analysis on cheeses

Physical and chemical parameters. The cheeses at d 0, 15 and 30 were sampled and analyzed for color, pH, water activity, maximum resistance to compression and chemical composition.

The color of external and internal surfaces was assessed using a Minolta Chroma Meter CR300 (Minolta, Osaka, Japan), measuring L* (lightness, from 0 = black, to 100 = white), a* (redness, from red = +a, to green = -a) and b* (yellowness, from yellow = +b, to blue = -b) according to the CIE L*a*b* system (CIE, 1986).

The pH was measured with a 70+DHS pH-meter equipped with an electrode XS Sensor 2-Pore NTC for penetration measurements (XS Instruments, Carpi, Modena - Italy).

Water activity (a_w) was detected with a dew-point hygrometer HygroLab 3 (Rotronic, Huntington, NY,

USA). Calibration was performed using 5 saturated solutions of known a_w .

The maximum resistance to compression (compressive stress, N/mm²), as index of cheese hardness, was measured on samples (2 cm x 2 cm x 2 cm) at room temperature (25°C) with an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milan, Italy).

For each cheese, DM, EE, CP ($N \times 6.38$) and ash content were determined according to International Dairy Federation (IDF) standards [4A:1982 (IDF, 1982), 5B:1986 (IDF, 1986), 25:1964 (IDF, 1964a) and 27:1964 (IDF, 1964b) respectively]. The determination of soluble nitrogen was carried out by treatment with a sodium citrate solution and subsequent precipitation of the proteins at pH 4.6 (DM, 1986). The proteolysis index (PI) was calculated as the percentage ratio between NPN and total nitrogen (TN).

Bioactive compounds and human health indices. The cheeses were also analyzed for fatty acids profile, determining also the total content of phenolic compounds, together with the antioxidant capacity.

Fatty acids (FA) in lyophilised cheese samples (100 mg) were directly methylated as described by Loo et al. (2002). Fatty acid methyl esters (FAME) were recovered in hexane (1.5 mL). An autosampler injected each sample (1 μ L) into an HP 6890 gas chromatography system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA, USA). The separation and identification of each FA were performed as described by Di Grigoli et al. (2022). The health-promoting index (HPI) was calculated as suggested by Chen et al. (2004), following the formula reported below:

$$HPI = \frac{PUFA + MUFA}{C12:0 + (4 \times C14:0) + C16:0}.$$

The thrombogenic index (TI) was calculated according to Ulbricht and Southgate (1991), as below reported:

$$TI = (C14:0 + C16:0 + C18:0) / \left[\frac{(0.5 \cdot \Sigma MUFA) + (0.5 \cdot \Sigma PUFA6) + (3 \cdot \Sigma PUFA3) + (\Sigma PUFA3 / \Sigma PUFA6)}{2} \right]$$

Sensory evaluation. The evaluation of the sensory traits on cheeses at d 0, 15 and 30 was performed following the ISO guidelines (2007) by 13 judges (7 females and 6 males, 21–65 years old), that were trained in preliminary sessions following the ISO 8589 (2007) indications.

The following 12 descriptors were included in the analysis: structure uniformity, holes, intensity of odor, odor of butter, odor of milk, unpleasant odor, salty, sweet, acid, bitter, spicy, chewiness, solubility, grittiness, overall acceptability (Costa et al., 2018), following the ISO guidelines. The judges scored the level of each attribute, adopting a 10-point hedonic scale (0 = extremely low; 10 = extremely high) as reported by Faccia et al. (2013).

Statistical Analysis

The data of individual milk production were statistically analyzed by the SAS 9.2 software [2010], using a mixed model that included the experimental phase (2 levels), group (G, 2 levels), lactation stage (LS, 3 levels) as fixed effects and the cow (C, 16 levels) as random effect used as error term. Results are reported as LSM and differences between means were tested by Tukey's *t*-test. Statistical significance was attributed to *p* values <0.05.

The data of bulk milk, used for cheese making, and cheeses were statistically analyzed by the SAS 9.2 software (2010), using a generalized linear model (GLM) that included the effects of group (G, 2 levels), the aging (A, 3 levels) and the interaction G*A. Results are reported as LSM and differences between means were tested by Tukey's *t*-test. Statistical significance was attributed to *p* values <0.05.

To evaluate the specific contribution of the physical, chemical and sensorial traits in explaining the differences between cheeses of 2 groups, a principal component analysis (PCA) was carried out, with the PRINCOMP SAS procedure. The variables used in the analysis were standardized by multiplying them by the inverse of the standard deviation (1/SD) and identified by gradual selection with the STEPDISC SAS procedure. The selection of the main components was carried out according to the Kaiser method, keeping those with Eigen values higher than 1.00.

RESULT AND DISCUSSIONS

Feed

The pabular resources selected by cows were analyzed, showing a low nutritional value for the high fiber, and the low crude protein and ethereal extract contents, respectively. These results are in line with those found by other authors in similar areas in summer, a season characterized by dry conditions influencing negatively the quality of natural resources (Pulina et al., 2006; Sitzia et al., 2015; Scano et al., 2019).

In general, the cladodes showed a chemical composition in line with those found by other authors (Villegas-Díaz et al., 2008; Abidi et al., 2009; Pessoa et al., 2020), with a high water content representing an important integration for animals rearing in dry areas, and a great ash percent, principally represented by calcium, potassium, and magnesium as found in other studies (Bakari et al., 2017; Naorem et al., 2022).

Considering the investigated bioactive compounds, the PUFAs in cladodes amounted to 46.12%, followed by SFAs (34.90%) and the MUFA (12.06%), respectively. The linoleic (C18:2 *cis* ω6, 27.00%), the palmitic (C16:0, 21.65%), the α-linolenic (C18:3 *cis* ω3, 19.12%), and the oleic (C18:1 *cis* ω9, 10.75%) fatty acids were those more represented, similarly as found by Makhalemele (2020). The cladodes also showed a discrete polyphenol content, as other authors observed in cladodes in equal maturity stages (Astello-Garcia et al., 2015; Figueroa-Pérez et al., 2018).

Individual milk

The physical, chemical, and technological parameters of individual milk yield are reported in Table 3.

The production and composition of milk were similar in the 2 groups, similarly to what was found on lactating cows and camels receiving cladodes of *Opuntia stricta* as integration (dos Santos et al., 2022; Ikanya et al., 2022). Moreover, both groups showed a decreasing productive trend, as generally observed in summer, when animals are in the last phases of lactation in correspondence with quantitative and qualitative deterioration of grazing (Alabiso et al., 2006; Maniaci et al., 2021).

The integration with cladodes affected the urea content reducing its level (*P* = 0.008) in milk. Similar effect was also observed in other studies on cow (de Oliveira Moraes et al., 2019) and sheep (de Albuquerque Saraiva et al., 2020). The urea content in milk is related to crude protein ingestion, and both groups fed low crude protein diet, reflecting the poor quality of pasture. Indeed, the urea showed a content in the

Table 3. Physical, chemical and technological parameters of individual milk yield

	Group		SEM	<i>p</i> Value
	Control (CON)	Experimental (OFI)		
Daily milk yield, kg/animal/d	5.54	5.64	0.443	0.754
pH	6.57	6.58	0.022	0.818
Titrateable acidity, °SH/50 mL	4.19	4.31	0.142	0.138
Ether extract, %	2.88	2.92	0.232	0.874
Crude protein, %	3.36	3.34	0.114	0.587
Casein, %	2.66	2.64	0.096	0.603
Lactose, %	5.10	5.06	0.062	0.297
Urea, mg/dl	18.55	15.47	1.174	0.008
Somatic cells, log ₁₀	5.01	5.08	0.181	0.122
r, min	17.13	18.54	2.045	0.502
k20, min	4.73	4.28	1.021	0.654
a30, mm	25.53	29.08	2.425	0.243

The results indicate the mean values of measurements performed for each group. SEM = standard error of the mean.

range usually found in milk obtained by animals fed a low- crude protein diet (16–20 mg/dl). On the other hand, the value of urea >16 mg/dl in milk is today considered acceptable, and low-protein diets could represent a sustainable productive system reducing waste and increasing the nitrogen efficiency of the animals (Burgos et al., 2010).

Microbiological investigation

Evolution of Microbial Population During Cheese Making. The Caciotta cheeses produced in this study were made with raw cows' milk and the addition of selected starter cultures was necessary for curd acidification and cheese ripening (Settanni e Moschetti, 2010). To this purpose, the microbiological investigations based on the plate counts of the main microbial groups associated with dairy productions and the isolation and typing of LAB populations from the final cheeses were necessary to evaluate the effect of cows' diet, supplemented with *O. ficus-indica* cladodes, on cheeses microbiology. In fact, is well known that *O. ficus-indica* cladodes represent a useful source of polyphenolic compounds (Rocchetti et al., 2018) able to exert an antibacterial activity (Liguori et al., 2022).

The results of the plate counts carried out throughout cheese production from cows' milk to finished product are reported in Table 4.

The specific search for coagulase positive staphylococci (CPS), *E. coli*, *L. monocytogenes* and *Salmonella* spp., responsible for food-borne diseases associated to cheese consumption (Kousta et al., 2010), did not reveal their presence in either of the samples analyzed (therefore these results are not included in Table 4). No significant differences ($P > 0.05$) were found for the levels of microorganisms object of investigation among

control (CON) and experimental (OFI) productions during all steps of cheese making. Raw cows' milk hosted levels of TMM at about 10^4 cfu/mL, both in CON and OFI productions, complied fully the European Regulation 853/2004 (Commission Regulation, 2004) establishing that the maximum level of total bacteria at 30°C is 100.000 cfu/mL. Thermophilic coccus LAB were found at the same level of TMM, while member of Enterobacteriaceae and Pseudomonadaceae family, commonly associated with poor hygiene of dairy productions (Claeys et al., 2013), were 2 Log cycles lower. Similar results were previously reported by Settanni et al. (2012) and by Busetta et al. (2023a) in raw cows' milk used for Caciocavallo Palermitano and Caciocavallo Podolico productions, respectively. The TMM and thermophilic coccus LAB were found in inoculated milk with NMSC used for CON and OFI productions at almost the same levels inoculated, confirming that *S. thermophilus* inoculums occurred at 10^7 cfu/mL. After curdling, TMM, *S. thermophilus*, member of Enterobacteriaceae and Pseudomonadaceae family increased of about one Log cycle as a direct consequence of whey draining (Settanni et al., 2013). The levels of TMM and *S. thermophilus* reached values of about 9 log cfu/g, while members of Enterobacteriaceae, but also that of pseudomonads remained almost constant (10^2 cfu/g) in all curds. Busetta et al. (2023b) observed an similar cell densities in acidified curds used for the production of PDO Provola dei Nebrodi cheese. The cheeses soon after production as well as after 15 d and 30 d of refrigerated storage at 10°C showed very high levels of TMM and *S. thermophilus*, while Enterobacteriaceae and pseudomonads disappeared from both productions confirming the sanitizing effect of heating step (such as curd stretching for the caciotta cheese production) (Stellato et al., 2015). These results highlighted that

Table 4. Microbial loads of samples collected during experimental cheese productions

Samples	Microbial counts			
	TMM	Enterobacteriaceae	Pseudomonadaceae	<i>S. thermophilus</i>
<i>Raw milk</i>				
Control (CON)	3.62	1.83	2.26	3.51
Experimental (OFI)	3.57	1.71	1.89	3.40
SEM	0.07	0.11	0.16	0.06
<i>p</i> value	0.751	0.629	0.288	0.389
<i>Inoculated milk</i>				
Control (CON)	7.26	1.36	1.77	7.02
Experimental (OFI)	7.19	1.44	1.81	7.10
SEM	0.09	0.06	0.12	0.05
<i>p</i> value	0.754	0.538	0.884	0.485
<i>Curd</i>				
Control (CON)	8.03	2.11	2.56	8.11
Experimental (OFI)	8.09	2.02	2.61	8.23
SEM	0.06	0.08	0.06	0.05
<i>p</i> value	0.666	0.638	0.707	0.216
<i>Acidified curd</i>				
Control (CON)	9.12	1.75	2.14	9.10
Experimental (OFI)	9.04	1.69	2.12	9.00
SEM	0.05	0.07	0.04	0.13
<i>p</i> value	0.498	0.709	0.827	0.741
<i>Cheese at T₀</i>				
Control (CON)	8.61	<2	<2	8.40
Experimental (OFI)	8.77	<2	<2	8.61
SEM	0.05	NE	NE	0.08
<i>p</i> value	0.151	NE	NE	0.234
<i>Cheese at 15 d</i>				
Control (CON)	8.70	<2	<2	8.72
Experimental (OFI)	8.65	<2	<2	8.58
SEM	0.05	NE	NE	0.07
<i>p</i> value	0.675	NE	NE	0.339
<i>Cheese at 30 d</i>				
Control (CON)	8.83	<2	<2	8.66
Experimental (OFI)	8.72	<2	<2	8.74
SEM	0.06	NE	NE	0.09
<i>p</i> value	0.442	NE	NE	0.720

Units are Log cfu/mL for milk samples and Log cfu/g for curd and cheese samples. Results indicate the mean values $\pm$ standard deviation (S.D.) of four plate counts (carried out in duplicate for two independent productions). Abbreviations: TMM, total mesophilic microorganisms; *S.*, *Streptococcus*; SEM, standard error of the mean; NE, not evaluated.

the supplementation of cows' diet with *O. ficus-indica* cladodes did not alter the fermentation process and the microbiological aspects of the cheeses.

Starter Culture Recognition. Thirty-six colonies of presumptive LAB were isolated from control and experimental cheeses at 30 d of refrigerated storage. All isolates were subjected to RAPD analysis, a technique commonly used to perform the strain typing and to monitor the added starter LAB strains (Fusco et al., 2019). The polymorphic profiles obtained were compared with those of *S. thermophilus* added as fermenting agent, to evaluate their ability to persist during cheese productions. The direct comparison of the RAPD profiles of the LAB isolated from the CON (Figure 1 a) and OFI (Figure 1 b) cheeses allowed the recognition of both *S. thermophilus* WVS18 and WVS271, at similar levels, clearly evidencing their dominance over indigenous milk LAB.

These results confirmed those obtained by microbial counts that excluded any negative influence of cows' diet supplemented with *O. ficus-indica* cladodes during the cheese making.

Bulk milk and cheeses compositions

The bulk milk showed physical, chemical, and technological parameters in line with those found in individual milk samples (Table S1). The physical characteristics and the chemical composition of cheeses in relation to the aging stage did not show differences (Tables S2, S3, S4, S5 and S6). Therefore, the data are reported and described considering only the 2 groups (Tables 5).

Considering the investigated bioactive compounds, the cheeses of group OFI, made from the milk of cow fed integrating with cladodes, showed higher polyphenols contents than group CON (6.01 vs 4.67 g GAE/

kg DM; $P = 0.041$), probably for the contribution of 15 kg of ingested cladodes (29.74 g GAE), confirming a transfer of these compounds from the diet to dairy products (Gama et al., 2022).

Different authors found a certain bio accessibility of polyphenols in cheeses, and the consequent antioxidant properties (Gladine et al., 2007; Di Trana et al., 2015). The polyphenols' content influenced the antioxidants capacity, expressed as TEAC, which was higher in cheeses of group OFI than group CON (50.71 vs 49.73 mmol trolox/kg DM; $P = 0.040$), in line with the results found by Ponte et al. (2022) on sheep fed with fresh sulla.

The polyphenols content and the TEAC did not influence the oxidative stability of cheeses fat, expressed as POV (primary oxidative fat index), and TBARS (secondary oxidative fat index), even if the POV tended to be lower in cheeses of group OFI, probably the extent of the differences found was not enough.

The fatty acid profiles of the 2 groups are reported in Tables 6, 7, and 8.

Considering the saturated fatty acids (Table 6), the cheeses of group OFI showed higher caprylic (C8:0) ($P = 0.014$), capric (C10:0) ($P < 0.001$), lauric (C12:0) ($P < 0.001$), myristic (C14:0) ($P < 0.001$), and palmitic (C16:0) ($P < 0.001$) acids than cheeses of group CON. Similar results were also observed by other authors in milk of sheep and goat fed integrating with cladodes (Costa et al., 2010; Cordova-Torres et al., 2017;).

Conversely, the stearic acid (C18:0) showed a lower value in cheeses of group OFI than in those of group CON ($P < 0.001$), as found by other authors reporting a linearly reduced stearic acid content in the milk of cows fed increasing levels of cladodes (Costa et al., 2010; Oliveira et al., 2019; Gama et al., 2021)

Considering the unsaturated fatty acids (Table 7), the oleic (C18:1c9) ($P = 0.008$) and the γ -linolenic acids (C18:3 ω 6) ($P < 0.001$) showed higher contents in the group C; conversely, the petroselinic (C18:1c6 n12) ($P < 0.001$), the vaccenic (C18:1 c11) ($P < 0.001$), the rumenic (C18:2 c9t11, RA) ($P = 0.008$) and the α -linolenic (C18:3 ω 3) ($P < 0.001$) acids were higher in group OFI than in group CON.

In general, the oleic fatty acid (C18:1 c9), is found in high amounts in milk, deriving from ruminal biohydrogenation of PUFA, body fat reserves, and principally from diet (Wood et al., 2008). Cladodes presented low oleic fatty acid content, therefore their integration in group OFI could have determined both lower ingestion of this fatty acid at pasture (rich in oleic fatty acid) than group CON. Other authors also observed similar results in milk of sheep and goat fed integrating with cladodes (Costa et al., 2010; Cordova-Torres et al., 2017). The increase in RA in cheeses of group OFI and

the contextual reduction of C18:0 were consistent with the trends found by other authors in cow milk (Gama et al., 2020 and 2021), including cladodes in animals' diet, that could promote an incomplete biohydrogenation of PUFA in the rumen.

The results obtained showed a certain worsening of the health fatty acid profile in cheeses of group OFI, in particular for the higher amount of myristic acid, which presents an evident hypercholesterolemic effect on humans (Santos-Silva et al., 2002). However, recent studies based mainly on a meta-analysis approach did not support in total the role of low consumption of SFA in reducing the risk of cardiovascular diseases (Astrup et al., 2020; Kang et al., 2020). Also, the high consumption of SFA seems to reduce the ictus risk (Kang et al., 2020).

Conversely, integration with cladodes resulted in an increase in the petroselinic, rumenic and α -linolenic acids, specific unsaturated fatty acids with positive effects on human health, for some anti-aging and anti-inflammatory properties, and also being able both to reduce cholesterol and to increase EPA and DHA fatty acids (Alaluf et al., 2002; Ferlay et al., 2017; Lordan et al., 2017; Kern et al., 2020; Djuricic et al., 2021).

The total fatty acids profiles and the health indices are reported in Table 8.

The cladodes integration determined a higher amount of SFA ($P = 0.002$) and lower MUFA ($P = 0.006$) in group OFI's cheeses than those of group CON, respectively. Similar results were found on milk of sheep and goat feed-integrated with cladodes by Cordova-Torres et al. (2017). The SFA and MUFA were lower and higher, respectively, than those found by Maniaci et al. (2021) on Caciocavallo cheeses made in summer, using milk of cows fed integrated with cladodes and wheat straw. In total the PUFA showed similar content compared with those found by Maniaci et al. (2021), even if the ω 6-fatty acids were higher in the present study.

As known, the contents of SFA influence the healthy indices, and cheeses of group OFI showed a higher thrombogenic (TI - $P = 0.001$) and a lower health-promoting indices (HPI - $P < 0.001$) than those of group CON. Similar values of HPI and TI were found in milk of goats fed with the integration of cladodes (El Otmani et al., 2021). However, TI index showed values in line with those generally found in dairy products, while the HPI index recorded higher values (Chen et al., 2020).

As know the fatty acid profiles are influenced by different factors, including the breed, the season, the lactation stages, the number of lactations, the animal age, but also by the diet and the ruminal biohydrogenation process (Jensen, 2002; Kelsey et al., 2003; Ellis et al., 2006).

Considering that cladodes contain different compounds, such as mucilages, and pectins, but also flavonoids and phenolic acids, able to retain the fat during the digestion, modulating also positively the ruminal microflora composition, the ruminal outflow velocity and the biohydrogenation processes on fat (Kennedy, 2005; Astello-García et al., 2015; Alves et al., 2017; Bayar et al., 2017; Izuegbuna et al., 2019; Vasta et al., 2019; Gama et al., 2021). Therefore, more investigations are required to better explain the obtained results.

The mean scores on sensorial profile of cheeses are reported in Table 9. The integration with cladodes determined in cheeses of group OFI the increase of the yellow color ($P = 0.013$) and of the odor intensities ($P < 0.001$), as well as of the butter flavor ($P = 0.001$). These parameters influenced positively the overall acceptability of group OFI products ($P < 0.001$).

The plot generated by PCA is shown in Figure 2. The length of each vector measures the contribution of each selected variable on the main components.

The first 2 principal components accounted for 79.46% of the total variance, discriminating the cheeses of the 2 group. In particular, the first and the second principal components explained the 60.60% and the

18.86% of the total variance, respectively. The separation generated by the PCA partially underlines the differentiation between the cheeses of the 2 groups. In fact, not all the variables showing statistical differences contributed to a great weight in the discrimination of cheeses. Otherwise, the internal redness (a^*) had a significant weight only in the PCA.

CONCLUSIONS

In general, the integration with cladodes of *Opuntia ficus indica* did not affect the qualitative and productive characteristics of milk. Only the urea content of individual milk by cows fed integrating with cladodes showed a lower value.

Considering the Caciotta cheeses, no differences were observed in relation to the storing stage.

From the microbiological point of view, the presence of cladodes in the cows' diet did not influence the fermentation process performed with 2 strains of *S. thermophilus*.

As bioactive compounds, the integration with cladodes determined a higher content of polyphenols in the cheeses and a consequent greater antioxidant capacity.

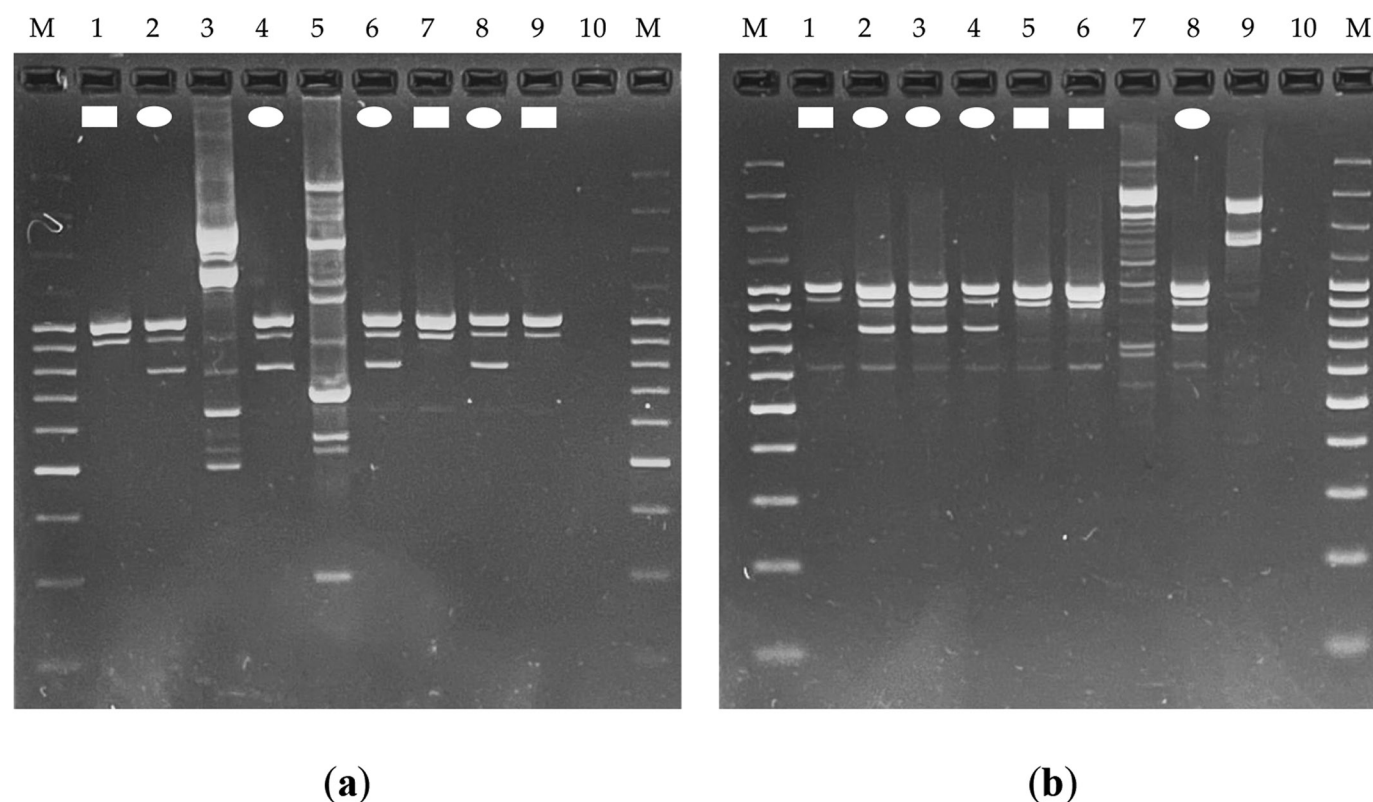


Figure 1. Randomly amplified polymorphic DNA profiles of lactic acid bacteria isolated from cheeses at 30 d of refrigerated storage 10°C. (a), control cheese production; (b), experimental cheese production: lanes: 1, *Streptococcus thermophilus* WVS18; 2, *S. thermophilus* WVS271; 3-9, cheese isolates; 10, negative control.

Table 5. Physical and chemical composition of cheeses

	Group		SEM	<i>p</i> Value
	Control (CON)	Experimental (OFI)		
24h yield %	8.89	8.70	0.584	0.841
<i>External color</i>				
L	69.52	66.88	1.145	0.134
a*	−3.54	−3.59	0.224	0.863
b*	16.10	16.94	1.013	0.575
<i>Internal color</i>				
L	83.34	81.96	1.416	0.506
a*	−2.78	−2.69	0.251	0.800
b*	15.42	15.81	0.402	0.506
pH	5.00	5.03	0.016	0.239
a _w	0.91	0.91	0.025	0.974
Hardness N/mm ²	0.55	0.51	0.112	0.812
<i>Chemical composition</i>				
Dry Matter (DM), %	59.60	59.68	0.333	0.876
Crude protein, % DM	47.13	46.31	0.847	0.511
Ether extract, % DM	43.74	44.86	0.711	0.292
Ash, % DM	6.04	5.99	0.035	0.392
PI %	1.44	1.51	0.132	0.692
Polyphenols, g GAE/kg DM	4.37	6.01	0.512	0.041
TEAC, mmol trolox/kg DM	49.73	50.71	0.314	0.040
POV, mEq O ₂ /kg fat	1.61	1.35	0.096	0.075
TBARS, mg MDA/kg DM	0.38	0.43	0.060	0.532

The results indicate mean values of three measurements performed on each sample. SEM = standard error of the mean. PI = proteolysis index. DE = delphinidin equivalent. GAE = gallic acid equivalent. TEAC = trolox equivalent antioxidant capacity. POV = Peroxide value. TBARS = thiobarbituric acid–reactive substances. MDA = malonylaldehyde.

The fatty acid profile in cheeses was influenced by the inclusion of cladodes in the diet. In particular, the caprylic, capric, lauric, myristic, and palmitic saturated fatty acids were higher in cheeses made by the milk of cows receiving cladodes, while stearic acid was lower. Considering the unsaturated fatty acids, the petroselinic, the vaccenic, the rumenic, and the α -linolenic acid showed higher contents in the experimental group, differently as observed for the oleic and the γ -linolenic acids that were lower.

The cheeses of group integrated with cladodes showed better overall acceptability, and a higher yellow color, odor intensities, and butter flavor. The multivariate analysis well distinguished the cheeses belonging to the 2 groups.

Specific investigations should be conducted on the effect of different levels of cladodes' supplementation on the production and metabolic parameters of Cinisara cows, to evaluate the nutritional value of the cladodes to be rationally included in their formulation diet. Moreover, further research should interest the content

Table 6. Effects of cladodes integration on saturated fatty acids profile (g/100 g FA) of Caciotta cheeses

	Group		SEM	<i>p</i> Value
	Control (CON)	Experimental (OFI)		
C4	2.60	2.62	0.106	0.899
C6	1.85	2.02	0.072	0.134
C8	1.00	1.16	0.036	0.014
C10:0	1.87	2.29	0.038	<0.001
C12:0	1.97	2.45	0.021	<0.001
C14:0	8.19	9.34	0.051	<0.001
C16:0iso	0.46	0.46	0.08	0.872
C16:0	24.32	25.73	0.123	<0.001
C17:0 anteiso	0.18	0.21	0.007	0.063
C17:0	0.94	0.95	0.014	0.069
C18:0	13.02	11.01	0.138	<0.001

The results indicate mean values of three measurements performed on each sample. SEM = standard error of the mean.

Table 7. Effects of cladodes integration on unsaturated fatty acids profile (g/100 g FA) of Caciotta cheeses

	Group		SEM	<i>p</i> Value
	Control (C)	Experimental (O)		
C14:1	1.24	1.26	0.038	0.976
C15:1	1.50	1.46	0.033	0.952
C16:1	2.28	2.39	0.055	0.299
C17:1	0.40	0.41	0.008	0.909
C18:1 c9 OA	25.03	22.97	0.444	0.008
C18:1 t11 TVA	2.28	2.21	0.068	0.488
C18:1 c6	0.63	0.78	0.011	<0.001
C18:1 c11	0.39	0.44	0.003	<0.001
Other C18:1	2.30	2.20	0.05	0.681
Other C18:2	0.84	0.85	0.041	0.775
C18:2 ω6 LA	2.79	2.92	0.057	0.136
CLA C18:2 c9t11 RA	0.78	0.81	0.007	0.008
Other CLA isomers	0.07	0.06	0.012	0.061
C18:3 ω3 ALA	0.33	0.47	0.004	<0.001
C18:3 ω6 GLA	0.29	0.24	0.005	<0.001
C20:1 n11	0.33	0.29	0.019	0.190
C20:3 ω3	0.14	0.15	0.002	0.058
C20:3 ω6 DGLA	0.13	0.11	0.003	0.051
C20:5ω3 EPA	0.12	0.12	0.006	0.707
C22:1	0.09	0.10	0.002	0.181
C22:4 ω6	0.07	0.09	0.022	0.085

The results indicate mean values of three measurements performed on each sample. SEM = standard error of the mean. OA = oleic acid. TVA = trans vaccenic acid. LA = linoleic acid. RA = rumenic acid. CLA = conjugated linoleic acid. ALA = α-linolenic acid. GLA = γ-linolenic acid. DGLA = Diomo-γ-linolenic acid. EPA = eicosapentaenoic acid.

of other important bioactive compounds in fresh but also in treated cladodes, as well as their effects on animals' welfare and their productions.

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Table 8. Effects of cladodes integration on total fatty acids profile (g/100 g FA) and health indices of Caciotta cheeses

	Group		SEM	<i>p</i> Value
	Control (CON)	Experimental (OFI)		
Total fatty acids, % DM	41.59	42.74	0.760	0.313
SFA	57.71	59.71	0.355	0.002
MUFA	36.65	34.51	0.431	0.006
PUFA	5.64	5.77	0.110	0.431
ω6	3.29	3.36	0.062	0.443
ω3	0.66	0.68	0.036	0.697
ω6/ω3	5.11	5.00	0.240	0.735
HPI	0.69	0.59	0.007	<0.001
TI	2.06	2.17	0.016	0.001

The results indicate mean values of three measurements performed on each sample. SEM = standard error of the mean. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; HPI = health-promoting index; TI = thrombogenic index.

Table 9. Effects of cladodes integration on sensorial profile of Caciotta cheeses

	Group		SEM	p Value
	Control (CON)	Experimental (OFI)		
Yellow color	4.10	4.62	0.177	0.013
Structure uniformity	7.35	7.41	0.176	0.769
Holes	0.58	0.46	0.096	0.318
Intensity of odor	5.17	6.04	0.157	<0.001
Odor of butter	4.53	5.12	0.126	0.001
Odor of milk	2.34	2.58	0.146	0.184
Unpleasant odor	0.19	0.14	0.024	0.109
Salty	2.44	2.58	0.161	0.492
Sweet	0.61	0.63	0.078	0.882
Acid	2.52	2.16	0.158	0.069
Bitter	0.43	0.29	0.059	0.063
Spicy	0.27	0.24	0.052	0.631
Chewiness	5.22	5.11	0.251	0.717
Solubility	4.67	4.82	0.300	0.679
Grittiness	3.90	3.51	0.251	0.201
Overall acceptability	4.95	5.72	0.185	0.001

The results indicate mean values. SEM = standard error of the mean.

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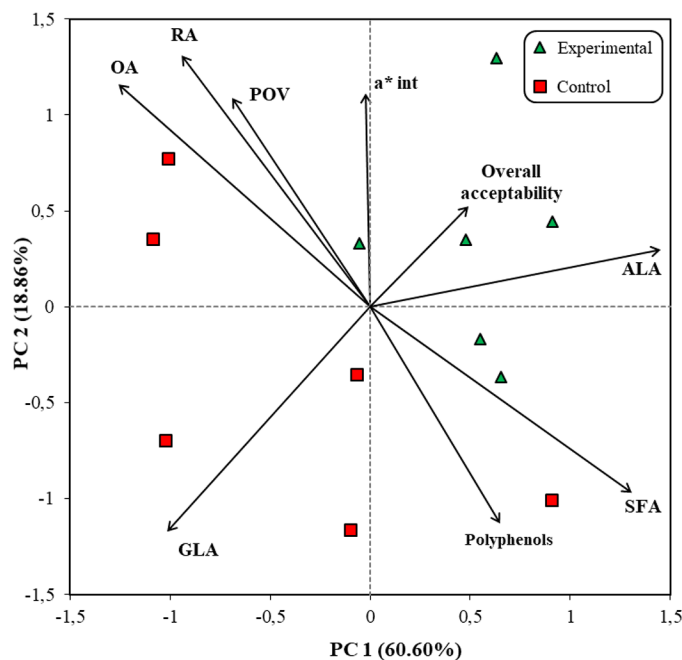


Figure 2. PCA analysis based on physical and chemical traits of cheese and FA for group. The length of each vector is proportional to its contribution to the main components. Abbreviations: POV = Peroxide value; a*int = internal redness; SFA = saturated fatty acids; OA = oleic acid; RA = rumenic acid; ALA = α -linolenic acid; GLA = γ -linolenic acid.

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