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

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ABSTRACT

Recently, the interest in improving livestock product nutraceutical profiles through sustainable feeding systems has increased. In this context, the overall quality and nutraceutical profiles were examined in dairy products obtained from 16 lactating Cinisara cows given an integrated feed in dry season with *Opuntia ficus-indica* cladodes. Two homogeneous groups of cows (milk yield: 6.3 ± 1.5 kg; body weight: 213 ± 55 kg) were 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 produced during the study was used to make Caciotta cheese and was analyzed at 0, 15, and 30 storage days. Milk and cheese samples were analyzed for chemical, physical, and microbiological traits. The nutraceutical and sensorial profiles, as well as the antioxidant capacity, were also determined in the final products. For milk, the urea content in individual samples was reduced in OFI but not in CON. In the cheese, integration of cladodes did not influence the starter cultures development with 2 strains of *Streptococcus thermophilus*, but it caused a higher content of polyphenols and a consequent greater antioxidant capacity and a change in the fatty acids profile. In particular, the caprylic, capric, lauric, myristic, and palmitic fatty acids were higher, as were the petroselinic, vaccenic, rumenic, and α-linolenic fatty acids. In contrast, the oleic and the γ-linolenic fatty acids were lower for OFI. The cheese from the OFI group showed better overall acceptability, and a higher yellow color, odor intensity, 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 that include cladodes for Cinisara lactating cows, but also to determine the content of other important bioactive compounds in fresh and treated cladodes, as well as their effects on animal welfare and production.
Key words: Cinisara cow, Caciotta, fatty acid, polyphenols, antioxidant capacity

INTRODUCTION

In recent years, interest has increased in the benefits for human health by consumption of functional foods as bioactive sources of antioxidants, vitamins, minerals, fiber, n-3 fatty acids, antioxidants, and phenolic compounds. Functional foods include vegetables, fruits, herbs, and oil seeds, but also animal products such as dairy products, fortified eggs, and seafood, in which functional components are transferred by dietary intake in a closed relationship with an animal's physiology and 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), as well as on industrial products and by-products in livestock feed, to improve the nutraceutical profile of foods (Todaro et al., 2017; Gaglio et al., 2021a; Şanta et al., 2021; Kholif and Olafadehan, 2022), while also taking 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 properties and plant adaptability of cladodes (Valentini et al., 2018; Gama et al., 2022), which can grow in arid, rocky, and steep places (Vastolo et al., 2020; Prisa, 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; there is a slowdown in winter and intense spring growth when most of the plants bloom. Finally, during the dry summer period, the growth is interrupted and almost all of the pabular essences wither. In the presence of optimal soil conditions and water availability,

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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., 2017; Oduor et al., 2022) for the administration of fresh forage in the dry period, when this plant presents high green matter production per unit of area and a low NDF content, therefore requiring an addition of a fiber source (Wanderley et al., 2012). The cladodes of *O. ficus-indica* change composition with 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, due to the high content of NFC (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 (Mashope, 2007; Felix et al., 2016).

Diverse compounds contained in cladodes can have positive effects on animal production results. The presence of compounds such as mucilage, pectin, and phenols in animal feeds can improve the fatty acids profile in ruminant milk, modulating the microbiota in the rumen or the transit rate (de Araújo et al., 2021; Netto et al., 2022). Valentini et al. (2018) found that the fermentation of *Opuntia* pectin in ruminants can improve the rumen environment by increasing 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 n-6/n-3 fatty acids ratio in milk, with a transfer of 18:2 n-6. Another study showed how the use of cladodes of *Opuntia* in a diet enriched with sorghum oil could reduce the biohydrogenation of PUFA in the rumen, increasing 18:1 *trans*-11 and C18:2 CLA *cis*-9,*trans*-11 in milk (Gama et al., 2021). Moreover, the use of cladodes in substitution of hay, such as *Cynodon* spp., or of floured corn, could decrease stearic acid (Moraes et al., 2019). Porto Filho 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 high levels of VFA caused an increase in the height and surface of the papillae in the ruminal epithelium, in an attempt to absorb the larger quantities of VFA produced. Another study conducted on dairy cows showed an increase in daily production of milk with a linear reduction of urea nitrogen in milk and urine due to the replacement of corn silage with increasing percentages of *O. ficus-indica* cladodes (Moraes et al., 2019). Kamble et al. (2017) showed that cladodes can replace hay to 60% with minimum effects on production in cows and sheep. Moreover, their use as forage integration seems to improve the taste and color of dairy products (Shetty et al., 2012).

In Italy, *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 Brazil, the integration of cladodes in feed 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 has adapted to be grazed in constrained environments, such as the marginal areas of northwestern 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 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. Specifically, the microbiological and sensorial profiles of cheeses, as well as the specific bioactive compounds, such as fatty acids and polyphenols, and the antioxidant capacity of final products, were investigated.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The experiment was carried out for 6 wk (June–July 2021), involving 16 Cinisara cows raised in a farm located at 540 m above sea level in Cinisi, Italy (38° 07′ 39″ N, 13° 07′ 42″ E), in the typical production area for this breed.

The selected animals (BW: 213 ± 55 kg; calving in autumn, winter, and spring) were divided into 2 homogeneous groups considering the lactation stage (154 ± 93 d) and 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 wk 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/d of commercial wheat bran (**CON**) or 4 kg/d of commercial wheat bran plus 15 kg/d of 1- and 2-yr-old fresh *Opuntia ficus-indica* cladodes (**OFI**). 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) 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 wheat bran and cladodes of *O. ficus-indica* were sampled, and the cows were observed during the grazing, recording and sampling the 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 in the stable was totally consumed by the animals, whereas the pasture ingestion was not evaluated. The samples were placed in sterile vacuum containers and refrigerated at 8°C for the transfer to laboratory, where they were processed as follows: pasture (being dry) and wheat bran were homogenized and stored at −20°C; the cladodes were freeze-dried, then homogenized and stored at −20°C.

All feeds were analyzed for DM (method 967.03), CP (N × 6.25; method 988.05), ether extract (**EE**; method 920.29), and ash (method 942.05) contents, following the recommendations of the AOAC International (2012). Neutral detergent fiber, NDFom, ADF, and ADL were determined in accordance with van Soest et al. (1991). Nonfiber carbohydrate 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. (2007), with C23:0 as the internal standard (Sigma-Aldrich, Milano, Italy). An autosampler injected each sample (1 μL) into an HP 6890 GC system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA). 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. (2014).

For the extract, the total concentration of polyphenols was measured using the Folin–Ciocalteau 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) 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 spectrophotometer. 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 m*M*) of Trolox in PBS obtained from a stock solution of 2.5 m*M* in PBS; these results were used to perform a calibration curve (R^2 = 0.99). The results were expressed as mmol of Trolox equivalent per 100 g of sample DM.

The diet formulation, gross chemical composition and the FA 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 calf suckling. Moreover, on d 3 and 5 of the same week, bulk milk from the morning milking of each group was sampled and used to make Caciotta cheeses.

Individual and bulk milk samples were analyzed for fat, protein, casein, lactose, urea, and SCS by infrared method (CombiFoss 6000; Foss Electric A/S, Hillerød, Denmark); pH was measured with a 70+DHS pH-meter (XS Instruments, Carpi, Modena, Italy); titratable acidity was assessed by the Soxhlet-Henkel method (°SH/50 mL).

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

Natural Milk Starter Culture Development

The natural milk starter culture (**NMSC**) was developed with 2 strains of *Streptococcus thermophilus* (WVS18 and WVS271) previously evaluated for their

Table 1. Diet formulation, chemical composition, and fatty acid profile of feed intake by control (CON) and experimental (OFI) groups (means ± SD)[1]

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

[1]Results indicate mean values of 3 measurements performed on each sample. NFC = 100 − (CP + ether extract + ash + NDFom). FA = fatty acids; OA = oleic acid; LA = linoleic acid; ALA = α-linolenic acid; GAE = gallic acid equivalent; TEAC = Trolox equivalent antioxidant capacity; ND = not determined; NDFom = NDF organic matter.

aptitudes in cheese production (Scatassa et al., 2015). These strains were first subcultured in Medium 17 broth (Biotec, Grosseto, Italy), washed and resuspended 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. The NMSC containing the multistrain culture was then used for cheese production.

Cheesemaking and Sampling

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

In each of 4 cheesemaking processes, 3 forms of cheese for each animal group (CON and OFI) were obtained. At the end, a total of 24 cheeses (12 for each group) were made and weighed after 24 h (yield at 24 h). After weighing, the cheeses were placed in saturated brine (NaCl) at 5°C for 24 h.

For each cheesemaking and for each animal group, 24 h after salting (d 0), one of the 3 wheels was sampled, and the other 2 wheels were vacuum packed and stored in a refrigerator at 10°C to be sampled at 15 or 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 suspensions were plated on selective agar medium 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 lactic acid bacteria (**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 a starter culture, over indigenous milk 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

Table 2. Growth conditions and selective agar media to enumerate the main microbial groups associated with dairy production

Microorganism[1]	Medium	Incubation conditions		Manufacturer
		Temperature (°C)	Time (h)	
TMM	Skim milk agar	30	72	Microbiol Diagnostici, Uta, Italy
Streptococcus thermophilus	Medium 17 agar	44	48	Biotec, Grosseto, Italy
Pseudomonas spp.	*Pseudomonas* agar base	25	48	Condalab, Madrid, Spain
Enterobacteriaceae	Violet red bile glucose agar	37	24	Condalab, Madrid, Spain
Listeria monocytogenes	*Listeria* selective agar base	37	24	Oxoid, Hampshire, UK
Escherichia coli	Chromogenic medium agar	37	24	Condalab, Madrid, Spain
Salmonella spp.	Hektoen enteric agar	37	24	Microbiol Diagnostici, Uta, Italy
CPS	Baird Parker agar	37	48	Oxoid, Hampshire, UK

[1]TMM = total mesophilic microorganisms; CPS = coagulase-positive staphylococci.

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 were sampled at d 0, 15, and 30 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 ($\mathbf{a_w}$) was detected with a dew-point hygrometer HygroLab 3 (Rotronic, Huntington, NY). Calibration was performed using 5 saturated solutions of known a_w.

The maximum resistance to compression (compressive stress, N/mm^2), as index of cheese hardness, was measured on samples (2 cm × 2 cm × 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 × 6.38), and ash content were determined according to International Dairy Federation standards [4A:1982 (IDF, 1982), 5B:1986 (IDF, 1986), 25:1964 (IDF, 1964a) and 27:1964 (IDF, 1964b) respectively]. The determination of soluble N was carried out by treatment with a sodium citrate solution and subsequent precipitation of the proteins at pH 4.6 (Ministro dell'Agricoltura e delle Foreste, 1986). The proteolysis index (**PI**) was calculated as the percentage ratio between NPN and total N.

Bioactive Compounds and Human Health Indices. The cheeses were also analyzed for FA profile, total content of phenolic compounds, and antioxidant capacity. Fatty acids in lyophilized cheese samples (100 mg) were directly methylated as described by Loor et al. (2002). Fatty acid methyl esters were recovered in hexane (1.5 mL). An autosampler injected each sample (1 μL) into an HP 6890 GC system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA). 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 and Liu (2020), using the following:

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

The thrombogenic index (**TI**) was calculated according to Ulbricht and Southgate (1991), using the following:

$$\mathrm{TI} = \left(\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}\right) / \left[\begin{array}{l}\left(0.5 \cdot \Sigma\mathrm{MUFA}\right) + \left(0.5 \cdot \Sigma\mathrm{PUFA6}\right) + \\ \left(3 \cdot \Sigma\mathrm{PUFA3}\right) + \left(\Sigma\mathrm{PUFA3}/\Sigma\mathrm{PUFA6}\right)\end{array}\right].$$

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

The following 15 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 (2007) 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 (SAS Institute Inc., 2010), using a mixed model that included the experimental phase (2 levels), group (2 levels), lactation stage (3 levels) as fixed effects and the cow (16 levels) as random effect used as error term. Results are reported as least squares means (**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 cheesemaking and cheeses were statistically analyzed by the SAS 9.2 software, using a generalized linear model that included the effects of group (2 levels), the aging (3 levels), and the interaction group × aging. 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 the 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 CP and ether extract contents. 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 reared in dry areas, and a high ash percentage, principally represented by calcium, potassium, and magnesium as found in other studies (Bakari et al., 2017; Naorem et al., 2022).

Of the investigated bioactive compounds, the PUFA in cladodes amounted to 46.12%, followed by SFA (34.90%), and the MUFA (12.06%). The linoleic (C18:2 *cis* n-6, 27.00%), palmitic (C16:0, 21.65%), α-linolenic (C18:3 *cis* n-3, 19.12%), and oleic (C18:1 *cis* n-9, 10.75%) FA were those more represented, similar to findings 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.

Table 3. Physical, chemical, and technological parameters of daily individual cow milk yield of control (CON) and experimental (OFI) groups[1]

	Group			
Item	CON	OFI	SEM	*P*-value
Daily milk yield, kg/animal	5.54	5.64	0.443	0.754
pH	6.57	6.58	0.022	0.818
Titratable acidity, °SH/50 mL	4.19	4.31	0.142	0.138
Ether extract, %	2.88	2.92	0.232	0.874
CP, %	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

[1]Results indicate the mean values of measurements performed for each group. r = clotting time; k20=curd-firming time; a30 = curd firmness.

The production and composition of milk were similar in the 2 groups, similar to what was found in 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 cows (Moraes et al., 2019) and sheep (Saraiva et al., 2020). The urea content in milk is related to CP ingestion, and both groups were fed low-CP diets, reflecting the poor quality of pasture. Indeed, the urea showed a content in the range usually found in milk obtained by animals fed a low-CP diet (16–20 mg/dL). In contrast, 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 Cheesemaking. The Caciotta cheeses produced in this study were made with raw cow 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 the cows' diet, supplemented with *O. ficus-indica* cladodes, on the microbiology of the cheese. In fact, is well known that *O. ficus-indica* cladodes represent a useful source of polyphenolic compounds (Rocchetti et al., 2018) able to exert antibacterial activity (Liguori et al., 2022). The results of the plate counts carried out throughout cheese production from cow milk to finished product are reported in Table 4.

The specific search for coagulase-positive staphylococci, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp., responsible for foodborne diseases associated with cheese consumption (Kousta et al., 2010) were not present 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 investigated among CON and OFI group products during all steps of cheesemaking. Raw cow milk hosted levels of total mesophilic microorganisms (**TMM**) at about 10^4 cfu/mL, in both CON and OFI products, which complies fully with 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, whereas 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 Busetta et al. (2023b) in raw cow milk used for production of Caciocavallo Palermitano and Caciocavallo Podolico, respectively. The analysis of milk after NMSC addition showed levels of TMM and thermophilic coccus LAB above 7 log cfu/mL, confirming that *S. thermophilus* inoculums occurred at 10^7 cfu/mL. After curdling, TMM, *S. thermophilus*, and members of *Enterobacteriaceae* and *Pseudomonadaceae* family increased by 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, whereas members of *Enterobacteriaceae* and pseudomonads remained almost constant (10^2 cfu/g) in all curds. Busetta et al. (2023a) observed a similar cell density in acidified curds used for the production of Protected Designation of Origin (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*, whereas *Enterobacteriaceae* and pseudomonads disappeared from both productions, confirming the sanitizing effect of a heating step (such as curd stretching for the Caciotta cheese production; Stellato et al., 2015). These results highlighted that 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 1a) and OFI (Figure 1b) 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 diet

Table 4. Microbial loads of samples collected during the cheese-making process using bulk milk of control (CON) and experimental (OFI) groups[1]

	Microbial count			
Sample	TMM	*Enterobacteriaceae*	*Pseudomonadaceae*	*S. thermophilus*
Raw milk				
CON	3.62	1.83	2.26	3.51
OFI	3.57	1.71	1.89	3.40
SEM	0.07	0.11	0.16	0.06
P-value	0.751	0.629	0.288	0.389
Inoculated milk				
CON	7.26	1.36	1.77	7.02
OFI	7.19	1.44	1.81	7.10
SEM	0.09	0.06	0.12	0.05
P-value	0.754	0.538	0.884	0.485
Curd				
CON	8.03	2.11	2.56	8.11
OFI	8.09	2.02	2.61	8.23
SEM	0.06	0.08	0.06	0.05
P-value	0.666	0.638	0.707	0.216
Acidified curd				
CON	9.12	1.75	2.14	9.10
OFI	9.04	1.69	2.12	9.00
SEM	0.05	0.07	0.04	0.13
P-value	0.498	0.709	0.827	0.741
Cheese at T_0				
CON	8.61	<2	<2	8.40
OFI	8.77	<2	<2	8.61
SEM	0.05	NE	NE	0.08
P-value	0.151	NE	NE	0.234
Cheese at 15 d				
CON	8.70	<2	<2	8.72
OFI	8.65	<2	<2	8.58
SEM	0.05	NE	NE	0.07
P-value	0.675	NE	NE	0.339
Cheese at 30 d				
CON	8.83	<2	<2	8.66
OFI	8.72	<2	<2	8.74
SEM	0.06	NE	NE	0.09
P-value	0.442	NE	NE	0.720

[1]Units are log cfu/mL for milk samples and log cfu/g for curd and cheese samples. Results indicate the mean values ± (SD) of 4 plate counts (carried out in duplicate for 2 independent productions). TMM = total mesophilic microorganisms; *S.* = *Streptococcus*; NE = not evaluated.

supplemented with *O. ficus-indica* cladodes during the cheesemaking.

Bulk Milk and Cheeses Compositions

The bulk milk showed physical, chemical, and technological parameters in line with those found in individual milk samples (Supplemental Table S1; https://data.mendeley.com/datasets/npcx3cw8zb/2, Maniaci, 2024). The physical characteristics and the chemical composition of cheeses in relation to the aging stage did not show differences (Supplemental Tables S2, S3, S4, S5, and S6; https://data.mendeley.com/datasets/npcx3cw8zb/2, Maniaci, 2024). Therefore, the data are reported and described considering only the 2 groups (Table 5).

Considering the investigated bioactive compounds, the cheeses of group OFIshowed 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 bioaccessibility of polyphenols in cheeses, and the consequent antioxidant properties (Gladine et al., 2007; Di Trana et al., 2015). The polyphenol content influenced the antioxidant capacity, expressed as TEAC, which was higher in cheeses of OFI than 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 cheese fat, expressed as peroxide value (primary oxidative fat index), and thiobarbituric acid–reactive substances (secondary oxidative fat index), even if the peroxide value tended to

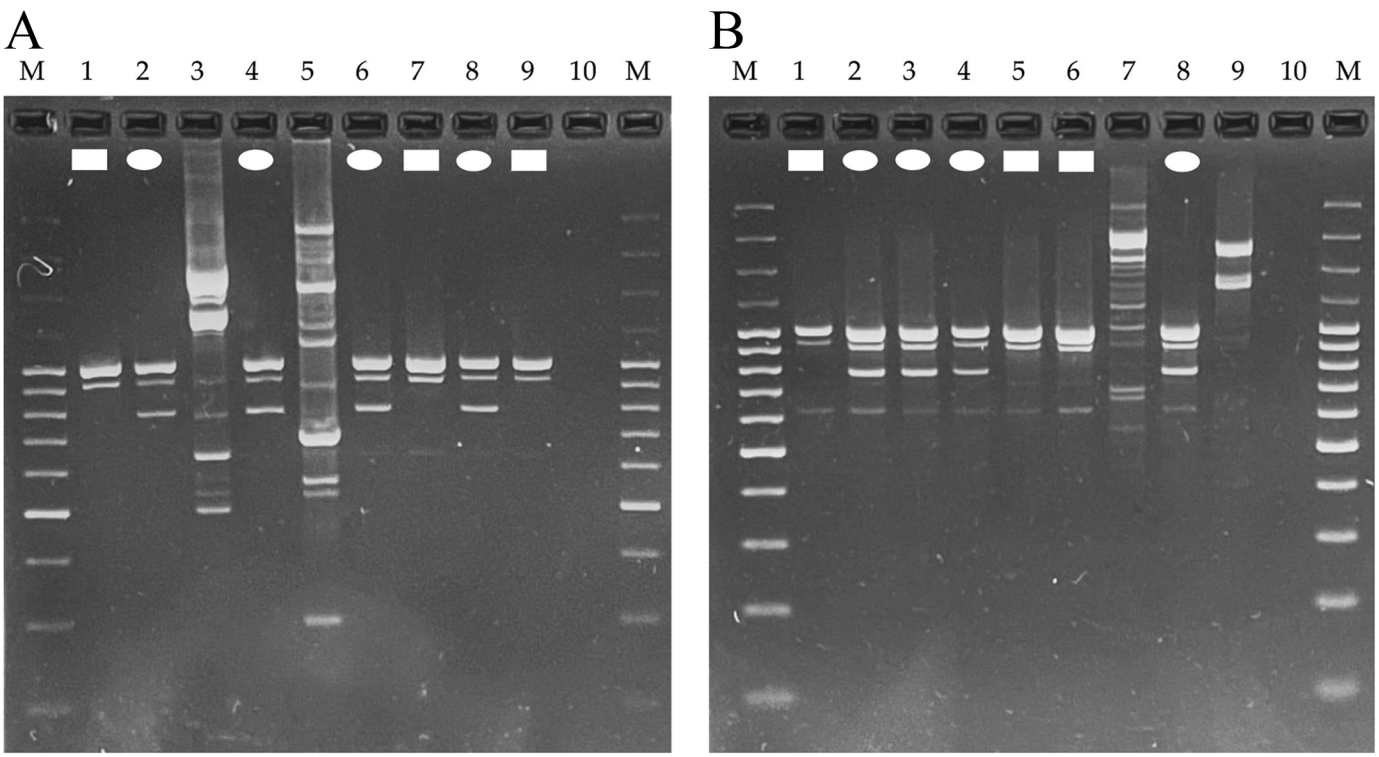

Figure 1. Randomly amplified polymorphic DNA profiles of lactic acid bacteria isolated from cheeses at 30 d of refrigerated storage at 10°C. (A) Control cheese production. (B) Experimental cheese production. Lane M: GeneRuler 100 bp plus DNA ladder (Thermo Fisher Scientific Inc., Vilnius, Lithuania). Lane 1: *Streptococcus thermophilus* WVS18; lane 2: *S. thermophilus* WVS271; lanes 3–9: cheese isolates; lane 10: negative control.

be lower in cheeses of group OFI, probably the extent of the differences found was not enough.

The FA profiles of the 2 groups are reported in Tables 6, 7, and 8. Considering the SFA (Table 6), the cheeses of 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 CON. Similar results were also observed by other authors in milk of sheep and goat fed an integration with cladodes (Costa et al., 2010; Cordova-Torres et al., 2017).

Conversely, the stearic acid (C18:0) showed a lower value in cheeses of OFI than in those of 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; Moraes et al., 2019; Gama et al., 2021).

Considering the UFA (Table 7), oleic (C18:1c9; $P = 0.008$) and γ-linolenic acids (C18:3n-6; $P < 0.001$) showed higher contents in group C; conversely, petroselinic (C18:1c6 n-12; $P < 0.001$), vaccenic (C18:1 c11; $P < 0.001$), rumenic (C18:2 c9t11; $P = 0.008$), and α-linolenic (C18:3n-3; $P < 0.001$) acids were higher in the OFI group than in CON.

In general, the oleic FA (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 FA content, therefore their integration in the OFI group could have determined both lower ingestion of this FA at pasture (rich in oleic FA) than CON. Other authors also observed similar results in milk of sheep and goats fed an integration with cladodes (Costa et al., 2010; Cordova-Torres et al., 2017). The increase in rumenic acid in cheeses of OFI and the contextual reduction of C18:0 were consistent with the trends found by other authors in cow milk (Gama et al., 2021, 2022), 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 FA 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 consump-

Table 5. Physical parameters and chemical composition of Caciotta cheeses made with bulk milk from control (CON) and experimental (OFI) groups[1]

	Group			
Item	CON	OFI	SEM	*P*-value
24-h yield, %	8.89	8.70	0.584	0.841
External color				
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
Internal color				
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
Chemical composition				
DM, %	59.60	59.68	0.333	0.876
CP, % 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

[1]The results indicate mean values of 3 measurements performed on each sample. PI = proteolysis index; GAE = gallic acid equivalent; TEAC = Trolox equivalent antioxidant capacity; POV = peroxide value; TBARS = thiobarbituric acid–reactive substances; MDA = malondialdehyde; L = lightness; a* = redness; b* = yellowness.

tion 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 UFA with positive effects on human health, for some anti-aging and anti-inflammatory properties, and the ability to reduce cholesterol and increase eicosapentaenoic and docosahexaenoic acid (DHA) FA (Alaluf et al., 2002; Ferlay et al., 2017; Lordan and Zabetakis, 2017; Kern et al., 2020; Djuricic and Calder, 2021). The total FA profiles and the health indices are reported in Table 8.

The OFI cheeses had higher SFA ($P = 0.002$) and lower MUFA ($P = 0.006$) than those of CON. Similar results were found on milk of sheep and goats fed an integration 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 an integration with cladodes and wheat straw.

Table 6. Effects of cladodes integration on SFA profile (g/100 g of fatty acids) of Caciotta cheeses[1]

	Group			
Item	CON	OFI	SEM	*P*-value
C4:0	2.60	2.62	0.106	0.899
C6:0	1.85	2.02	0.072	0.134
C8:0	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
C16:0 iso	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

[1]Results indicate mean values of 3 measurements performed on each sample. CON = control group; OFI = experimental group.

Table 7. Effects of cladode integration on unsaturated fatty acids profile (g/100 g of fatty acids) of Caciotta cheeses[1]

	Group			
Item	CON	OFI	SEM	*P*-value
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 n-6 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 n-3 ALA	0.33	0.47	0.004	<0.001
C18:3 n-6 GLA	0.29	0.24	0.005	<0.001
C20:1 n11	0.33	0.29	0.019	0.190
C20:3 n-3	0.14	0.15	0.002	0.058
C20:3 n-6 DGLA	0.13	0.11	0.003	0.051
C20:5 n-3 EPA	0.12	0.12	0.006	0.707
C22:1	0.09	0.10	0.002	0.181
C22:4 n-6	0.07	0.09	0.022	0.085

[1]Results indicate mean values of 3 measurements performed on each sample. OA = oleic acid; TVA = transvaccenic acid; LA = linoleic acid; RA = rumenic acid; ALA = α-linolenic acid; GLA = γ-linolenic acid; DGLA = diomo-γ-linolenic acid; EPA = eicosapentaenoic acid; CON = control group; OFI = experimental group.

In total, the PUFA showed similar content compared with those found by Maniaci et al. (2021), even if the n-6 FA were higher in the present study.

As known, the contents of SFA influence the healthy indices, and cheeses of group OFI showed higher TI ($P = 0.001$) and lower HPI ($P < 0.001$) than those of 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 showed values in line with those generally found in dairy products, whereas HPI recorded higher values (Chen and Liu, 2020).

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

Given that cladodes contain compounds like mucilages, pectins, flavonoids, and phenolic acids, which possess the ability to retain fat during digestion, they play a positive role in modulating the composition of ruminal microflora, ruminal outflow velocity, and the biohydrogenation processes associated with fat (Kennedy, 2005; Astello-Garcia et al., 2015; Alves et al., 2016; 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 results.

Table 8. Effects of cladodes integration on total fatty acids profile (g/100 g of fatty acids) and health indices of Caciotta cheeses[1]

	Group			
Item	CON	OFI	SEM	*P*-value
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
n-6	3.29	3.36	0.062	0.443
n-3	0.66	0.68	0.036	0.697
n-6/n-3	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

[1]Results indicate mean values of 3 measurements performed on each sample. HPI = health-promoting index; TI = thrombogenic index; CON = control group; OFI = experimental group.

Table 9. Effects of cladodes integration on sensorial profile of Caciotta cheeses[1]

	Group			
Attribute	CON	OFI	SEM	*P*-value
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

[1]Results indicate mean values of attribute scores. CON = control group; OFI = experimental group.

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

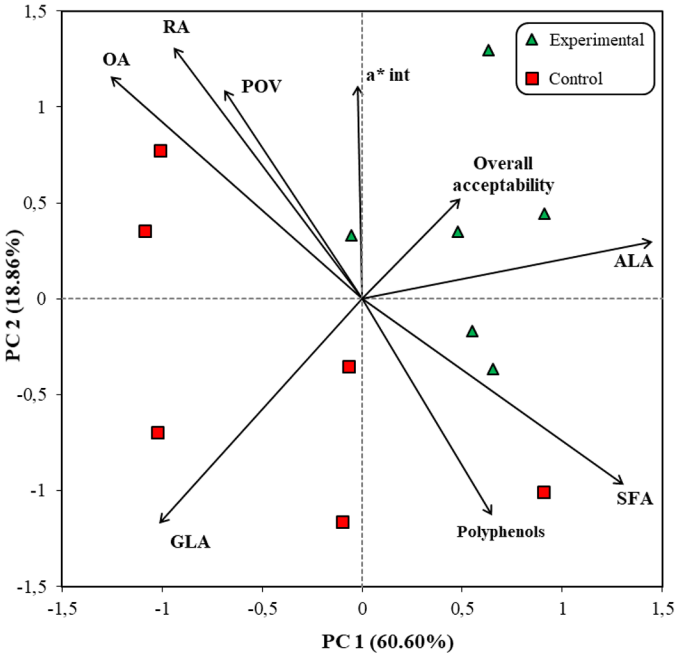

Figure 2. Principal component (PC) analysis based on physical and chemical traits of cheese and fatty acids for group. The length of each vector is proportional to its contribution to the main components. POV = peroxide value; a*int = internal redness; OA = oleic acid; RA = rumenic acid; ALA = α-linolenic acid; GLA = γ-linolenic acid.

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 groups. 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 the discrimination of cheeses; the internal redness (a*) had a significant weight only in the PCA.

CONCLUSIONS

The integration of *Opuntia ficus-indica* cladodes into the diet of cows did not significantly affect the qualitative and productive aspects of milk, except for a lower urea content. Caciotta cheeses derived from this milk exhibited no differences during storage. Microbiologically, the presence of cladodes did not affect the fermentation process in the cheeses. However, the inclusion of cladodes led to higher polyphenol content in the cheeses, enhancing their antioxidant capacity. The fatty acid profile in cheeses was influenced by cladode inclusion, with certain SFA increased and stearic acid decreased. Unsaturated fatty acids displayed variations, with higher levels of petroselinic, vaccenic, rumenic, and α-linolenic acids. Overall, cheeses from cladode-fed cows demonstrated superior acceptability, along with heightened yellow color, odor intensities, and butter flavor. Multivariate analysis effectively differentiated cheeses from the 2 groups. Future research

should explore varied cladode supplementation levels in Cinisara cows to assess nutritional value and delve into the content of other bioactive compounds in fresh and treated cladodes, considering effects on animal welfare and production.

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