










## Article

# Perennial Common Basilisk (*Prangos ferulacea* (L.) Lindl.): Ecological Aspects, Forage Value, and Assessment of Its Effects on Chemical and Microbiological Properties of Raw Milk and Ricotta—A Case Study in Sicily (Italy)

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## Abstract

This paper illustrates the results of a case study conducted in the Madonie Regional Park (Sicily, Italy) focusing on *Prangos ferulacea* (L.) Lindl. This species spontaneously grows in the area and plays an important role as forage plant, contributing to the production of traditional dairy products. A multidisciplinary approach was adopted to investigate the ecological characteristics and the chemical composition of the species, and to assess its effects on chemical and microbiological properties of raw milk and ricotta from grazing animals. Indices of bioindication were used to analyse the ecological features of the study area, and a change in the landscape has been observed. Samples of *P. ferulacea* were collected in the wild in specific plot areas. Chemical analyses were carried out to determine the main nutritional parameters of the species. Chemical and microbiological analyses were performed on raw milk and ricotta samples to evaluate their nutritional composition and quantify the main microbial groups. Raw milk showed no significant microbial differences between samples, with low levels of lactic acid bacteria (LAB) and some Enterobacteriaceae and *Escherichia coli* (~10<sup>2</sup> CFU/mL), while pathogens like *Listeria monocytogenes* and *Salmonella* spp., as well as spoilage yeasts were undetectable. Ricotta cheese showed a high hygienic profile, with LAB around 10<sup>4</sup> CFU/g and no spoilage or pathogenic microbes detected, including STEC-negative *E. coli*. Additionally, SPME-GC/MS and LC/MS analyses were carried out to identify the phenolic compounds of the species with those of dairy products and showed how the contribution of *P. ferulacea* to ricotta was effective for the aromatic profile and negligible for the polyphenolic component.

**Keywords:** bioindication indices; dairy products; ecology; forage quality; Madonie Regional Park; nutrient content; volatile compounds



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## 1. Introduction

*Prangos* belongs to the *Apiaceae* family and includes 106 species spread throughout Europe and Western and Central Asia [1]. The Irano-Turanian region is considered the main centre of diversity for this genus [2]. Downie et al. [3] affirmed that the *Prangos* genus is included in the subfamily *Apioideae* within the *Cachrys* clade. It was split from *Cachrys* and related genera due to differences in fruit morphology [4], its classification later confirmed through genetic analysis [2,3]. Perennial common basilisk (*Prangos ferulacea* (L.) Lindl.) is one of the most widespread species of the genus in Europe and occurs from Italy to Iran [1]. *P. ferulacea* is known for its high forage quality [5–7] and contains several chemical compounds with beneficial properties, including flavonoids, phenolic compounds, coumarins, and terpenoids [8–11]. These compounds are primarily associated with antioxidant and antimicrobial properties [9,12]. In Turkey, *P. ferulacea* is known as “*heliz*”; it is used to enhance the aroma and flavour of traditional cheese such as Otlu [6]; and it has antimicrobial properties [13]. It is also used as a seasoning in yogurt and other food [14]. It is well known that the extensive grazing systems and summer transhumance to high altitude pastures positively impact the chemical, microbiological, and aromatic properties of milk and dairy products. In particular, the botanical biodiversity of pastures and the intake of wild forage species have been shown to improve the lipid profile by increasing the levels of unsaturated fatty acids (CLA and omega-3) and other bioactive compounds [15,16]. However, despite that the Madonie area can boast a long tradition of cattle, sheep, and goat production based on grazing, a progressive abandonment of this practice has occurred in recent decades due to the low profitability of agricultural businesses, the limited structures of the area, and the generational turnover [17]. On the other hand, in recent years, from the side of the consumers there is a growing interest in animal products derived from extensive grazing systems. The production of ricotta from the milk of animals grazing on *P. ferulacea* represents a significant example of local agro-biodiversity and, at the same time, a potential living lab where local knowledge, ecological resources, and academic research can be integrated to foster sustainable rural development [18]. Considering the extensive range of properties, additional studies are needed to improve the knowledge of the impact of *P. ferulacea* forage on dairy products from grazing livestock animals. With this aim, a case study was carried out at a local farm in the Madonie Regional Park (Central Sicily, Italy), where *P. ferulacea* has long been used as a forage species and is traditionally associated with the production of typical dairy products such as “*ricotta di basilisco*” [19]. In particular, the following hypotheses were tested: (1) *P. ferulacea* is a key component of plant biodiversity in the Madonie Regional Park; (2) *P. ferulacea*-based forage greatly affects the aromatic profile and the nutritional value of milk, whey, and ricotta; and (3) the hygienic safety of the final products is not modified.

### 1.1. Taxonomy, Distribution, and Ecology of *P. ferulacea*

*P. ferulacea* is commonly found in the Central and Eastern Mediterranean, the Near and Middle East, in the central part of the Italian Apennines, in Sicily, in the Balkan Peninsula (Croatia, Montenegro, Albania, Serbia, Bulgaria, Greece), Romania, Anatolia, southwest Asia (as far south as Israel), and the Middle East (Iran, Turkmenistan). According to Walter and Gillett [20], *P. ferulacea* is listed as a vulnerable species in the IUCN Red List of Heartened Plants. It typically grows in mountainous pastures ranged from 1000 to 2000 m a.s.l. It lives in rocky and sunny places and prefers limestone substrate [21]. Most suitable climatic conditions for the growth of *P. ferulacea* include moist environment, cold temperatures, and periods of frost to complete the life cycle; the species tends to reproduce more successfully on clay soils than on other soil types. However, despite the well documented taxonomy and extensive geographical distribution of *P. ferulacea*, there

is limited research on its ecological characterisation in Mediterranean mountain pastoral systems. On this basis, the first aims of this case study were to provide an ecological characterisation of *P. ferulacea* in the Madonie Regional Park.

### 1.2. Forage Quality and Nutritional Value of *P. ferulacea*

In vitro studies using *P. ferulacea* have shown that the dried forage contains a high level of metabolisable energy (ME): 12.2 MJ/kg dry matter (DM) for the whole plant, 11.9 MJ/kg DM for leaves, and 12.7 KJ/kg DM for stems. These values are comparable to those of high-quality forages commonly used in ruminant nutrition. In terms of digestibility, in vitro dry matter and organic matter digestibility were measured at 0.769 and 0.806 for the whole plant, 0.773 and 0.790 for the leaves, and 0.740 and 0.840 for the stems, respectively. These results show that *P. ferulacea* can be considered a high-energy forage [22]. In some areas of Iran, the plant is not directly grazed by livestock, likely due to the strong aroma of its aerial parts; instead, it is harvested, dried, and stored for use during the winter season [23]. In this regard, Ahmed et al. [24] report that the high content of coumarins in fresh shoots and persistent smell likely deter grazing at different stages of growth. However, ensuring a consistent supply of high-quality forage, the species plays a fundamental role in the development of livestock farming systems [25], particularly in the Mediterranean and Middle Eastern regions, contributing to enhance the welfare, health, and productivity of livestock animals. Coşkun et al. [6] reported that the forage quality of *P. ferulacea* is comparable to *Medicago sativa* L., although it contains less crude protein (CP) but more metabolisable energy and lower fibre content. For these reasons, *P. ferulacea* is considered a valuable resource for animal nutrition, particularly in Turkey, Iran, and Italy, where it grows wild in natural pastures, giving aroma and flavour to different dairy products. Atamanalp et al. [26] found that the most significant minerals in *P. ferulacea* were calcium ( $68,350 \pm 0 \mu\text{g}/\text{kg}$ ), potassium ( $112,550 \pm 3309.2 \mu\text{g}/\text{kg}$ ), phosphorus ( $1305 \pm 5.7 \mu\text{g}/\text{kg}$ ), copper ( $1291 \pm 18.4 \mu\text{g}/\text{kg}$ ), iron ( $6371 \pm 2159.9 \mu\text{g}/\text{kg}$ ), magnesium ( $24,605 \pm 2166.3 \mu\text{g}/\text{kg}$ ), sodium ( $1216 \pm 45.4 \mu\text{g}/\text{kg}$ ), and zinc ( $2690 \pm 17.6 \mu\text{g}/\text{kg}$ ). Despite *P. ferulacea* being recognised for its nutritional and forage quality, the effects it has on the composition of milk, whey, and ricotta in Mediterranean grazing systems remain poorly studied. In this context, the second aim of the research was to evaluate the nutritional value of the species and to assess the effects of this plant on chemical composition of raw milk and ricotta compared to the same products obtained without *P. ferulacea*.

### 1.3. Phytochemical Composition and Bioactive Compounds of *P. ferulacea*

Extracts obtained from *P. ferulacea* exhibit antimicrobial activity against various bacteria and fungi [9]. In traditional medicine, the plant is used to facilitate digestion and as a natural solution for urinary disorders [11,27]. Preliminary studies suggest that *P. ferulacea* extracts may inhibit the growth of cancer cells due to bioactive compounds that affect cellular proliferation [10,28]. In Persian folk medicine, this plant has been used as a carminative and emollient tonic for gastrointestinal and liver disorders and as an anti-inflammatory, antiviral, antifungal and antibacterial agent [29]. In Italy and other areas, leaves and flowers of *P. ferulacea* are traditionally used to treat many diseases [30]. The essential oil of *P. ferulacea* contains monoterpenes, sesquiterpenes, coumarines, flavonoids, alkaloids, tannins, saponins, and terpenoids [31]. The extracts are also known to modulate inflammatory pathways, making them useful for managing chronic inflammatory conditions [10,11,28,32]. *P. ferulacea* contains coumarins such as scopoletin, umbelliferone, and bergapten, which are recognised for their anti-inflammatory, antimicrobial, and antioxidant properties [8,9,33,34]. Scopoletin may regulate blood pressure and blood glucose levels [35], while umbelliferone exhibits antioxidant and hepatoprotective effects [36]. Flavonoids like quercetin and its

derivatives have antioxidant, antiallergic, and cardioprotective properties [12,37]. Additionally, the essential oil of *P. ferulacea* is rich in terpenoids such as  $\beta$ -myrcene, limonene, and  $\alpha$ -pinene, known for their antimicrobial, calming, and insect-repellent effects [11,28,38,39]. Furthermore, some studies report that the dried plant components of *P. ferulacea* can be used in dyeing processes, using natural dyes derived from flavonoids to colour wool fibres, representing an innovative and more sustainable methodology in the textile production sector [40]. Although the phytochemical composition of *P. ferulacea* has been studied, little is known about the potential influence of its bioactive compounds on the aromatic and microbiological characteristics of dairy products of traditional grazing conditions. The third aim of this study was to assess the effects of this plant on aromatic and microbiological characteristics of raw milk, whey, and ricotta compared to the same products obtained without *P. ferulacea*.

## 2. Materials and Methods

### 2.1. Test Site

The study was conducted in 2023 in the Madonie Regional Park (37°45' and 38°03' N latitude, and 13°50' and 14°12' E longitude), situated to the east of the Province of Palermo (Sicily, Italy). It covers an area of 39,936 ha with altitudes ranging from 0 to 1979 m a.s.l. [41]. The study area (37°51'54" N, 14°04'28" E) is located at 1270 m a.s.l.; it is characterised by an average temperature of 8–9 °C and average precipitation of 1200–1300 mm; the bioclimate [42] is a lower oromediterranean thermotype with lower humid ombrotype, and the substrate is carbonate. The Soil map of Sicily [43] together with data (<https://zenodo.org/records/7072306>, accessed on 17 April 2025) provided by the Council for Agricultural Research and Economics (CREA) was used to obtain information regarding the soil type of the sampling locations. An association Lithosols—rock outcrop—Andic brown soils, equivalent to the RSG Leptosols, was determined. The closest CREA data corresponds to S250-P-871, also described during the same 2008 campaign. The classification, according to the WRB (World Soil Information), second edition, is the same: Regosols Eutri-Endoleptic. In the study area, wild plants were identified according to Pignatti et al. [44] and by direct comparison with the samples of the *Herbarium Mediterraneum Panormitanum* or/and those of the *Herbarium*. New voucher specimens were housed in the *Herbarium* of the Department of Agricultural, Food and Forestry Sciences, at University of Palermo. Nomenclature was based on Bartolucci et al. [45]. With the aim to study the phytosociological aspects of the area, one plot of 25 m<sup>2</sup> was established in 2014 in a homogeneous area considering the topography and vegetation. Within this plot, identified by the GPS coordinates of the vertices, the presence of all vascular plant species was recorded, and the cover percentage of each species was visually estimated in 2014. The relevés was repeated in 2023. The study was carried out following the phytosociological method of Braun-Blanquet [46]. The ecological features were analysed using the Pignatti–Ellenberg bioindication indices [47–49]. For each activity, the response to abiotic factors, such as light condition (L), temperature (T), and climatic continentality (K), and to edaphic conditions, such as moisture (F), reaction (R), nutrient availability (N), and salinity (S), were calculated [50]. The Ellenberg indicator values were determined in accordance with [51]. For each relevé, the Ellenberg indicator average values and basic stationery and biodiversity indices are presented: canopy density, total number of taxa, percentage of endemics, percentage of taxa characteristic of the mountain belt, percentage of cosmopolitans, Shannon Index [52]. A comparison was done with an area located in the lower Madonie area, near the municipality of Castelbuono (Palermo, Italy) (37°54'33" N 13°57'34" E). There, a local farm raises crossbred cows and goats (100 and 250, respectively) under a continuous grazing system and processes the milk at the company's facilities. During the second ten-day period of May, samples of raw

milk, whey, and ricotta were taken three times during the cheesemaking performed in three successive days from milk of animals reared on pastureland in the absence of *P. ferulacea* (P0). In the third ten days of May, following transhumance, the herd was transferred to the high mountain pastures of the Madonie Regional Park, characterised by the presence of *P. ferulacea* (P1); also in this time, raw milk, whey, and ricotta were sampled during cheesemaking in three successive days. In both P0 and P1 collections, samples were stored at  $-20\text{ }^{\circ}\text{C}$  until lyophilisation. In the area characterised by the presence of *P. ferulacea* (P1), plant samples were collected and freeze-dried for subsequent analysis.

## 2.2. Ricotta Production and Sample Collection

Ricotta was produced using the whey derived from cheese manufacture with bulk raw milk. The process was performed in accordance with knowledge “customised” by the farmers’ know-how that was handed down among generations. In detail, after curd separation, the resulting whey was filtered, placed in a large vat, heated to  $45\text{ }^{\circ}\text{C}$ , and added with salt ( $0.4\text{ g L}^{-1}$ ). Raw milk (10% *v/v*) was then added at  $\sim 60\text{ }^{\circ}\text{C}$ . Then, it was heated to  $\sim 85\text{ }^{\circ}\text{C}$  until protein flocculates to the surface. Once fully emerged, ricotta was manually collected and placed in perforated plastic cylindrical containers called “fucelle”, for draining the remaining “scotta” (de-proteinised whey), left to drain at room temperature, and sampled 30 min after production.

## 2.3. Chemical Composition of Forage, Raw Milk, and Ricotta

The freeze-dried samples of *P. ferulacea* plants were analysed according to AOAC [53] procedures for dry matter (DM) (method 934.01), ether extract (method 920.39), crude protein (CP) (method 2001.11), and ash (method 942.05). The fibrous fractions, as neutral detergent fibre (NDF) inclusive of ash (method 2002.04), acid detergent fibre (ADF) (method 973.18), and acid detergent lignin (ADL) (method 973.18), were determined in accordance with AOAC [53] and Van Soest et al. [54]. Raw milk samples were analysed for lactose, fat, protein, and casein by infrared method (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark). The freeze-dried samples of ricotta were analysed following the method of International Dairy Federation (IDF) for DM IDF [55], fat IDF [56], protein ( $\text{N} \times 6.38$ ) IDF [57], and ash IDF [58]. Chemical composition of plants, raw milk, and ricotta is reported in Table 1.

**Table 1.** Chemical composition of plants (% DM), raw milk (%), and ricotta (% and % DM).

Parameters	<i>P. ferulacea</i>	Raw Milk		Ricotta	
		P0	P1	P0	P1
Dry matter (DM)	18.48			28.44	27.08
Lactose		5.26	6.67		
Ether extract/fat	6.91	7.08	4.53	53.51	51.44
Crude protein (CP)	16.34	4.50	5.32	33.46	33.86
Casein		3.39	4.08		
Ash	10.87			3.00	3.16
NDF	25.88				
ADF	23.78				
ADL	3.44				
Cellulose	19.88				
Hemicellulose	2.10				

Abbreviation: P0, absence of *P. ferulacea*; P1, presence of *P. ferulacea*.

#### 2.4. Microbiological Analyses on Raw Milk and Ricotta

Microbiological analyses were performed on raw milk samples and ricotta. Liquid samples (1 mL) were directly serially diluted in Ringer's solution (Oxoid) in a 1:10 proportion. Solid samples (20 g) were added to 180 mL of sterile Ringer's solution (0.9% *v/v*) and homogenised with a stomacher (Bag-Mixer 400, Interscience, Saint Nom, France) at the maximum speed for 2 min and then serially diluted in Ringer's solution. Microbial suspensions were then seeded in the corresponding culture media for the enumeration of the following microbial groups: total mesophilic microorganisms (TMM); lactic acid bacteria (LAB) cocci and rods, both thermophilic and mesophilic; enterococci; coagulase-positive staphylococci (CPS); *Listeria monocytogenes*; *Escherichia coli*; *Salmonella* spp.; pseudomonads, yeasts, and moulds. Culture media and incubation conditions were provided in accordance with Gaglio et al. [59]. Microbiological counts were threefold. The detection limit of the plate count method was estimated to be <2 CFU/g according to the volume of the plated sample and the minimum number of countable colonies.

#### 2.5. Shiga-Toxigenic *Escherichia Coli* (STEC) Detection

*Escherichia coli* colonies detected were analysed for their STEC genes following the multiplex PCR approach described by Osek [60], and—designed on the basis of the genes—coding for Shiga toxins 1 and 2 (*stx*<sub>1</sub> and *stx*<sub>2</sub>) was applied to the *E. coli* isolates developed at the highest dilutions of the raw milk samples.

#### 2.6. SPME-GC/MS Analysis of *P. ferulacea* Biomass, Raw Milk, Whey, and Ricotta

Solid-phase microextraction (SPME) is a solvent-free extraction technique, now widely used and established for studying volatile compounds in foods. It provides qualitative and quantitative information on individual molecules that contribute to flavour, thus correlating them with consumer perception and appreciation. The experimental conditions for the extraction phase, temperature, choice of SPME fibre, desorption, and subsequent GC/MS analyses were optimised after several trials. Six grams of *P. ferulacea* biomass sample were transferred to a 40 mL glass vial with a silicone septum.

All aroma component analyses were conducted using SPME-GC/MS with a 50/30 µm divinylbenzene/carboxyl/polydimethylsiloxane fibre (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA). The experimental conditions for SPME extraction, the GC method, and all mass spectroscopy parameters were optimised to perform the qualitative and semi-quantitative identification of over 100 compounds using mass spectral data (NIST library) according to the established protocol, as reported by Gannuscio et al. [61].

All compounds were grouped into the following 11 classes: hydrocarbons, alcohols, fatty acids, esters, ketones, aldehydes, terpenes, dienes, phenols, ethers, and unknown compounds. This allows for easier assessment and definition of the variations and contributions of each class of compounds in the different matrices analysed, from plants to ricotta.

#### 2.7. LC/MS Analysis of *P. ferulacea* Biomass, Raw Milk, Whey, and Ricotta

Extraction of phenolic compounds from *P. ferulacea* biomass sample in addition to the two sets (P0 and P1) of raw milk, whey, and ricotta were extracted and quantified following the indications reported in consolidated literature and/or modified according to appropriate adjustments. Two grams of the sample was weighed and 5 mL of methanol/water (80:20 *v/v*) was added. The mixtures were shaken for 1 min, treated in an ultrasonic bath for 15 min at room temperature, and centrifuged at 5000 rpm for 25 min at 20 °C. The resulting supernatant was filtered using PTFE filters (0.45 µm). The filtered samples were then diluted 100-fold with methanol, and a 5 µL aliquot of the sample was finally injected into the LC/MS system. Twenty polyphenols were searched in the matrices to quantify them;

for all samples the tests were conducted in triplicate, and for each polyphenol appropriate calibration curves were constructed. The analytical method used for the identification of polyphenols was based on the same experimental, chromatographic, and mass spectrometric conditions described by Scirba et al. [62]. Quantification was carried out using the following pure standards: Apigenin 7-Glucoside, Apigenin, Quercetin, Gallic Acid, L-Mandelic Acid, Chlorogenic Acid, Hydroxycinnamic Acid, Kaempferol, Caffeic Acid, Vanillic Acid, Catechin, Rutin, Coumaric Acid, Syringic Acid, Gentisic Acid, Ferulic Acid, and Luteolin. An external calibration method was employed for the phenolic compound quantification. A 5 ppm methanolic solution of each standard was prepared and five calibration solutions were subsequently prepared at concentrations of 1 ppm, 500 ppb, 250 ppb, 100 ppb, 50 ppb, and 5 ppb. The linear correlation coefficient ( $R^2$ ) for all compounds was on average 0.99. Quantitative data were processed using Quan/Qual Browser Trace Finder 4.0 (Thermo Fisher Scientific, San José, CA, USA). Each calibration point represented the average of three independent injections. The limits of detection (LOD) and quantification (LOQ) were estimated from the blank measurements and the calibration curve. The LOD corresponds to the blank signal plus three times its standard deviation, while the LOQ corresponds to the blank signal plus ten times its standard deviation.

### 2.8. Statistical Analyses

Regarding microbiological data, plate count data were subjected to one-way analysis of variance (ANOVA) using XLStat software version 7.5.2 for Excel (Addinsoft, New York, NY, USA). The Tukey's test was applied for pairwise comparison between the different samples analysed. Statistical significance was attributed to  $p$ -values  $< 0.05$ . Concerning chemical data, the same statistical methodology was applied for the aromatic fraction.

## 3. Results and Discussion

### 3.1. Ecological Aspects

The study area appears as a barren surface in which all the plants are grazed up to a few centimeters from the ground, while a few years ago the area appeared as a pasture dominated by *P. ferulacea* (Figure 1).



**Figure 1.** Comparison between Piano Battaglia on the Madonie Mountains in June 2014 and June 2023.

Currently, the aerial part of *P. ferulacea* has almost disappeared. This species has begun to be appreciated by farmed herbivores and is highly sought by feral deer that avidly graze the leaves of the plant as soon as they are emitted. The relevé P1 carried out on the Madonie in 2014 consisted of 28 taxa and a canopy density of 80%, that of 2023 (P1—2023) consisted of 34 taxa and a canopy density of 60% (Table 2). The six species that appeared are, for the

most part, with a wide range, while *P. ferulacea* and *Secale strictum* have decreased from a numerical and biomass point of view.

**Table 2.** Basic stationery and biodiversity indices: canopy density, total number of taxa, percentage of endemics, percentage of taxa characteristic of the mountain belt, percentage of cosmopolitans, Shannon Index (SH), Pignatti–Ellenberg bioindication indices. (L: light conditions; T: temperatures; K: climatic continentality; F: moisture; R: reaction; N: nutrient availability; S: salinity.)

	Canopy Density	No. Taxa	% Endemics	% Mt. Belt	% Cosmop.	Shannon_H Index	L	T	K	F	R	N	S
P1—2014	80	28	13.79	31.03	6.90	2.59	8.4	6.6	4.4	2.8	5.6	2.8	0.0
P1—2023	60	34	11.76	23.53	8.82	2.67	8.4	6.5	4.5	3.1	5.2	3.1	0.0
P0—2023	100	31	3.22	6.45	12.90	3.1	8.1	7.3	4.3	3.5	6.0	3.4	0.0

In 2023, a higher Shannon index value than in 2014 resulted (2.67 vs. 2.59); although the overall number of species decreased, the number of endemics and the relative abundance of each species increased. This is presumably due to an increase in the available surface area following the reduction of *Prangos* biomass. Similarly, there has been a decrease in the number of species strictly linked to the mountain habitat in favor of more thermophilous species. As regards the Ellenberg indicators, the two relief showed similar values of light, continentality (4.4 vs. 4.5), and moisture requirements (2.8 vs. 3.1) and the same light and temperature index. In 2023, the indices related to soil reaction decreased and nutrient availability increased. The area without *P. ferulacea* (P0) appears poorer in terms of plant diversity. Although it shows a higher density and similar number of taxa (31), it has a lower rate of endemism and of taxa characteristic of the mountain belt and a higher number of cosmopolitan taxa. The temperature index and the nutrient indices are higher.

### 3.2. Chemical Analysis of Forage, Raw Milk and Ricotta

In late spring, the *P. ferulacea* forage showed high protein level, intermediate between those found in the forage of a more widespread legume species, as sulla (*Sulla coronaria* (L.) Medik), in the same environment and period by Di Trana et al. [63] (CP 18.6% DM) and Gannuscio et al. [64,65] (CP 14.8% DM). However, compared to sulla forage, the *P. ferulacea* plants were also characterised by lower fibre contents (NDF < 6–15% DM) and higher lipids (ether extract, >4.5–5% DM), that contribute to improving its nutritive value. Coskun et al. [6], who analysed *P. ferulacea* plants harvested in late May, report lower levels in protein and ether extract and slightly higher NDF content, attributable to the drying to which the forage was submitted in the field. The raw milk processed to ricotta was different in the absence of *P. ferulacea* (P0) and in the presence of *P. ferulacea* (P1) for all components, presumably due to the pasture composition, besides than to the diverse incidence of the raw milk from the different animal species. The amounts of the main nutrients detected in ricotta, as protein and fat, reflect those of origin milk; indeed, ricotta sampled in P0 showed more fat, reaching a level equal to 15.2% of the fresh product. However, both protein and fat were within the ranges reported for different types of ricotta manufactured in artisanal dairy farms from milk of different livestock animals [66,67].

### 3.3. Microbiological Evolution

The levels of the different microbial groups investigated in raw milk of both P0 and P1 samples are reported in Table 3. No statistically significant differences ( $p > 0.05$ ) were observed among both milk bulks for all the analysed microbial groups. Excluding coccus mesophilic LAB that were found to be at the same level ( $10^4$  CFU mL<sup>-1</sup>) of TMM, the remaining LAB groups showed a lower load of about one log cycle. Within the undesired bacteria enterococci, CPS, *L. monocytogenes*, *Salmonella* spp., pseudomonads, yeasts, and

moulds were undetectable ( $<1$  log CFU mL<sup>-1</sup>), while members of the Enterobacteriaceae family and *E. coli* were around 10<sup>2</sup> CFU mL<sup>-1</sup> in both P0 and P1 milk bulks.

**Table 3.** Microbiological counts of raw milk.

Microbial Counts	P0	P1	p-Value
TMM	4.91 ± 0.35	4.48 ± 0.35	0.328
Mesophilic coccus LAB	4.94 ± 0.42	4.48 ± 0.26	0.300
Thermophilic coccus LAB	3.89 ± 0.18	3.70 ± 0.28	0.500
Mesophilic rod LAB	3.99 ± 0.52	3.78 ± 0.29	0.668
Thermophilic rod LAB	3.74 ± 0.59	3.26 ± 0.18	0.373
Enterobacteriaceae	1.78 ± 0.21	1.68 ± 0.16	0.646
Enterococci	<1	<1	n.e.
CPS	<1	<1	n.e.
<i>L. monocytogenes</i>	<1	<1	n.e.
<i>E. coli</i>	1.38 ± 0.37	1.42 ± 0.51	0.937
<i>Salmonella</i> spp.	<1	<1	n.e.
Pseudomonads	<1	<1	n.e.
Yeasts	<1	<1	n.e.
Moulds	<1	<1	n.e.

Units are Log CFU/mL. Results indicate mean values ± S.D. of six plate counts (carried out in triplicates for two independent productions). Abbreviations: P0, absence of *P. ferulacea*; P1, presence of *P. ferulacea*; TMM, total mesophilic microorganisms; LAB, lactic acid bacteria; CPS, coagulase-positive staphylococci; *L.*, *Listeria*; *E.*, *Escherichia*; n.e., not evaluated.

The analysis of ricotta (Table 4) showed a high hygiene profile of the final products from both trials, indicating the absence of the main spoilage and pathogenic microorganisms. The four LAB groups (mesophilic and thermophilic rods and cocci) investigated were recorded at about 10<sup>4</sup> CFU g<sup>-1</sup>.

**Table 4.** Microbiological counts of ricotta.

Microbial Counts	P0	P1	p-Value
TMM	5.40 ± 0.32 a	5.30 ± 0.19 a	0.742
Mesophilic coccus LAB	4.40 ± 0.42 a	4.10 ± 0.49 a	0.577
Thermophilic coccus LAB	4.48 ± 0.19 a	4.18 ± 0.45 a	0.470
Mesophilic rod LAB	4.85 ± 0.29 a	4.48 ± 0.36 a	0.362
Thermophilic rod LAB	4.48 ± 0.49 a	4.54 ± 0.31 a	0.898
Enterobacteriaceae	<1	<1	n.e.
Enterococci	<2	<2	n.e.
CPS	<2	<2	n.e.
<i>L. monocytogenes</i>	<2	<2	n.e.
<i>E. coli</i>	<2	<2	n.e.
<i>Salmonella</i> spp.	<2	<2	n.e.
Pseudomonads	<2	<2	n.e.
Yeasts	<2	<2	n.e.
Moulds	<2	<2	n.e.

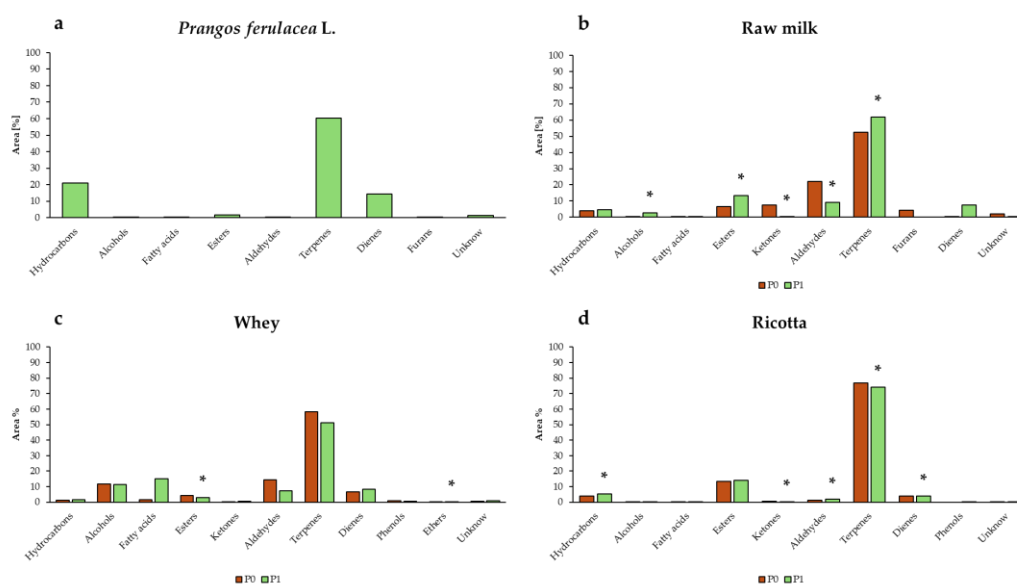
Units are Log CFU g<sup>-1</sup>. Results indicate mean values ± S.D. of six plate counts (carried out in triplicates for two independent productions). Data within a row followed by the same letter are not significantly different according to Tukey test. Abbreviations: P0, absence of *P. ferulacea*; P1, presence of *P. ferulacea*; TMM, total mesophilic microorganisms; LAB, lactic acid bacteria; CPS, coagulase-positive staphylococci; *L.*, *Listeria*; *E.*, *Escherichia*; n.e., not evaluated.

Ricotta is a typical Italian dairy product, widely used in many traditional dishes, both sweet and salty [66]. In the area of the Madonie mountains, the production of “basilisk” ricotta has almost been lost, and few cheesemakers continue to hand it down. Since the idea of this work was to give value to a product that embodies authentic flavours of Sicily, ricotta produced from livestock animals fed with *P. ferulacea* was microbiologically

investigated. According to these results, no differences were found between the two productions performed in relation to the analysed microbial groups. The intake of *P. ferulacea* leaves did not affect the development of protechnological microorganisms or encourage the growth of pathogens or alterative ones. Unlike other Italian artisanal ovine ricotta production where the LAB, rod, and cocci were found at lower levels [68,69], the samples analysed throughout this study recorded two log cycles higher LAB levels. The observed presence of microorganisms of the *Enterobacteriaceae* family, considered to be indicator bacteria of the microbiological quality of foodstuffs and of the hygienic status of a production process, can easily occur due to its high nutritious content [70]. Their presence has been detected in several works, so they are a recognised part of the natural microbiota of many dairy products [71–73]. However, their presence was lower than  $2 \log \text{CFU mL}^{-1}$ , which means in the range of the detection limit established by EU Regulation 2073/2005 and thus safe for consumption. Within the *Enterobacteriaceae* family belongs *E. coli*, a common contaminant of raw milk and usually associated to faecal contamination; however, certain strains may exhibit virulence factors that can lead to human disease. Based on the EU One Health 2019 Zoonoses Report [74], STEC infections are the most frequently reported zoonoses after campylobacteriosis and salmonellosis. For this reason, the presence of STEC was specifically investigated. The PCR approach applied clearly indicated that none of the *E. coli* colonies had this virulence factor. In addition, no alterative or pathogenic microorganisms were detected in both ricotta samples, supporting the hygienic safety of the product.

### 3.4. SPME-GC/MS Analysis of *P. ferulacea* L., Raw Milk, Whey, and Ricotta

The relative abundance of each compound was evaluated according to a quantitative approach with limitations, as the areas of each peak do not reflect the real quantities of the different compounds. However, these percentages are very useful as a comparison tool and give indications to evaluate the contribution of *P. ferulacea* L., in this case, for raw milk, whey, and ricotta flavour (Figure 2). As is evident from the graph in Figure 2, the greatest contribution of the aroma is due to the following classes: terpenes, dienes, and hydrocarbons.



**Figure 2.** The aroma of *P. ferulacea* (a) and of raw milk (b), whey (c), and ricotta (d) obtained in the absence of *P. ferulacea* (P0) and in the presence of *P. ferulacea* (P1). Data obtained by HS-SPME-GC/MS analysis for each class of compound. All the values marked with \* of the first and second samples are significantly different ( $p < 0.05$ ).

The aroma profiles of raw milk samples without (P0) or with (P1) *P. ferulacea* L. show the same classes of compounds present in plant samples of *P. ferulacea*, except for furans absent in milk. In detail the most abundant classes change; the terpenes class remains the predominant class among all, but the aldehyde and ester classes increase in raw milk, with the highest values in raw milk obtained from animals reared on pastureland in the absence of *Prangos ferulacea* L. (P0). As demonstrated so far, the aromatic contribution of volatile and semi-volatile compounds present in the *P. ferulacea* plant is more or less maintained but certainly also enriched by the classes of substances that are well present in raw milk.

In fact, as reported in Figure 2, whey shows an aromatic profile, with all classes of compounds present in samples of raw milk. It should be noted that the class of terpenes remain the more abundant in both samples, but in whey P1 fatty acids and alcohols become more plentiful followed by aldehydes, dienes, and esters. However, whey is the raw material from which ricotta is obtained, which influences the final product. So finally, as shown in Figure 2, in the aromatic profile of ricotta there are a few classes of compounds, and the most significant were, for both sets of samples, terpenes followed by esters, hydrocarbons, and dienes. In detail, it is important to note that the contribution of the aromatic profile of ricotta is very similar in terms of classes of compounds to that of the plant in which terpenes are always the most abundant, followed by dienes, hydrocarbons, and esters in the plant. In ricotta, after terpenes, the contribution of esters stands out, followed by hydrocarbons and dienes. Therefore, we can assert that it is evident that the contribution of terpenes remains the most abundant class, correlating it more directly to the contribution of the plant, while the presence of esters in ricotta is correlated to fermentation processes typical in milk and in general in dairy production. Overall, although the analysis is semi-quantitative (peak areas do not reflect absolute values), the data is effective for comparing the aromatic contributions among matrices (raw milk, whey, ricotta). Terpenes are the most abundant class of volatile compounds across all analyzed matrices (plant, milk, whey, ricotta), highlighting their direct transfer from the plant to dairy products. In the samples without *P. ferulacea* (P0), aldehydes and esters increase, whereas in the presence of the plant (P1), terpenes, fatty acids, and alcohols become more prevalent, especially in whey. The aromatic profile of ricotta is more similar to that of the plant compared to raw milk and whey, suggesting that the plant's volatiles are relatively stable and persist into the final dairy product. The presence of esters in ricotta may also be attributed to fermentation processes typical of dairy production.

### 3.5. LC/MS Analysis of *P. ferulacea*, Raw Milk, Whey, and Ricotta

The SRM chromatogram of the *P. ferulacea* sample (Figure S1) shows the presence of several targeted phenolic compounds. Their quantification was reported in Table 5. In plants, approximately seven polyphenols with varying contributions were identified; while very few were found in raw milk, whey, and ricotta.

Several tests were conducted to optimise the extraction procedure using different MeOH/H<sub>2</sub>O ratios, and the selected protocol proved to be the most effective among those evaluated. Nevertheless, the extraction was not exhaustive, particularly for milk samples in which no phenolic compounds were detected. This limitation is likely attributable to the presence of lipids and casein, which can interact with polyphenols and hinder their transfer into the extract [75]. Only coumaric acid was found in the whey and ricotta. This area requires further investigation, and additional tests will be conducted. Ultimately, the plant shows a significant phenolic content, with rutin being the most abundant compound (161.26 µg g<sup>-1</sup>). Phenolic compounds are virtually absent in the dairy products analysed, except for trace amounts of coumaric acid in whey and ricotta. These results suggest that phenolic compounds from the plant do not effectively transfer into dairy

derivatives, possibly due to low solubility or degradation during animal metabolism or dairy processing.

**Table 5.** Polyphenolic compounds detected in samples.

Active Substances	<i>P. ferulacea</i>	Raw Milk		Whey		Ricotta	
		P0	P1	P0	P1	P0	P1
Cumaric acid	3.21	n.d.	n.d.	0.20	0.23	0.20	0.18
Ferulic acid	0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Luteonin	0.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	1.52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hydroxycinnamic acid	7.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rutina	161.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin	0.48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σpolyphenols	174.56	n.d.	n.d.	0.20	0.23	0.20	0.18

Abbreviation: P0, absence of *P. ferulacea*; P1, presence of *P. ferulacea*; n.d., not detected.

#### 4. Conclusions

This study provided a multidisciplinary evaluation on perennial common basilisk (*Prangos ferulacea* (L.) Lindl.), a spontaneous species of the pastures in the Madonie mountains (Sicily). The *P. ferulacea* forage sampled in late spring denoted a good nutritional value, being well provided in protein, similarly to legume forages. The main nutrients amount detected in ricotta, as protein and fat, fully reflected that of the typical product manufactured in local artisanal dairy farms. The microbiological evaluation confirmed the safety of the final product, thereby indirectly contributing to the development of livestock farming in a sustainable manner, maintaining indigenous breeds and supporting small local cheese makers. Comparing the volatile compounds present in the plant, milk, and ricotta clearly indicates a direct transfer of aromatic markers from *P. ferulacea*, which supports its role in shaping the sensory profile of ricotta. However, phenolic compounds showed limited transfer, suggesting they contribute less to the nutraceutical properties of the final product.

The results highlight the ecological importance of these pastures and their impact on the quality and flavour of traditional dairy products. The presence of *P. ferulacea* as forage species can support traditional pastoralism, a fundamental element for sustainable development of mountain areas. Depth studies are necessary both to improve extraction methods and to clarify the mechanisms involved in the transfer of bioactive compounds.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16010066/s1>. Table S1: Conditions employed for the SPME-GC/MS analysis; Table S2: LC-MS/MS parameters for phenolic compounds identified in samples. Figure S1: SRM chromatogram of the *P. ferulacea* biomass. Table S3: Volatile aromatic compounds (%) in *P. ferulacea*, raw milk, whey and ricotta under P0 and P1 conditions.

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