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Influence of feeding Murciano-Granadina Goats with *Posidonia oceanica* Banquettes on the Resulting Milk and Cheese

Anne van Eldik[1], María Isabel Hortelano[2], Daniel Álvarez[2], Juan Sotillo[3], Cándido Gutiérrez[3], Víctor Garcia[2], Margherita Bacchiocchi[4], María Belén López[2,*]

[1]HAS Hogeschool. Postbus 90108, 5200 MA 's-Hertogenbosch, Netherlands
[2]Food Science and Technology Department, International Excellence Campus for Higher Education and Research "Campus Mare Nostrum", Veterinary Faculty, University of Murcia, Campus de Espinardo, 30100, Murcia, Spain
[3]Animal Medicine and Surgery Department, International Excellence Campus for Higher Education and Research "Campus Mare Nostrum", Veterinary Faculty, University of Murcia, Campus de Espinardo, 30100, Murcia, Spain
[4]Veterinary Science Department, University of Pisa, Viale delle Piagge, 2, 56124 Pisa, Italy
*Corresponding author: mbelen@um.es

Abstract The inclusion in the diet of biological products of a biodegradable nature is a highly valued strategy with many advantages. *Posidonia oceanica* is a seagrass species which may be used as a substitute for straw. In this study, one group of goats (control) was fed a normal diet that contained straw, while the straw in the diet of the other group was replaced by *P. oceanica*. No significant differences were found between the control and experimental group regarding the main physicochemical and sensorial parameters of the resulting milk and cheese; although the cheese from the group fed *P. oceanica* was firmer and chewier. When the milk-clotting time was determined under different assay conditions, the origin of the milk was found to have a significant effect, the milk from animals fed with algae showing greater technological suitability. In conclusion, *P. oceanica* can be used as a straw substitute, promoting a sustainable livestock system and contributing to the maintenance of meadows.

Keywords: *Posidonia oceanic, goat milk, goat fresh cheese, milk clotting time*

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1. Introduction

Goat milk is increasingly regarded an interesting alternative to cow milk due to its characteristic taste and technological and health-related benefits [1]. Some authors [2,3] suggest that goat milk can be prescribed for lactose intolerant children and as an alternative for people who are allergic to cow .milk, since about 40 % of people allergic to cow milk can tolerate goat milk proteins

According to FAOSTAT [4], both in Spain and the rest of Europe, goat milk production grew between 2000 and 2010, decreased between 2010 and 2012 and increased again in 2013, suggesting an improvement in the sector after the recent economic crisis. However, to continue promoting this sector, new strategies need to be adopted.

Due to the poor quality and shortage of pasture in Mediterranean countries, farmers are forced to use cereal and grain concentrates for animal feed. Using agricultural by-products may be of interest in this respect for two reasons: reduced feeding costs and alleviation of the environmental problems associated with their accumulation [5]. As a consequence, interest is growing in using these residues as a partial replacement in feed for ruminants, as long as there is no effect on the characteristics of the final product: milk, cheese, yogurt and meat products [6].

As an extension of this idea, it might be interesting to develop a sustainable livestock system by using marine waste from the Mediterranean Sea, due to its importance for environmental conservation [7].

From a nutritional point of view, by-products can be included in the diet to provide energy and protein, and they are also often characterized by their high fibre content. This is the case of *Posidonia oceanica*, a seagrass that may be used as a substitute for straw, due to the ability of ruminants to ferment it in the rumen precisely because of its high fibre content [8]. *P. oceanica* has a neutral detergent (NDF) and acid detergent (ADF) fibre content of 473 and 359 g kg^{-1} of dry matter, respectively [7]. This seagrass is rich in lignite and has an alkaline pH when degraded, while the level of heavy metals it contains are not problematic according to raw materials legislation.

At present, significant quantities of dead leaves of *P. oceanica* are washed up on beaches, where they are mainly considered waste destined for incineration. Several applications have been developed for *Posidonia* oceanic leaves including their use as shock-absorbing material for

glassware transportation, maintaining the moisture level of fresh fish [9], as well as for methane production, conversion into cellulose or as animal fodder [10].

Previous studies [11] indicated that the production of *P. oceanica* is approximately 5×10^6-5×10^7 tonnes per year, making the Mediterranean Sea a source of cheap material for use as feed component. Furthermore, the use of *P. oceanica* as a peat substitute in substrates would help reduce the over-exploitation of peat lands, with consequent environmental advantages [12]. Moreover, it might also help farmers to reduce their dependence on grain for use as animal feed, bringing down costs while using a by-product that might even generate additional income for producers.

However, changes in the animal diet could cause changes in any derived food products, like meat and milk, which should be analysed to ensure that their quality is unaffected. In a study carried out to investigate the metabolic effect of *P. oceanica* on the milk of Murciano-Granadina goats [12], the authors concluded that using banquettes of *P. oceanica* has potential for use in ruminant feeding as a fibrous forage source.

The aim of this study was to determine the influence of incorporating *P. oceanica* in the diet of Murciano-Granadina goats on milk production, milk coagulation and milk and fresh cheese products.

2. Material and Methods

2.1. Posidonia Oceanica Collection

P. oceanica was collected from Mediterranean beaches of La Manga del Mar Menor (San Javier, Murcia Spain) and transported to the farm of the University of Murcia for cleaning and storage. For this, the seagrass was washed with clean fresh water and then dried in the sun for 48 hours before packing into plastic bags. The composition of the *P. oceanica* diet used in this study is described in a previous study [12].

2.2. Experimental Design

Experiments were conducted with 48 lactating Murciano-Granadina goats from the IMIDA (Murcia, Spain). The animals were divided into two groups: group C (control), and group P (given a diet that included *P. oceanica*). Both groups can be considered homogeneous as each consisted of 24 animals, of which 6 were in second lactation, 6 in third lactation, 6 in fourth and 6 in fifth lactation.

Goats in the control group (Group C) were fed a basal diet of 2.3 kg·day^{-1} per animal, prepared and balanced (isoenergetic and isoproteic) by Cargill Animal Nutrition S.A. (Martorell, Barcelona, Spain), using the following ingredients: molasses (1.00 %), vitamins and mineral additive (1.04 %), straw (26.65 %), calcium carbonate (1.49 %), corn flour (3.67 %), honey bean (4.16 %), sunflower oil (7.00 %), rye (6.99 %), malt rootlet (8.00 %), barley (15 %) and soybean hulls (25.00 %).

In the other group (Group P), the goats were fed the same diet but with the barlew straw completely replaced by *P. oceanica*. The experiment was carried out during February to March 2015, the first 15 days being considered as a period of adaptation to the new diet. Goats were machine-milked in the morning and bulk milk samples from each group were taken twice a week for one month (10 sampling days) and kept cooled at 4°C until delivery to the Food Technology Pilot Plant of the University of Murcia a transportation time of less than 2 hours.

2.3. Goat Milk and Cheese Making

Bulk milk was collected twice a week and one sample of each group (C and P) was taken each day. The milk was pasteurized (78°C for 15 s) by a plate heat exchanger (100L Alfa Laval, Lund, Sweden) in the pilot plant of the Food Technology Department the same day of milking.

Fresh cheese was made as described by Garcia [13] at the Food Technology Pilot Plant the day after collection. For this, the pasteurized Murciano-Granadina goat milk was introduced in a double-zero cheese-vat (Type 10 L, Pierre Guerin Technologies, Mauze, France) and tempered until a constant temperature of 32°C was reached. Stirring slowly, 3 mL of $CaCl_2$ (Chr. Hansen, France) at a concentration of 510 g·L^{-1} were added. Then, 3 mL of calf liquid rennet (Caglio Star Spain S.A., Cieza, Murcia, Spain) was added and the cutting time (T_{cut}) was determined by multiplying T_{max} by $\beta = 3$, as a modification of the method described by Fagan [14]. T_{max} is an optical parameter derived from a CoAguLite^{TM} optical sensor coupled to the vat, and is useful for predicting milk clotting time.

When milk clotting time was reached, the curd was cut in a first cut of 20 s, followed by a pitching of 10 minutes and second cut of 5 minutes. Another pitching of 3minutes was performed before finally stirring for 15 minutes. The curds were placed in square moulds, unpressed, and brined (17°Be for 30-40 minutes). Cheese yield was defined as the amount of milk needed to obtain a given number of kilograms of cheese (L kg^{-1}).

2.4. Milk Clotting Time Determination

The Berridge milk clotting time in pasteurized goat milk was measured according to IDF 110A [15], introducing 10 mL of milk into a standard test tube for tempering. When the milk reached the target temperature, 0.2 mL of enzyme dilution was added and stirred. The timer was started and when the first flocks appeared on the wall of the tubes, it was stopped. The elapsed time was considered the clotting time [16].

A factorial design was applied with two levels for milk type (C and P), three levels for temperature (20, 30 and 40°C) and four levels for enzyme concentration (0.005, 0.01, 0.015 and 0.02 mg·mL^{-1}). All analyses were made in quadruplicate.

2.5. Physicochemical Analysis

Dry matter, fat, protein and lactose were measured with an infrared spectrophotometer (Milko Skan in a Combi Foss 5000, Foss Electric, Hillerød, Denmark) according to IDF standard 141B [17]. The pH measurements were made using a Crison® pH meter (micro pH 2001,

Barcelona) connected to a Crison® glass combined electrode (1952-2002) previously calibrated at room temperature. All analyses were made in duplicate.

Cheeses were analysed after 24 hours. Total fat content was measured according to Van Gulik's method [18]. The dry matter content of cheese (3 ± 0.1 g) was measured by oven-drying at 105°C until constant weight [19]. The total nitrogen concentration was determined (0.5 ± 0.01 g of cheese) according Kjeldahl method [20]. The pH was measured by suspending 5 ± 0.1 g of grated cheese in 30 mL of distilled water and stirring for 10 minutes using a Crison® pH meter (micro pH 2001, Barcelona) connected to a previously calibrated Crison® glass-combined electrode (1952-2002).

To measure the fatty acid composition of both types of milk and cheese, lipids were extracted according Röse-Gottlieb, using 3 mL of 30 % ammonia, 10 mL of pure ethanol, 25 mL of ethyl ether and 25 mL of 40 % petroleum ether in 10 mL of milk or 1 g of cheese. Methylation was carried out in 0.01 g of sample with 5.7 µL of internal standard (undecanoic acid methyl ester). Three millilitres of 0.2 N sodium methylate were added and heated for 5 min. After cooling, 3 mL of sulfuric acid: methanol (3 %) was added and the mixture was reheated for another 5 min. Then, 3 mL of hexane were added and heated for 1 min before pouring the product into a 10 mL volumetric flask, which was flushed with salt water. The supernatant was used for chromatographic analysis.

Finally, total fatty acid were quantified by gas chromatography (IDF 184, 2002), using a Finnigan Trace GC ULTRA gas chromatograph (Thermo Finnigan, España), equipped with an AS3000 auto-sampler (Thermo Finnigan, España), a capillary column with cross-linked 70 % Cyanopropyl Polysilphenylene-siloxane, 60 m long, 0.25 mm internal diameter and 0.25 µm film thickness (BPX70, SGE, Australia) and an ionization flame detector. An aliquot of 1 µL was injected into the gas chromatograph and the following ramp was used: 50°C for 5 minutes; increase of 7°C·min^{-1} to 165°C. This temperature was maintained for 12.5 min before a second increase of 2°C·min^{-1} to 225°C, which was held for 4 min.

The methyl esters of the fatty acids were quantified using the methyl ester of undecanoic acid (Sigma U 0250, Madrid, Spain) as internal standard. This standard was also used in the calibration curves for each fatty acid standard (Sigma-Aldrich, San Luis, Missouri, USA) analysed.

The integration of fatty acids was processed by software from Chrom Card Fisons Instruments (Italy), and the concentration of each fatty acid was expressed in mg·100mg^{-1} of total fatty acids. The injections were performed in duplicate.

2.6. Analysis of Minerals and Metals in Milk and Cheese

HNO_3 and H_2O_2 were of supra-pure quality and double de-ionized water was used for all dilutions. Samples (5 mL of milk and 0.3 g of cheese) were digested with 3 mL of HNO_3 (65 %) and 2 mL of H_2O_2 (30 %) in a microwave digestion system for 31 min and diluted to 10 mL with de-ionized water. The digestion conditions for the microwave system were: 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400W, 8 min at 550 W, vent 8 min). This procedure was preferred because of its greater accuracy with respect to both time and recovery values, which were nearly quantitative (95 %) for the above-mentioned digestion method.

As, Cr, Hg, Si, Fe, Cu, Zn and Sr were determined by inductively coupled plasma-mass spectrometry (ICP-MS). The analysis was performed using an Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an Integrated Autosampler (I-AS). Samples were introduced into a Scott spray chamber using a MicroMist glass concentric nebulizer and then into a Fassel type torch. An octopole reaction system (ORS) using He as collision gas, was used to remove polyatomic interferences. The sample temperature was set at 2°C and the radio frequency power was set to 1500 W. Argon was used as the carrier gas, at a flow rate of 0.97 L·min^{-1}. The sample was inserted and nebulized with a pump operated at 0.1 rotations per second. The argon gas used was of spectral purity (> 99.998 %). For the quantification of S, Ca, Na, Mg, P and K an inductively coupled Plasma system equipped with a Perkin Elmer Optima 8300 DV Optical Emission Spectrophotometer was used.

Concentrations of elements in the samples were determined in triplicate and an external standard calibration method was applied. Seven-point calibration curves were constructed by analysing multi-element reference standards prepared from stock solutions (High-Purity Standards, Charleston, SC, USA).

2.7. Sensory Analysis

A prescribed descriptive test [21] was carried out after previous training of the panellists, when every parameter was defined and quantified. Granny Smith apples, crackers and natural mineral water were served for cleaning the palate between samples.

Sensory milk and cheese parameters were analysed by the 8 panellists trained in dairy products analysis in a laboratory equipped for sensory analysis, at the Department of Food Technology of the University of Murcia. Refrigerated fluid milk was tempered at 16°C and the samples (approximately 35 mL) were presented to the testers in plastic cups with random code numbers. The sensory analysis of the cheeses was performed 24 hours after cheesemaking and each cheese was split into two halves labelled using randomly chosen digits. One was divided into wedges of approximately 1 cm thickness. The other whole half was used for the visual phase.

The score set included eight descriptors for milk, related with odour and flavour and ten descriptors for cheese, related with odour, flavor and texture, all of them with a structured intensity scale ranging between 1 and 10. Both milk and cheese tests included an overall quality score in the same range.

2.8. Cheese Texture Analysis

Texture profile analysis (TPA) was carried out as described by Garcia *et al.* (2012), using a QTS-25 texture analyser (Brookfield CNS Farnell, Borehamwood, Hertfordshire, England) equipped with a load cell of 25 kg and Texture Pro V. 2.1 software. For TPA, three cube

shaped samples (3 cm^3) were cut from a rindless cheese, wrapped in aluminium foil and equilibrated at 20 ± 0.5°C for 3 h before testing. The testing conditions were: room temperature of 20°C; two consecutive cycles of 50% compression; cross-head moved at a constant speed of 30 $mm \cdot min^{-1}$ and a trigger point of 0.05 N. Texture variables, hardness (expressed as N), gumminess (expressed as N), chewiness (expressed as N*mm), cohesiveness (adimensional), springiness (expressed as mm) and adhesiveness (expressed as N*s) were calculated as described by Bourne [22].

2.9. Statistical Analysis

Statistical treatment of the data was performed using the statistical software IBM SPSS Statistics 19 (IBM Spain, S.A., Madrid, Spain). One-way ANOVA was used to assess significant differences and non-linear regression analysis was used to obtain the kinetics of the clotting time.

3. Results and Discussion

3.1. Milk

As shown in Table 1, no significant differences ($P > 0.05$) were observed between groups as regard milk production, fat, dry matter and pH. However, protein and lactose showed significant differences ($P < 0.05$), with a higher protein content and lower lactose level in the milk from group P. The absence of differences in dry matter confirmed that the protein and lactose are affected by diet.

The origin of the milk significantly influenced the magnesium and calcium contents (Table 1), with magnesium showing lower values in the C milk and calcium showing lower values in the P milk. The other elements were not influenced by the origin of the milk, although the values of zinc were slightly higher in P milk, which agrees with the higher values determined by Castillo [7] in the *P. oceanica* leaves. The concentration of macro-minerals may not fluctuate much, but they vary depending on the breed, diet, individual animal and status of udder health [23]. Other minerals (As, Cr, Hg, Si) were analysed but, since no trace of any was found, they are not included in the table. A previous study by Castillo [7] revealed higher levels of two heavy metals (Pb and Cr) in *P. oceanica* than were found in other studies, except as study carried out by Sanz-Lazaro [24] in the Aegean Sea. The fact that none of these metals was found in the milk despite their relatively high concentrations in the Mediterranean Sea confirms that *P. oceanica* can be considered a product presenting a low risk of heavy metal toxicity.

Table 2 shows that the inclusion of seagrass in goat feed affected the levels of C13, C15, C16:1, C18:2t9t12 and C20, the level of the last being lower in P milk, while the others fatty acids showed lower levels in C milk. Fatty acids, grouped according to their bonds, did not show significant differences. The fatty acid analysis was performed because it is known that the food that goats consume may influence the composition of these compounds in the milk [25]. However, the differences were minimal, since the fatty acids that showed differences were present in low amounts and, even together, represented less than 3 % of the total fatty acids. Also of note was the increase in linolelaidic acid observed in the P milk since trans- isomers of linoleic acid are associated with heart diseases [25]. The fact that fatty acids grouped according to their bonds did not show significant differences indicates that the food type had little influence in this respect. However, there is little information on this topic to make comparisons. Previous studies [26] indicated that *P. oceanica* from the Mediterranean Sea is characterized by long-chain fatty acids (C22-C34) and that the major fatty acids in the leaves are C16, C18:2 and C18:3. In the present study only C16:1 was seen to increase in P milk.

In the sensory analysis (Figure 1), no statistically significant differences were found for any parameter. However, tasters detected that P milk had a slightly less intense goat milk odour, sweetness and flavor, but with no significant differences. Both milks had the same final score. These results indicate that the use of *P. oceanica* as a straw substitute in goat feed will provide milk with the same sensory features as found for the control diet.

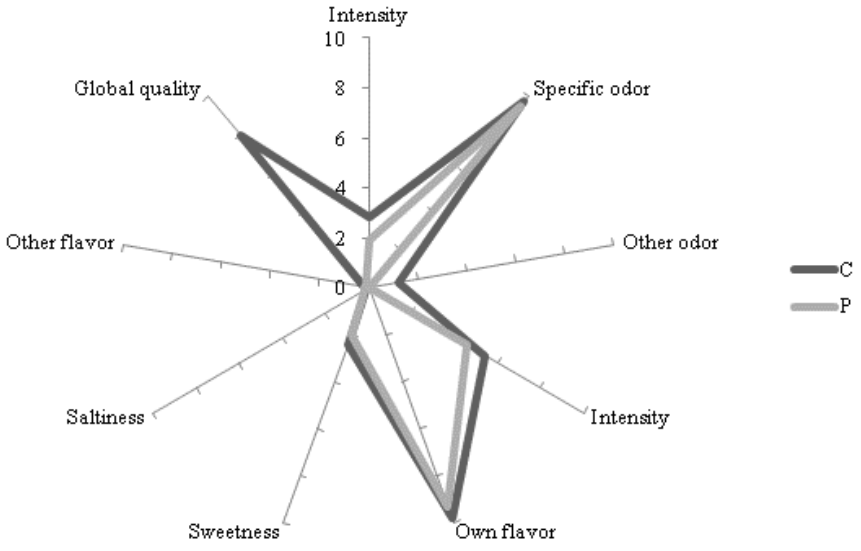

Figure 1. Sensory profile for both types of milk (C and P)

Table 1. Physicochemical parameters and mineral content (mean ± SD) of both types of goat milk [‡]

	C	P
Milk production (kg)	33.2±1.8[a]	33.8±4.8[a]
Fat (g kg^{-1})	59.9±1.7[a]	59.1±2.5[a]
Protein (g kg^{-1})	39.1±0.3[b]	40.0±1.0[a]
Lactose (g kg^{-1})	48.4±0.7[a]	47.9±0.8[b]
Dry matter (g kg^{-1})	155.2±2.2[a]	154.8±3.8[a]
pH	6.79±0.07[a]	6.79±0.10[a]
Fe (µg g^{-1})	0.32±0.04[a]	0.31±0.04[a]
Cu (µg g^{-1})	0.11±0.03[a]	0.09±0.02[a]
Zn (µg g^{-1})	5.11±0.20[a]	5.27±0.36[a]
Sr (µg g^{-1})	2.27±0.66[a]	2.26±0.67[a]
Ca (mg g^{-1})	1.54±0.02[a]	1.48±0.04[b]
Na (mg g^{-1})	0.34±0.01[a]	0.33±0.01[a]
K (mg g^{-1})	1.51±0.05[a]	1.52±0.06[a]
Mg (mg g^{-1})	0.14±0.00[b]	0.15±0.00[a]
P (mg g^{-1})	1.14±0.02[a]	1.13±0.04[a]
S (mg g^{-1})	0.37±0.01[a]	0.38±0.02[a]

[‡] C: Goats fed with control feed; P: Goats fed with *Posidonia oceanica*. Different superscripts in the same row mean significant differences between values ($P < 0.05$).

Table 2. Fatty acid profile (mg g^{-1} of total fatty acids) of goat milk (mean ± SD) [‡]

	C	P
C4	6.7±4.2[a]	7.9±6.0[a]
C6	18.9±2.8[a]	18.7±2.4[a]
C8	28.8±2.7[a]	28.6±2.4[a]
C10	105.2±4.2[a]	105.8±4.6[a]
C12	52.4±12.3[a]	50.1±2.8[a]
C13	1.0±0.2[b]	1.1±0.1[a]
C14	94.7±2.8[a]	95.0±4.1[a]
C14:1	2.6±1.2[a]	2.4±1.2[a]
C15	6.6±0.4[b]	7.0±0.5[a]
C15:1	2.3±0.2[a]	2.3±0.1[a]
C16	253.3±12.6[a]	256.9±10.4[a]
C16:1	5.8±0.3[b]	6.1±0.5[a]
C17	4.9±0.2[a]	4.9±0.2[a]
C17:1	1.5±0.2[a]	1.7±0.8[a]
C18	127.1±15.9[a]	126.5±10.2[a]
trans 9 - C18:1	37.5±14.2[a]	32.0±12.4[a]
C18:1	201.1±12.5[a]	208.4±15.4[a]
trans, *trans* 9, 12 - C18:2	3.6±0.2[b]	3.8±0.2[a]
C18:2	35.4±01.8[a]	36.2±2.4[a]
γ - C18:3	0.4±0.0[a]	0.4±0.0[a]
C18:3	2.6±0.4[a]	2.5±0.4[a]
C20	11.4±1.1[a]	10.3±1.5[b]
C20:1 - *cis* 11	0.7±0.1[a]	0.7±0.1[a]
C21	0.3±0.1[a]	0.3±0.0[a]
C20:4	1.9±0.1[a]	1.9±0.1[a]
C22	0.5±0.1[a]	0.5±0.1[a]
C24	0.2±0.1[a]	0.2±0.1[a]
SFA [†]	708.6±15.5[a]	708.7±13.5[a]
MUFA [†]	247.5±15.0[a]	246.5±12.1[a]
PUFA [†]	43.9±2.1[a]	44.8±2.8[a]

[‡] C: Goats fed with control feed; P: Goats fed with *Posidonia oceanica*. Different superscripts in the same row mean significant differences between values ($P < 0.05$).
[†] SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

2.2. Milk Coagulation

Figure 2 shows the results obtained for milk-clotting time according to rennet concentration, temperature and milk origin. The data indicate that temperature significantly ($P < 0.05$) influenced the clotting time, which agrees with other authors [27,28]. This was expected because milk coagulation is known to be strongly dependent on temperature [29]. The same authors showed that the velocity of coagulation increases as temperature increases from 20°C to 40-42°C, above which the coagulation process may slow down. Enzyme concentration also had a significant effect ($P < 0.05$) on clotting time, which diminished as the rennet concentration increased. This, too, was expected and it is related with higher levels of κ-casein proteolysis [30].

The milk origin significantly influenced milk coagulation time, the milk from goats fed *P. oceanica* showing lower values. Nájera [31] pointed to an inverse relationship between Ca^{+2} content and coagulation time, so that our results were unexpected because the milk with the lower Ca^{+2} content had a lower milk clotting time. This may have been related with the fibre present in *P. oceanica*, which would play a significant role in the induction kinetics of milk clotting, decreasing the milk-clotting time [32]. However, to confirm this, a fibre analysis should be included in future studies. The higher protein content (Table 1) in the milk of goats fed seagrass could also have been partially responsible for this increase in coagulant activity.

In order to better understand the coagulation kinetics in both milks under the different conditions, different kinetic models were applied to the data. As can be seen from Table 3, the results for both samples (C and P) perfectly fit the kinetic models proposed by Payens [33] and van Hooydonk & Walstra [34], while the model proposed by Hyslop [35] showed a lower fit for both samples, with a lower R^2 throughout the range of temperature (R^2 = 0.983-0.986). Taking as reference the best fitting models, it can be seen that the kinetic fit was similar in both types of milk and was best at 30-40°C.

2.3. Fresh Cheese

Table 4 depicts the total fat and protein contents, dry matter, pH, cheese yield and the optical parameter T_{max}. None of the parameters showed a significant difference between groups, confirming the homogeneity of the product regardless of the origin of the milk. Although no significant difference were found between T_{max} values, the same behaviour was observed as in Figure 2, where the clotting times for P milk were lower than those obtained for the control milk.

The mineral content (Table 4) of cheeses is, in general, higher than in milk, mainly due the loss of water, thereby increasing the concentration of solids. However, the K content was lower in cheese and showed significant variations ($P < 0.05$) that depended on the origin of the milk, with lower values measured in the P cheese. This may be because a large amount of K was lost when the whey was removed during processing, which would have altered the relative concentrations in the cheeses studied.

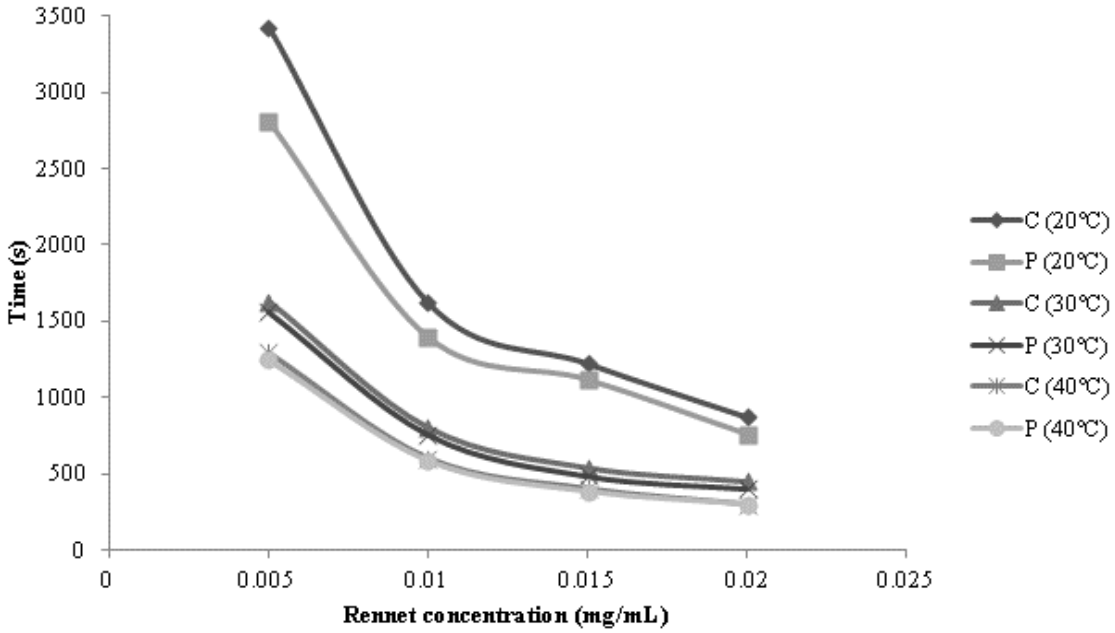

Figure 2. Milk clotting time at different temperatures (20°C, 30°C and 40°C)

Table 3. Kinetic models in both types of goat milk

Kinetic model[‡]	Reference	Temperature	Type of milk[†]	Fitted equation[1]	R^2
$t=AC_0^{-B}$	Payens *et al.*	20°C	C	$t=18.084C_0^{-0.989}$	0.996
			P	$t=21.240C_0^{-0.921}$	0.993
		30°C	C	$t=8.978\ C_0^{-0.981}$	0.997
			P	$t=6.995\ C_0^{-1.021}$	0.998
		40°C	C	$t=4.567\ C_0^{-1.065}$	0.999
			P	$t=4.555\ C_0^{-1.059}$	0.999
$t=AC_0^{-0.5}-B$	Hyslop *et al.*	20°C	C	$t=361.308\ C_0^{-0.5}-1768.908$	0.983
			P	$t=286.620\ C_0^{-0.5}-1303.770$	0.983
		30°C	C	$t=171.618\ C_0^{-0.5}-833.567$	0.984
			P	$t=168.363\ C_0^{-0.5}-852.458$	0.986
		40°C	C	$t=142.550\ C_0^{-0.5}-751.526$	0.986
			P	$t=137.244\ C_0^{-0.5}-719.170$	0.985
$t=AC_0^{-1}+B$	van Hooydonk and Walstra	20°C	C	$t=16.836\ C_0^{-1}+34.282$	0.996
			P	$t=13.332\ C_0^{-1}+129.109$	0.993
		30°C	C	$t=7.998\ C_0^{-1}+22.853$	0.997
			P	$t=7.842\ C_0^{-1}-11.891$	0.998
		40°C	C	$t=6.639\ C_0^{-1}-39.718$	0.999
			P	$t=6.394\ C_0^{-1}-34.096$	0.998

[‡] A and B are adjustable parameters. t: time; C_0: concentration.
[†] C: Goats fed with control food; P: Goats fed with *Posidonia oceanica*.

As regard the fatty acid profile of fresh cheese (Table 5), only C13, C15, *t*9-*t*12-C18:2 and C20 showed significant differences ($P < 0.05$), with higher values for C13, C15 and *t*9-*t*12-C18:2 and lower for C20 in the P cheeses. These differences reflected those observed in milk (Table 2), indicating that fatty acids did not vary during the cheesemaking process. No significant differences between cheeses were found for the fatty acids grouped according to their double bonds (Table 5), as occurred in the milk (Table 2), underlining the low impact that the feed had on the profile of the fatty acids studied.

The texture analysis (Figure 3) performed on the cheeses pointed the greater hardness and chewiness of the P cheese, while other parameters did not show significant differences. The lack of differences in the physicochemical parameters of cheeses (Table 4) makes it difficult to identify the reasons for the differences in hardness and chewiness. The use of *P. oceanica* leaves in the diet could

be associated with the structural modifications of the para-κ-casein due to their hydrocolloidal properties as result of the cellulose they contain, which would increase the water-holding capacity and reduce the syneresis [36]. However, further analysis is necessary to confirm this hypothesis.

As regard the sensory analysis of the cheeses (Figure 4), only saltiness showed significant differences ($P<0.05$) between cheese groups, with higher values in C cheese. Differences in the salt content would probably be due to the lower amount of potassium and sodium in these cheeses (Table 4). The typical sweetness of goat milk was not detected in either group, and both obtained good final score with no significant differences, although the P cheese had slightly greater hardness (as in the instrumental texture profile).

Table 4. Physicochemical and cheesemaking parameters and mineral content (mean ± SD) of cheeses [‡]

	C	P
Fat (g kg^{-1})	194.0±14.9^{a}	202.6±18.4^{a}
Protein(g kg^{-1})	112.3±24.1^{a}	110.2±23.5^{a}
Dry matter (g kg^{-1})	378.0±8.3^{a}	384.4±10.7^{a}
pH	7.10±0.09^{a}	7.07±0.05^{a}
T_{max} (min)	6.69±0.86^{a}	6.21±0.43^{a}
Cheese yield (L kg^{-1})	3.97±0.10^{a}	3.94±0.10^{a}
Fe (µg g-^{1})	1.31±0.24^{a}	1.35±0.29^{a}
Cu (µg g-^{1})	0.39±0.07^{a}	0.37±0.11^{a}
Zn (µg g-^{1})	20.04±2.17^{a}	21.52±1.64^{a}
Sr (µg g-^{1})	8.63±2.19^{a}	8.92±2.44^{a}
Na (mg g^{-1})	3.92±0.68^{a}	3.39±0.59^{a}
K (mg g^{-1})	1.13±0.08^{a}	1.05±0.04^{b}
Ca (mg g^{-1})	5.13±0.56^{a}	5.01±0.38^{a}
Mg (mg g^{-1})	0.28±0.03^{a}	0.28±0.03^{a}
P (mg g^{-1})	3.32±0.46^{a}	3.27±0.37^{a}
S (mg g^{-1})	1.01±0.11^{a}	1.05±0.06^{a}

[‡] C: Goats fed with control food; P: Goats fed with *Posidonia oceanica*. Different superscripts in the same row mean significant differences between values ($P < 0.05$).

Table 5. Fatty acid profile (mg g^{-1} of total fatty acids) of both types of cheese (mean ± SD) [‡]

	C	P
C4	6.0±5.2^{a}	5.2±4.1^{a}
C6	19.0±2.8^{a}	18.8±2.6^{a}
C8	33.4±16.3^{a}	28.9±2.6^{a}
C10	107.2±6.3^{a}	106.7±6.3^{a}
C12	50.2±2.6^{a}	50.5±3.5^{a}
C13	1.1±0.2^{b}	1.2±0.2^{a}
C14	95.2±2.7^{a}	95.5±4.0^{a}
C14:1	2.9±1.1^{a}	2.9±1.0^{a}
C15	6.6±0.4^{b}	7.0±0.5^{a}
C15:1	2.3±0.2^{a}	2.3±0.1^{a}
C16	253.5±12.5^{a}	258.8±8.7^{a}
C16:1	6.0±0.2^{a}	6.1±0.6^{a}
C17	4.9±0.2^{a}	4.9±0.3^{a}
C17:1	1.5±0.1^{a}	1.5±0.2^{a}
C18	123.0±9.3^{a}	125.5±9.6^{a}
trans 9 - C18:1	32.5±11.4^{a}	31.4±12.5^{a}
C18:1	202.1±7.0^{a}	201.2±8.4^{a}
trans, *trans* 9, 12 - C18:2	3.6±0.2^{b}	3.8±0.2^{a}
C18:2	35.4±1.6^{a}	35.9±2.0^{a}
γ - C18:3	0.4±0.1^{a}	0.4±0.0^{a}
C18:3	2.6±0.3^{a}	2.5±0.4^{a}
C20	11.0±1.8^{a}	9.9±1.5^{b}
C20:1	0.7±0.0^{a}	0.8±0.4^{a}
C21	0.3±0.0^{a}	0.3±0.0^{a}
C20:4	1.9±0.1^{a}	1.9±0.1^{a}
C22	0.5±0.0^{a}	0.5±0.0^{a}
C24	0.2±0.1^{a}	0.3±0.0^{a}
SFA [†]	708.4±13.3^{a}	709.4±16.2^{a}
MUFA [†]	247.9±13.0^{a}	246.1±14.9^{a}
PUFA [†]	43.8±1.7^{a}	44.4±2.3^{a}

[‡] C: Goats fed with control food; P: Goats fed with *Posidonia oceanica*. Different superscripts in the same row mean significant differences between values ($P < 0.05$).
[†] SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

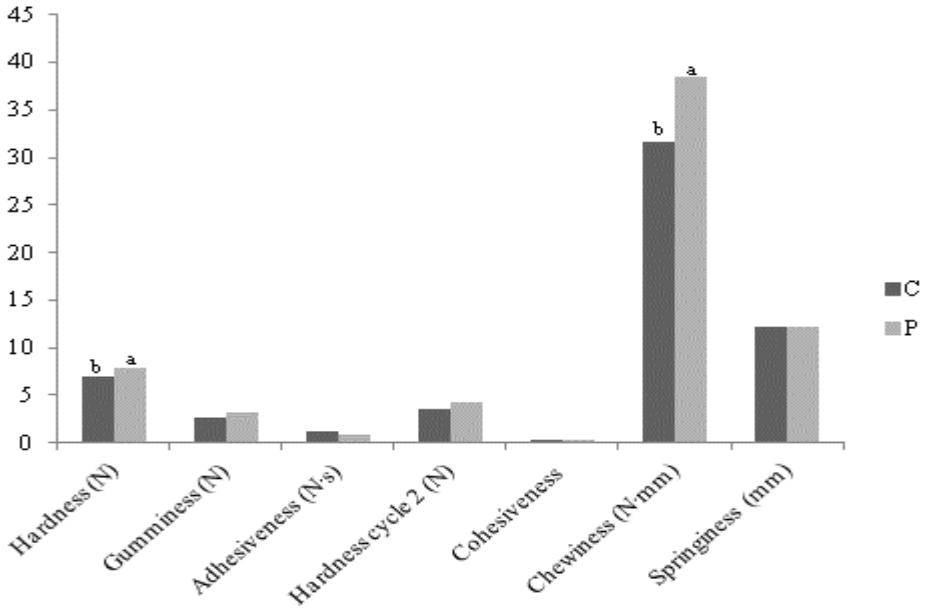

Figure 3. Cheese texture profile parameters. Different letters mean significant differences between values ($P < 0.05$)

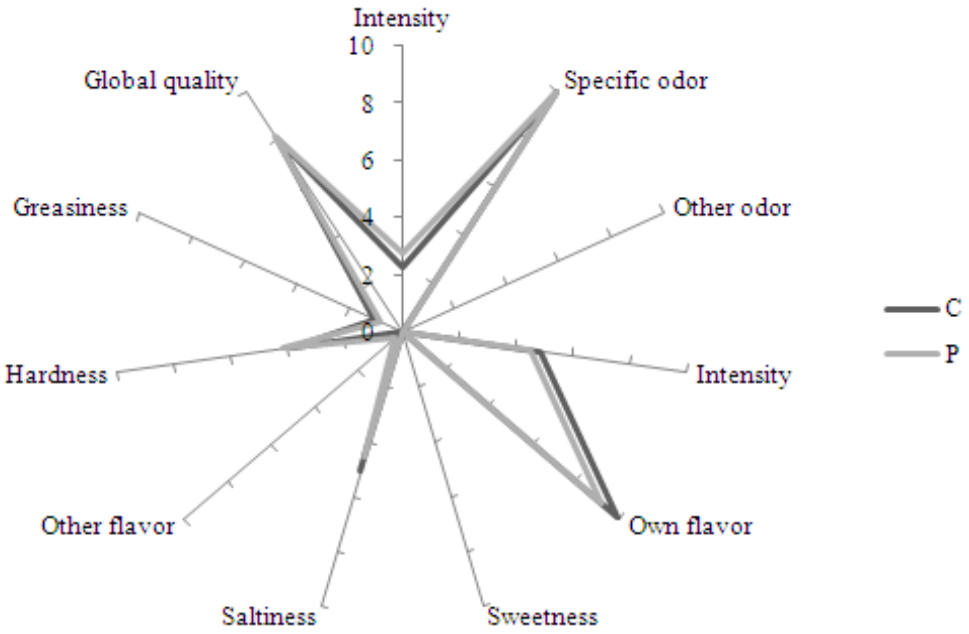

Figure 4. Sensory profile for both types of cheese (C and P)

4. Conclusions

As already mentioned, the lack of any related literature means that it is difficult to provide a detailed discussion. However, the results indicate that the replacement of straw by *P. oceanica* provides milk and cheese with very similar characteristics to those obtained from animals fed with straw. Moreover, the sensory analysis of both milks and cheeses led to similar scores being obtained regardless of the origin of the milk and processed cheese. For cheesemaking, both milks could be used to obtain similar curds (although with different coagulation times). The best temperature range for coagulation was 30-40°C. More studies should be carried out to characterize in greater depth the cheese made with milk from goats fed *P. oceanica*. However, the results are promising, suggesting that using this seagrass as a substitute for straw in animal feed for dairy goats could help reduce the over-exploitation of peat lands and decrease the amount of waste destined for incineration, thus reducing its environmental impact, while helping reduce production costs and optimize production costs.

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