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Changes in the Concentration and Profile of Free Fatty Acids during the Ripening of a Spanish Blue-Veined Cheese Made from Raw and Pasteurized Cow and Goat Milk

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Abstract: Blue-veined cheeses in general undergo a pronounced lipolysis. The aim of this work was to determine the evolution of free fatty acids (FFA) in Valdeón cheese during ripening, comparing cheeses made from raw and pasteurized milk. The effect of season on the evolution of FFA in pasteurized milk cheeses was also studied. Cheeses made with raw milk showed the highest concentrations of FFA, reaching values of 23,081.9 mg 100 g^{-1} dry matter at the end of ripening, compared to the values of cheeses made with pasteurized milk (7327.1 mg 100 g^{-1} dry matter), in both cases with a predominance of oleic and palmitic acids. However, pasteurization did not affect the FFA profile of the cheeses. Regarding the cheeses made with pasteurized milk in different seasons, the highest FFA concentration was reached in cheeses made in summer after 30 days of ripening. The season also influenced the FFA profile and thus the concentration of short-, medium- and long-chain fatty acids in relation to total FFA. There were no significant differences in sensory analysis between cheeses made from raw and pasteurized milk.

Keywords: blue-veined cheese; lipolysis; raw milk; pasteurized milk; ripening time; season

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1. Introduction

Hydrolysis of triglycerides, the major component of the fat fraction in milk, by lipases results in the release of fatty acids (FA), partial glycerides (mono- and diglycerides) and in some cases glycerol [1]. They may be derived from milk, rennet, starters and additional cultures [2,3]. Free fatty acids (FFA), especially short- and medium-chain FAs, contribute to the development of the sensory characteristics of blue-veined cheeses and can also act as precursors for other compounds such as ketones, which also contribute to the flavor of the cheese [4,5]. In fact, ketones are the main aroma compounds in blue-veined cheeses, although their concentration may vary during cheese ripening due to their conversion to secondary alcohols [6,7]. In mold-ripened cheeses, fungal lipases are very active and, specifically in blue-veined cheeses, lipolysis is due to the metabolic activity of *Penicillium roqueforti*. This activity depends on its growth range and the strain used [3]. In addition, ripening time, salt concentration, pH and milk characteristics (determined by species, breed, lactation status and feeding), among other factors, will influence the intensity of lipolysis and thus the proportion of FFA in blue-veined cheeses [8,9]. It is therefore expected that the degree of lipolysis of cheeses will vary according to the season of production, as reported by other authors for other types of cheese [10,11]. Furthermore, the reduction of the milk microbiota as a result of pasteurization and the inactivation of endogenous milk protein lipase are aspects that will influence the lipolysis and flavor of cheeses [12].

Valdeón cheese is a traditional blue-veined cheese made in the province of León (Spain) from raw or pasteurized cow's milk or a mixture of cow's, sheep's and/or goat's milk. Since 2003 the authenticity of Valdeón cheese has been protected by a Protected Geographical

Indication (PGI). Previous studies [13–16] have been carried out on microbiological and proteolytic aspects of this cheese, but this is the first study on lipolysis Valdeón cheese. Knowledge of the FFA content is of particular interest in Valdeón cheese because of the intensity of lipolysis that generally occurs in blue cheese varieties and because of its impact on the final quality of the cheese [17]. Therefore, the aim of this study was to determine the influence of ripening time on the concentration and profile of free fatty acids in Valdeón cheeses made with raw and pasteurized milk. The influence of the season on these lipolytic parameters was also studied in cheeses made with pasteurized milk. The results obtained will allow us to know how the variables studied influence the composition and profile of the FFA and to what extent they affect the aroma and flavor of Valdeón cheese.

2. Materials and Methods

2.1. Cheese Making and Sampling

Twelve batches of Valdeón cheese, made using 90% cow's milk and 10% goat's milk, were made following the procedure described by Diezhandino et al. [13] (see Figure 1). Of these twelve batches, eight were made from pasteurized milk (two in each season of the year), using a commercial mesophilic starter culture (FD-DVS CHN-19, Chr. Hansen SL, Madrid, Spain) and a liquid spore suspension (1.6 × 10^8 spores mL^{-1}) of *P. roqueforti* (Biostar, Toledo, Spain). The other four batches were made from raw milk in the same cow/goat ratio, but using only the liquid suspension of *P. roqueforti* spores, as cheesemakers do not add the starter. The effect of seasonality has not been taken into account for the production of raw milk cheeses, which are produced in much smaller quantities and at specific times of the year. Commercial liquid calf rennet (NATUREN liquid 140 S/S, 90% Chymosin; 140 ± 5 IMCU ml^{-1}; Chr. Hansen SL, Madrid, Spain) was added. A total of 48 cheeses were made with pasteurized milk and 24 with raw milk, giving a total of 72 cheeses. The cheeses were ripened for 4 months in a drying room at 10 °C and 90% relative humidity. Cheese samples were taken after 2, 15, 30, 60, 90 and 120 days of ripening. Each sample consisted of a whole cheese (2.4 kg). The cheeses were completely crushed and homogenized before sampling.

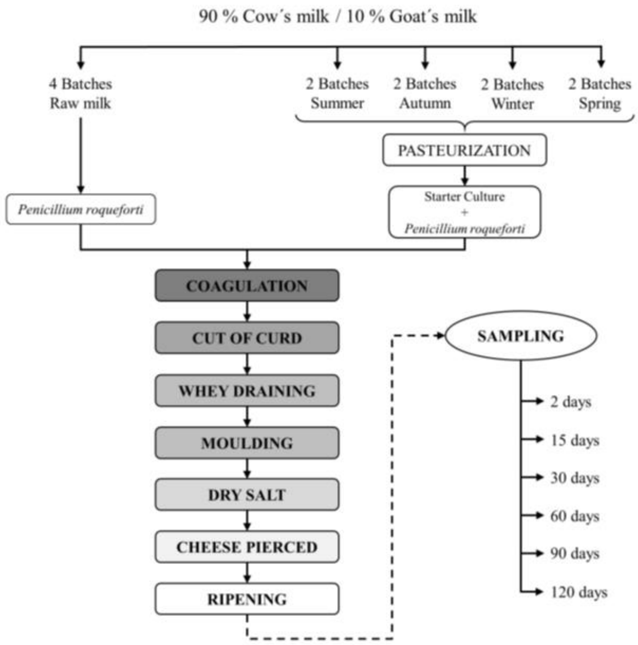

Figure 1. Production of Valdeón cheese using pasteurized or raw milk, ripening period and sampling procedures.

2.2. Physico-Chemical Analysis

Dry matter was determined following FIL-IDF standard 004 [18]. Protein and fat content were determined according to FIL-IDF standard 20-1 [19] and standard 22 [20], respectively. Lactose and L- and D-lactic acid contents were determined using an enzyme kit from Boehringer Mannheim (R-biopharm, Roche, Mannheim, Germany). NaCl content and pH were determined according to AOAC standard 935.43 [21] and standard 14.022 [22], respectively. Water activity was determined using an Aqualab Dew Point Analyzer CX-2 (Decagon Devices Inc., Pullman, WA, USA).

2.3. Free Fatty Acids Analysis

The extraction, separation and identification of FFA were carried out according to the method described by De Jong and Bandings [23]. To extract the FFA, 1 g of cheese was weighed together with 3 g of anhydrous sodium sulfate, and then 0.3 mL of 2.5 M sulfuric acid and 1 mL of internal standard solution (pentanoic acid [C5:0], nonanoic acid [C9:0] and heptadecanoic acid [C17:0] in heptane, 0.5 mg ml^{-1} (w/v)) was added. Fat was extracted by adding 3 mL of a 1:1 ether diethyl-heptane mixture, performing centrifugation at 804$\times$ g for 2 min at 20 °C, and repeating this process three times. Finally, the organic phase was collected and the FFA were separated by means of solid phase extraction (SPE) using polypropylene columns containing Sep-Pak® Aminopropyl 3 cc Vac cartridges (500 mg) (Waters, Milford, Ireland). FFA were separated and identified using a Hewlett Packard 6890 Series GC System gas chromatograph (Hewlett Packard, Wilmington, DE, USA) equipped with a Hewlett Packard 7683 Series injector and a Hewlett Packard 5973 Mass Selective Detector. Separation of FFA was performed in a Thermo Scientific column measuring 30 m $\times$ 0.32 mm $\times$ 0.50 µm (Thermo Fisher Scientific Inc., Madrid, Spain). The oven temperature was programmed to start at 50 °C and increase at a rate of 15 °C min^{-1} until reaching a temperature of 200 °C, which was maintained for 2 min. Then, the temperature was increased at a rate of 2 °C min^{-1} to 220 °C, at which point it was maintained for 5 min. The temperatures of both the injector and detector were 230 °C. The injector volume was 4 µL (Split 10:1).

Eight standard solutions were prepared with increasing concentrations of FA butyric acid [C4:0], caproic acid [C6:0], caprylic acid [C8:0], capric acid [C10:0], lauric acid [C12:0], myristic acid [C14:0], myristoleic acid [C14:1], palmitic acid [C16:0], palmitoleic acid [C16:1], stearic acid [C18:0], oleic acid [C18:1], linoleic acid [C18:2] and linolenic acid [C18:3] (Sigma-Aldrich, St. Louis, MO, USA)) and fixed concentrations of internal standards to calculate the calibration curves (Table 1).

Table 1. Parameters of calibration standard solutions of fatty acids (FA) studied: concentration range, equation of calibration curves, correlation coefficients (R2), limit of detection (LOD) and limit of quantification (LOQ).

FA	Concentration Range (mg L^{-1})	Equation	R2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
C4:0	25–750	y = 1.0979x − 0.0182	0.997	0.43	1.45
C6:0	25–750	y = 0.713x + 0.4033	0.993	1.21	4.02
C8:0	25–800	y = 1.1521x − 0.0572	0.998	0.42	1.39
C10:0	25–850	y = 0.9686x − 0.1051	0.998	0.56	1.88
C12:0	30–850	y = 0.7736x + 0.1735	0.996	0.89	2.97
C14:0	30–950	y = 0.7191x + 0.2375	0.997	0.99	3.31
C14:1	30–900	y = 0.8274 + 0.1774	0.996	0.82	2.74
C16:0	30–850	y = 0.764x + 0.8585	0.992	1.30	4.33
C16:1	30–900	y = 0.7019x + 0.7422	0.994	1.26	4.18
C18:0	30–850	y = 0.8442x + 0.8477	0.993	1.10	3.66
C18:1	30–900	y = 0.6342x + 0.9672	0.989	1.85	6.16
C18:2	40–1100	y = 0.7475x + 1.1192	0.992	1.69	5.62
C18:3	30–850	y = 1.1381x + 1.0715	0.991	0.89	2.95

Identification of FFA was performed by comparing retention times with those of patterns followed by confirmation against the mass spectra of FA peaks in the Hewlett Packard Willey database 275 L Mass Spectral Library. The chromatograms were processed using HP G1701BA version B.01.00 Chemstation Software (Hewlett Packard, Wilmington, DE, USA).

2.4. Sensory Analysis

The sensory analysis was carried out by a panel of 20 tasters trained in the characteristics of blue-veined cheeses and following ISO 8586 [24]. The aroma and flavor of cheeses made from raw and pasteurized milk at different stages of ripening, as well as cheeses made from pasteurized milk at different times of the year and throughout the ripening process, were evaluated. Five sensory attributes for aroma and seven for flavor were rated on a scale of 1 to 7 [15], with 1 being no perception of aroma or flavor, 4—moderate perception and 7—very high perception.

2.5. Statistical Analysis

Significant differences between means of FFA with respect to ripening time, season and milk treatment were evaluated by analysis of variance (ANOVA) with a 95% confidence interval, using SPSS v.25 (SPSS, Chicago, IL, USA).

3. Results and Discussion

3.1. Physico-Chemical Parameters

Table 2 shows the principal chemical and physico-chemical parameters of Valdeón cheeses made with pasteurized or raw milk, throughout ripening. It should be remembered that only pasteurized milk cheeses were produced at different times of the year. Differences between the values as a function of ripening time, season and heat treatment of the milk are also shown in the same table.

Table 2. Means ± standard deviation of the chemical and physico-chemical parameters of Valdeón cheeses, made with pasteurized (P) or raw (R) milk, throughout ripening.

	Milk	Ripening Time (Days)						Difference		
		2	15	30	60	90	120	A	S	T
Dry matter	P	51.93 ± 2.32 **	57.15 ± 2.98 **	58.61 ± 2.60 **	60.87 ± 2.79 **	61.78 ± 2.87 *	62.74 ± 2.41 *	***	*	***
	R	44.65 ± 0.29	48.84 ± 0.00	49.33 ± 2.24	51.49 ± 0.12	54.25 ± 3.89	58.04 ± 2.11	**		
Protein	P	34.53 ± 2.33	33.56 ± 1.27	33.82 ± 1.61	33.93 ± 1.73	34.35 ± 1.31	33.98 ± 1.39	NS	***	***
	R	36.86 ± 1.46	34.78 ± 0.28	36.33 ± 0.28	36.59 ± 0.85	35.99 ± 0.39	36.22 ± 0.29	NS		
Fat	P	53.62 ± 1.19 **	53.16 ± 1.10 **	56.09 ± 2.16 *	55.50 ± 2.24	56.33 ± 1.10 **	57.29 ± 1.92	***	NS	***
	R	49.84 ± 1.90	49.14 ± 1.45	51.99 ± 2.00	51.70 ± 0.91	52.33 ± 0.82	55.12 ± 0.43	*		
Lactose	P	0.78 ± 0.30 **	0.18 ± 0.12 *	0.11 ± 0.10	0.14 ± 0.15	0.09 ± 0.10	0.07 ± 0.10	***	NS	NS
	R	1.89 ± 0.12	0.72 ± 0.58	0.20 ± 0.21	ND	ND	ND	**		
L-lactic acid	P	3.24 ± 0.33 *	2.23 ± 0.51	1.21 ± 0.48	0.49 ± 0.24	0.43 ± 0.23	0.38 ± 0.21	***	NS	NS
	R	2.51 ± 0.06	2.02 ± 0.38	1.16 ± 0.15	0.56 ± 0.13	0.55 ± 0.30	0.31 ± 0.12	***		
D-lactic acid	P	0.33 ± 0.19	0.64 ± 0.39	0.36 ± 0.24	0.16 ± 0.14	0.17 ± 0.15	0.10 ± 0.10	***	NS	NS
	R	0.38 ± 0.12	0.77 ± 0.15	0.72 ± 0.21	0.39 ± 0.17	0.15 ± 0.14	0.20 ± 0.09	*		
Salt/moisture	P	2.03 ± 0.87	6.25 ± 0.47 *	6.79 ± 0.59	7.60 ± 0.96	7.79 ± 1.24	8.92 ± 1.12	***	NS	NS
	R	1.64 ± 0.37	5.40 ± 0.07	5.87 ± 0.38	6.57 ± 0.45	7.39 ± 1.55	8.81 ± 1.29	**		
aw	P	0.98 ± 0.01	0.96 ± 0.00	0.95 ± 0.01 **	0.94 ± 0.01 **	0.93 ± 0.01	0.92 ± 0.01	***	NS	NS
	R	0.98 ± 0.01	0.95 ± 0.01	0.97 ± 0.01	0.96 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	*		
pH	P	5.03 ± 0.14 *	5.19 ± 0.13	5.97 ± 0.27	7.07 ± 0.39	6.83 ± 0.37	6.85 ± 0.41	***	NS	NS
	R	5.47 ± 0.48	5.32 ± 0.22	5.85 ± 0.11	6.57 ± 0.06	6.59 ± 0.01	6.53 ± 0.18	**		

Dry matter (g 100 g^{-1} cheese); Protein (g 100 g^{-1} dry matter); Fat (g 100 g^{-1} dry matter); Lactose (g 100 g^{-1} dry matter); L-lactic acid (g 100 g^{-1} dry matter); D-lactic acid (g 100 g^{-1} dry matter). P: pasteurized milk. R: raw milk. Last column shows the significant differences (A—Age; S—Season; T—Treatment). NS: no significant differences; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. The differences between the values of each parameter and at each sampling point between cheeses made with pasteurized and raw milk are indicated next to the numerical value.

Dry matter, protein and fat contents showed significant differences ($p < 0.001$) between cheeses made with raw and pasteurized milk (see Table 2, column T). Dry matter and protein contents also showed significant differences ($p < 0.05$ and $p < 0.001$, respectively) according to the season of the year (Table 2, column S). The influence of seasonality on milk composition is well known and has also been reported for cheeses of different types [10,25]. Dry matter and salt/moisture content also showed significant differences in their evolution during the ripening period. (Table 2, column A). The differences were more pronounced for cheeses made with pasteurized milk, possibly due to the differences in composition which, as already mentioned, introduce seasonality in these cheeses. The lactose content decreased during ripening, with a more pronounced decrease in pasteurized milk cheeses during the first month of ripening. After 60 days, lactose was no longer detectable in raw-milk cheeses and remained at very low levels in pasteurized milk cheeses. This decrease is related to the metabolic activity of the starter lactic acid bacteria added in the production of pasteurized milk cheeses and the indigenous lactic acid bacteria in raw milk cheeses to form L-lactic acid. From the beginning of ripening, L-lactic acid gradually decreased while D-lactic acid increased during the first 15 days of ripening. Lactobacilli are responsible for the racemization of L-lactic acid into D-lactic acid. On the other hand, fungal metabolism contributed to the decrease in lactic acid content during cheese ripening. The aw and pH values showed no significant differences between raw and pasteurized milk cheeses (column T), although the aw values showed significant differences ($p < 0.01$) between pasteurized and raw milk cheeses at 30 and 60 days of ripening. No significant differences in the evolution of pH and aw were found with respect to the season, but significant differences were observed during the ripening process (column A), and again these were more pronounced in cheeses made with pasteurized milk. The course of acidification was different for each type of cheese. The initial acidification was slower in the raw milk cheeses, which was to be expected since the production of the cheeses with pasteurized milk included the addition of a lactic acid starter to the milk. However, after 30 days of ripening, the pH was lower in the raw milk cheeses. From this point on, the pH increase due to the metabolic activity of *Penicillium* was slower in the raw milk cheeses, and at the end of the ripening period (120 days) the pH was lower than in the pasteurized milk cheeses.

3.2. Free Fatty Acids

Table 3 shows the average concentrations of the different FFA during the ripening of Valdeón cheese made with raw and pasteurized milk, as well as the differences between the values depending on the ripening time, the season (for cheeses made with pasteurized milk) and the heat treatment of the milk. Most of the FFA studied increased in concentration with ripening, as was found in other studies on blue-veined cheeses such as Gorgonzola [26] or Stilton [27]. However, during the last month of ripening, the values remained practically constant. Oleic (C18:1) and palmitic (C16:0) acids predominated throughout ripening. In raw and pasteurized milk cheeses the concentration of C16:0 showed significant differences ($p < 0.001$) as a function of ripening time, as well as for several FFA (C4:0, C10:0, C18:1 and C18:3). However, in other FFA (C6:0, C8:0, C12:0, C14:0 and C18:0) significant differences were only found between cheeses made with pasteurized milk over the ripening period (Table 3). In pasteurized milk cheeses, FFA C16:0, C18:0 and C18:1 showed the highest values at 90 days of ripening, whereas in cheeses made from raw milk, the highest values for the concentration of these FFA were obtained at 120 days. The evolution of C16:0 and C18:0 values during ripening differed significantly ($p < 0.001$) between raw and pasteurized milk cheeses, whereas that of C18:1 showed no significant differences between the two types of cheese. However, taking into account specific ripening points such as 120 days, the C18:1 content showed significant differences ($p < 0.01$) between raw and pasteurized milk cheeses. The C18:2 content also reached higher values in raw milk cheeses compared to pasteurized milk cheeses, showing significant differences between the two types of cheeses ($p < 0.001$) throughout the ripening process (Table 3). The C18:3 content did not show significant differences between raw and pasteurized milk cheeses over the whole ripening period, but at the last sampling point (120 days) raw milk cheeses differed significantly from pasteurized milk cheeses ($p < 0.01$). The observed decrease in FFA content may be associated with the hydrolysis of FFA to form other compounds such as methyl ketones, alcohols, lactones, aldehydes, etc. [28]. Methyl ketones are important fatty acid catabolites in blue-veined cheeses, due to the action of *P. roqueforti* lipases [3]. Other authors have also observed this effect in Gorgonzola and Picón Bejes-Tresviso [28,29].

Table 3. Means ± standard deviation of the free fatty acid concentration (mg 100 g^{-1} dry matter) found in Valdeón cheese made from pasteurized and raw milk throughout the year and during the ripening.

FFA	Milk	Ripening Time (Days)						Difference		
		2	15	30	60	90	120	A	S	T
C4:0	P	10.46 ± 4.53	14.46 ± 5.55	217.71 ± 223.70	635.16 ± 308.14	788.03 ± 319.50	678.34 ± 231.91	***	NS	NS
	R	11.27 ± 1.52	13.41 ± 3.31	40.55 ± 6.94	1093.42 ± 517.63	1094.58 ± 178.77	1082.82 ± 214.84	**		
C6:0	P	ND	ND	108.81 ± 110.11	317.88 ± 181.61	406.23 ± 165.72 *	379.88 ± 89.67 *	**	NS	**
	R	ND	ND	30.44 ± 18.46	709.87 ± 380.50	768.75 ± 284.60	912.92 ± 554.01	NS		
C8:0	P	ND	ND	37.90 ± 36.72	97.90 ± 46.94 *	122.06 ± 44.77 *	127.87 ± 39.89 *	***	*	*
	R	13.25 ± 3.43	15.97 ± 2.79	18.43 ± 2.39	340.64 ± 268.99	362.44 ± 286.89	459.13 ± 360.31	NS		
C10:0	P	25.38 ± 4.93	30.02 ± 12.38	108.33 ± 77.54	252.65 ± 107.90 **	301.05 ± 106.88 *	331.76 ± 120.88 ***	***	NS	**
	R	32.21 ± 8.01	41.54 ± 18.13	70.62 ± 31.27	605.89 ± 223.55	662.42 ± 299.89	1147.21 ± 473.27	*		
C12:0	P	ND	ND	86.49 ± 59.38	182.96 ± 74.58 **	236.81 ± 93.24 *	247.67 ± 108.17 ***	**	NS	***
	R	ND	ND	26.02 ± 14.42	484.44 ± 169.44	561.75 ± 325.11	1049.91 ± 351.43	NS		
C14:0	P	ND	14.53 ± 10.34	198.92 ± 148.63	370.70 ± 159.84 **	488.71 ± 216.26 *	525.60 ± 336.46 ***	***	*	***
	R	ND	30.13 ± 0.00	55.08 ± 5.94	1090.39 ± 508.60	1328.58 ± 803.54	2499.28 ± 591.10	NS		
C14:1	P	ND	ND	32.25 ± 26.23	59.92 ± 26.01 **	73.82 ± 37.56 *	94.03 ± 62.43 **	NS	NS	***
	R	ND	ND	ND	152.75 ± 57.34	169.95 ± 99.68	343.69 ± 107.42	NS		
C16:0	P	34.77 ± 38.50	76.77 ± 54.60	571.38 ± 409.09	1175.23± 646.45 **	1778.97±519.88 ***	1372.69±576.76 ***	***	NS	***
	R	68.62 ± 0.00	181.80 ± 0.00	195.79 ± 118.21	3240.48 ± 664.37	4171.81 ± 234.47	5844.36 ± 45.94	***		
C16:1	P	ND	ND	126.07 ± 60.90	169.12 ± 111.71	247.07 ± 66.04	164.60 ± 65.75 **	NS	**	**
	R	ND	ND	ND	325.67 ± 45.31	345.40 ± 19.95	369.79 ± 123.38	NS		
C18:0	P	0.38 ± 0.00	43.81 ± 16.05	103.03 ± 51.47	262.04 ± 110.05 **	383.12 ± 267.15	293.52 ± 211.75 **	*	NS	***
	R	ND	ND	ND	552.46 ± 7.36	679.31 ± 133.96	864.75 ± 122.84	NS		
C18:1	P	14.81 ± 0.00	130.01 ± 192.01	1495.2 ± 1221.9	3097.6 ± 2266.5	4103.4 ± 2275.8	2909.6 ± 1204.3 ***	**	NS	NS
	R	0.56 ± 0.00	174.67 ± 85.47	481.45 ± 175.55	5183.5 ± 54.64	6905.9 ± 2834.3	7897.2 ± 811.70	**		
C18:2	P	ND	ND	79.98 ± 47.46	128.11 ± 88.06	145.74 ± 70.03	118.60 ± 78.43 **	NS	*	***
	R	ND	ND	ND	267.38 ± 97.75	242.74 ± 67.97	456.39 ± 290.98	NS		
C18:3	P	ND	ND	36.19 ± 20.04	66.46 ± 26.00	86.26 ± 35.19	82.92 ± 18.94 **	**	*	NS
	R	ND	ND	19.95 ± 5.20	83.58 ± 19.58	106.88 ± 30.69	154.47 ± 14.17	*		

P: pasteurized milk. R: raw milk. ND: Not detected. Last column shows the significant differences (A—Age; S—Season; T—Treatment). NS: no significant differences; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. The differences between the values of each parameter and at each sampling point between cheeses made with pasteurized and raw milk are indicated next to the numerical value.

Table 4 shows the evolution of total FFA (C4:0–C18:3), saturated FFA (SFA), mono-unsaturated FFA (MUFA) and poly-unsaturated FFA (PUFA) and the ratios PUFA/SFA and n−6/n−3. The total FFA reached at the end of ripening was much higher for cheeses made from raw milk than for those made from pasteurized milk (23,081.9 versus 7327.1 mg FFA 100 g^{-1} dry matter). In general, a higher increase of all FFA was observed in raw milk cheeses after 60 days of ripening (Figure S1). However, the values obtained in raw milk cheeses were lower than those observed in other blue-veined cheeses such as Gamonedo, Cabrales and Roquefort [30–32]. Cheeses made from pasteurized milk showed higher salt/moisture content and lower aw (at 30 and 60 days of ripening), whereas salt/moisture values of raw milk cheeses were lower (see Table 2). High salt concentrations can affect the metabolic activity of molds [26,33], in this case limiting the production of lipases by *P. roqueforti* and probably their activity in pasteurized milk cheeses. Moreover, milk lipoprotein lipase (LPL), a potent endogenous milk lipase, remains active in cheeses made from raw milk, whereas heating at 72 °C for 15 s almost completely inactivates the enzyme [34]. Lactic acid bacteria (LAB) are generally low lipolytic, but some strains of lactobacilli or enterococci may have some activity as they are specific for short-chain fatty acids. [35]. In this respect, it should be noted that cheeses made from raw milk showed

ROGOSA and KAA counts in the order of 2 to 4 log units higher than those present in cheeses made from pasteurized milk [15]. Other authors reported that non-starter lactic acid bacteria contribute to taste and flavor development during lipolysis and proteolysis [12]. Ripening time (A) had a significant influence ($p < 0.001$) on total FFA in both raw and pasteurized milk cheeses. The SFA and MUFA content in raw and pasteurized milk cheeses showed significant differences ($p < 0.001$ and $p < 0.01$, respectively) throughout ripening, whereas the evolution of PUFA content only showed significant differences ($p < 0.01$) in pasteurized milk cheeses. Table 4 also shows that SFA predominated, representing around 50% of the total FFA after 90 days of ripening in cheeses made with pasteurized milk and between 55 and 60% in raw milk cheeses. Comparing pasteurized and raw milk cheeses at 60 days of ripening, the MUFA/total FFA ratio was slightly higher in pasteurized milk cheeses (≈46% versus 40%), with a decrease after 90 days of ripening in both cases. PUFA/total FFA values at 60 days of ripening were slightly higher in pasteurized milk cheeses (2.71% versus 2.37%, taking into account average values from 2 to 4 months of ripening), although at the end of ripening the PUFA/total FFA ratio in raw milk cheeses increased to values close to those of pasteurized milk (2.75 versus 2.64). The PUFA/SFA ratio was similar for both types of cheese at 30 days of ripening, with slightly higher values for raw milk cheeses, while the ratio was maintained for the rest of the ripening period, with higher values for pasteurized milk cheeses, although they were not significantly different in any case. Excessive n−6 PUFA content and a high n−6/n−3 ratio are associated with health problems (immunological, inflammatory, cancer, etc.) [36,37]. In this sense, the value of the n−6/n−3 ratio at the end of ripening was around 1.0 for pasteurized milk cheeses and around 0.4 for raw milk cheeses, with the n−6 and n−3 PUFA content increasing in the latter at the end of ripening. Comparing cheeses made from pasteurized milk in different seasons, summer cheeses showed the highest concentration of total FFA after 30 days of ripening, reaching values of 10,149.8 mg 100 g^{-1} dry matter at the end of ripening (Figure S2). On the other hand, the lowest concentration was found in winter cheeses (5879.1 mg 100 g^{-1} dry matter, after 120 days of ripening). However, the differences observed were not statistically significant.

Table 4. Means ± standard deviation of the total free fatty acid, saturated fatty acids, mono- and poly-unsaturated concentration (mg 100 g^{-1} dry matter); and PUFA/SFA, n−6/n−3 and atherogenicity index (AI) found in Valdeón cheese made from pasteurized and raw milk throughout the year and during the ripening.

FFA	Milk	Ripening Time (Days)						Difference		
		2	15	30	60	90	120	A	S	T
Total FFA	P	55.13 ± 36.59	214.76 ± 246.73	3013.32 ± 2346.89	6815.71 ± 3505.87 *	9161.27 ± 3349.41 *	7327.09 ± 2550.09 ***	***	NS	*
	R	91.31 ± 61.87	351.56 ± 259.55	938.32 ± 364.50	14130.45 ± 2909.73	17400.46 ± 906.95	23081.89 ± 3723.84	***		
SFA	P	53.28 ± 36.81	133.50 ± 94.27	1368.80 ± 1018.70	3294.52 ± 1419.37 **	4504.97 ± 1253.68 **	3957.33 ± 1403.18 ***	***	NS	**
	R	91.03 ± 61.48	176.89 ± 174.09	406.48 ± 165.29	8117.59 ± 2725.72	9629.63 ± 1810.37	13860.38 ± 2376.19	***		
MUFA	P	1.85 ± 5.24	81.26 ± 159.98	1578.34 ± 1300.22	3326.64 ± 2340.98	4424.30 ± 2334.26	3168.25 ± 1276.44 ***	**	NS	NS
	R	0.56 ± 0.00	174.67 ± 85.47	481.45 ± 175.55	5661.90 ± 66.68	7421.21 ± 2754.60	8610.65 ± 1042.50	**		
PUFA	P	ND	ND	66.18 ± 66.57	194.56 ± 95.00	232.00 ± 90.83	201.52 ± 84.37 **	**	*	**
	R	ND	ND	19.95 ± 5.20	350.96 ± 117.33	349.62 ± 37.29	610.86 ± 305.15	NS		
PUFA/SFA	P	NC	NC	0.047 ± 0.021	0.064 ± 0.033	0.050 ± 0.010	0.051 ± 0.016	NS	NS	NS
	R	NC	NC	0.051 ± 0.008	0.043 ± 0.000	0.037 ± 0.003	0.043 ± 0.015	NS		
n-6/n-3	P	NC	NC	1.075 ± 0.853	0.727 ± 0.466	0.710 ± 0.348	1.061 ± 0.729	NS	NS	NS
	R	NC	NC	NC	0.321 ± 0.044	0.477 ± 0.260	0.412 ± 0.232	NS		
AI	P	NC	2.345 ± 1.700	0.928 ± 0.369	0.899 ± 0.247	0.924 ± 0.252	1.131 ± 0.342 *	**	NS	*
	R	NC	1.286 ± 0.000	0.883 ± 0.006	1.338 ± 0.436	1.456 ± 0.935	1.830 ± 0.022	***		

FFA: free fatty acids. SFA: saturated fatty acids. MUFA: mono-unsaturated fatty acids. PUFA: poly-unsaturated fatty acids. AI: atherogenicity index. P: pasteurized milk. R: raw milk. NC: not calculated. ND: not detected. Last column shows the significant differences (A—Age; S—Season; T—Treatment). NS: no significant differences; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. The differences between the values of each parameter and at each sampling point between cheeses made with pasteurized and raw milk are indicated next to the numerical value.

Figure 2 shows the evolution of short-chain (SCFA: C4:0−C6:0), medium-chain (MCFA: C8:0−C12:0) and long-chain fatty acids (LCFA: ≥ C14:0) during ripening, expressed as a ratio of total FFA. The SCFA/total FFA ratio decreased significantly ($p < 0.05$) at the beginning of ripening and then showed a slight increase until 60 days, after which it remained stable until the end of ripening. The MCFA/total FFA ratio showed a significant decrease ($p < 0.05$) during the first month of ripening and then remained fairly stable until the end. In contrast, the LCFA/total FFA ratio showed the opposite trend, increasing significantly ($p < 0.05$) during the first month of ripening and then remaining fairly stable. With the exception of two days of ripening, LCFAs were consistently found to be the predominant fatty acids. It should be noted that LCFAs are thought to play a minor role in the flavor of cheese due to a high LCFA perception threshold [38], although in some cases they may contribute to the development of soapy flavors. In contrast, the perception threshold for SCFA and MCFA is much lower and each imparts a characteristic flavor note. Butanoic acid gives "rancid" and "cheesy" flavors. Hexanoic acid has a "pungent", "blue cheese" flavor and octanoic acid has a "waxy", "soapy", "goaty", "musty", "rancid" and "fruity" flavor. In addition, FFA can generate secondary compounds of great flavor importance such as methyl ketones, lactones, esters and secondary alcohols [34,38]. In fact, LCFA such as C18:2 and C18:3 are in turn precursors of other flavor compounds such as alcohols [39].

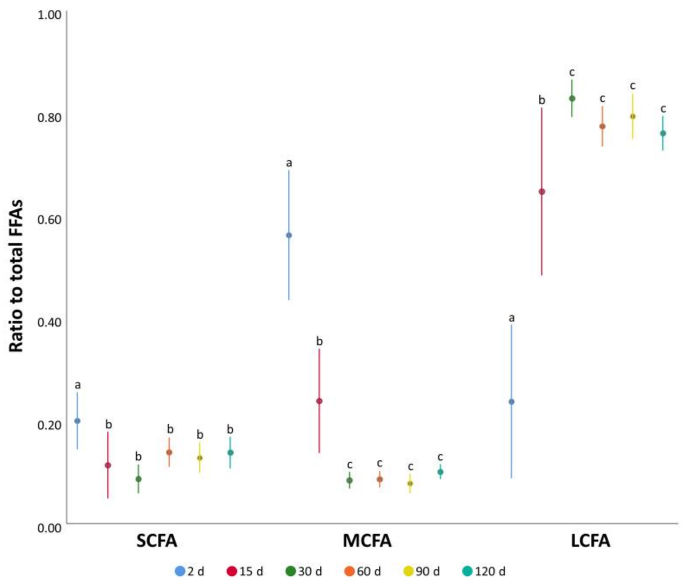

Figure 2. Ratios of short−, medium−, and long−chain fatty acids (SCFA: C4:0−C6:0, MCFA: C8:0−C12:0 and LCFA: ≥ C14:0, respectively) to total free fatty acids in Valdeón cheese during ripening. Means with the same superscript are not significantly different ($p > 0.05$).

Figures 3 and 4 show a representation of the same ratios by the season of production and heat treatment of the milk, respectively. Figure 3 shows the evolution of the ratios of SCFA, MCFA and LCFA to total FFA in Valdeón cheeses made with pasteurized milk in different seasons of the year. The values of the ratio LCFA/total FFA were higher than those of SCFA/total FFA and MCFA/total FFA, with the highest values for cheeses produced in spring. The greater abundance of grasses in spring favors cattle feeding and the incorporation of unsaturated fatty acids in the diet and may influence the differences observed in the LCFA ratio. With regard to the SCFA/total FFA content, spring and winter cheeses differed from summer cheeses, whereas the differences with autumn cheeses were not significant. The MCFA/total FFA content of summer cheeses differed from that of spring cheeses. However, autumn and winter cheeses did not differ significantly from summer and spring cheeses. The same behavior was observed for the LCFA/total FFA ratio.

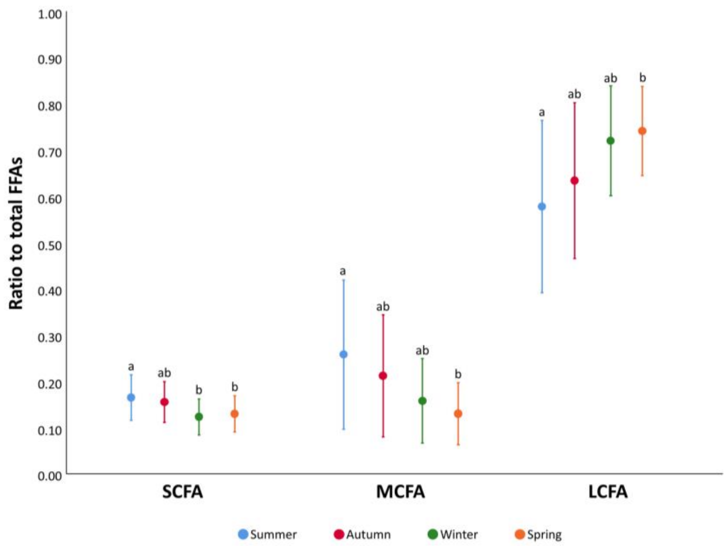

Figure 3. Ratios of short−, medium− and long−chain fatty acids (SCFA: C4:0−C6:0, MCFA: C8:0−C12:0 and LCFA: ≥ C14:0, respectively) to total free fatty acids (means ± standard deviation) in Valdeón cheese throughout the year. Means with the same superscript are not significantly different ($p > 0.05$).

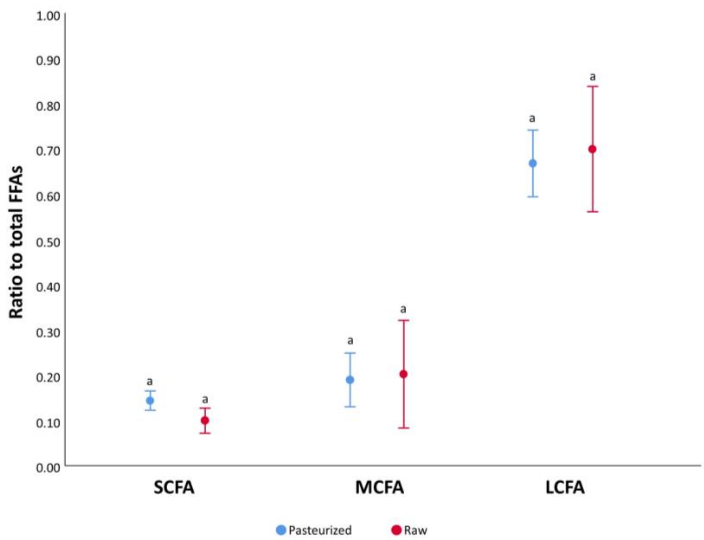

Figure 4. Ratios of short−, medium− and long−chain fatty acids (SCFAs, MCFAs and LCFAs, respectively) to total free fatty acids (FFA) (mean ± standard deviation) in Valdeón cheese made from pasteurized and raw milk. Means with the same superscript are not significantly different ($p > 0.05$).

The evolution of the ratios of SCFA, MCFA and LCFA to total FFA in Valdeón cheeses made with pasteurized or raw milk is shown in Figure 4. The results of the three indices studied were consistent with those observed by Fernández-García et al. [10] in another type of cheese. However, no significant differences were observed between raw and pasteurized milk cheeses in any of the ratios studied (SCFA/total FFAs, MCFA/total FFAs and LCFA/total FFAs). This suggests that although this factor affected the total concentration of FFA in Valdeón cheese, it did not affect the FFA profile.

3.3. Sensory Analysis

The sensory scores for the aroma and flavor of cheeses made from pasteurized and raw milk after two months of ripening (60–120 days) are shown in Table 5. The scores were very similar for both types of cheese, with no significant differences between them, except for bitterness ($p < 0.05$). Cheeses made from pasteurized milk showed a higher bitterness after

60 days of ripening than those made from raw milk, which may be related to the greater extent of proteolysis and accumulation of low molecular weight hydrophobic peptides that occurs in pasteurized milk cheeses, as indicated by Diezhandino et al. [15]. On the other hand, cheeses made with raw milk had higher spiciness scores than those made with pasteurized milk after two months of ripening (Figure S3). In these raw milk cheeses, the "mold" aroma was lower and the "fruity" aroma was higher than in pasteurized milk cheeses of the same ripening period. The "lactic" aroma was slightly more intense in the raw milk cheeses, which were also characterized by a more acidic and less bitter taste and a more pronounced spiciness than in the pasteurized milk cheeses. The scores for aftertaste and persistence were higher for cheeses made with raw milk from the third month of ripening. Significant differences were observed between pasteurized milk cheeses during ripening for salt, spiciness and persistence (Table 5, column A). The overall impression was higher for cheeses made with raw milk, although there were no significant differences; however, differences were again observed for this parameter between cheeses made with pasteurized milk during ripening.

Table 5. Sensory scores for aroma and taste of Valdeón cheeses made from pasteurized or raw milk from 60 days of ripening.

Descriptor		Milk	Ripening Time (Days)			Difference	
			60	90	120	A	T
Aroma (1–7)	Lactic	P	3.08 ± 0.47	3.54 ± 0.65	3.32 ± 0.39	NS	NS
		R	3.39 ± 0.07	3.68 ± 0.09	3.55 ± 0.09	NS	
	Spicy	P	3.04 ± 0.56	3.03 ± 0.50	3.99 ± 0.58	**	NS
		R	2.91 ± 0.11	3.26 ± 0.28	3.90 ± 0.19	NS	
	Musty	P	4.27 ± 0.35	3.97 ± 0.75	4.41 ± 0.37	NS	NS
		R	3.91 ± 0.18	3.82 ± 0.29	4.30 ± 0.42	NS	
	Sour	P	2.69 ± 0.37	2.72 ± 0.44	3.54 ± 0.38	**	NS
		R	3.23 ± 0.11	2.71 ± 0.18	3.44 ± 0.27	NS	
	Fruity	P	2.07 ± 0.49	2.22 ± 0.30	2.69 ± 0.66	NS	NS
		R	2.48 ± 0.37	2.46 ± 0.65	2.74 ± 0.67	NS	
Taste (1–7)	Salty	P	4.39 ± 0.46	4.52 ± 0.40	4.74 ± 0.33	***	NS
		R	4.11 ± 0.19	4.48 ± 0.73	4.65 ± 0.18	NS	
	Bitter	P	4.01 ± 0.40 *	3.40 ± 0.35	3.65 ± 0.57	**	*
		R	2.83 ± 0.21	3.15 ± 0.44	3.27 ± 0.26	NS	
	Astringent	P	3.47 ± 0.60	3.13 ± 0.40	3.57 ± 0.61	NS	NS
		R	2.61 ± 0.17	2.96 ± 0.40	3.82 ± 0.31	NS	
	Spicy	P	3.27 ± 0.47	3.21 ± 0.63	4.09 ± 0.63	***	NS
		R	3.11 ± 0.26	3.55 ± 0.87	4.99 ± 0.12	NS	
	Sweet	P	1.86 ± 0.35	2.17 ± 0.29	2.11 ± 0.49	NS	NS
		R	2.61 ± 0.08	2.28 ± 0.04	2.22 ± 0.22	NS	
	Acid	P	3.29 ± 0.52	3.22 ± 0.59	3.49 ± 0.29	NS	NS
		R	3.48 ± 0.19	3.07 ± 0.23	3.83 ± 0.38	NS	
	Persistence	P	4.54 ± 0.42	4.48 ± 0.32	5.13 ± 0.33	**	NS
		R	4.43 ± 0.23	4.75 ± 0.14	5.55 ± 0.11	*	
Global impression (1–10)		P	6.83 ± 0.49	7.16 ± 0.27	7.11 ± 0.53	***	NS
		R	7.57 ± 0.22	7.19 ± 0.27	7.43 ± 1.12	NS	

P: pasteurized milk. R: raw milk. Last column shows the significant differences (A—Age; T—Treatment). NS: no significant differences; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. The differences between the values of each parameter and at each sampling point between cheeses made with pasteurized and raw milk are indicated next to the numerical value. Scale for aroma and taste: 1—no aroma or taste perception, 4—moderate perception and 7—very strong perception.

4. Conclusions

During the ripening of Valdeón cheese, there was an increase in the content of FFA. It was observed that oleic and palmitic acids were the predominant fatty acids throughout the ripening process, except for cheeses aged 2 days. The use of raw or pasteurized milk influenced the FFA content from the first month of ripening. Cheeses made from raw milk had higher total FFA values than those observed in cheeses made from pasteurized milk. The season of production of pasteurized milk cheeses also influenced the concentration of FFA in these cheeses. In general, the highest FFA concentrations corresponded to cheeses produced in summer. The SCFA/total FFA, MCFA/total FFA and LCFA/total FFA ratios were indicative of ripening time and season of production. However, they were not influenced by milk pasteurization. The higher accumulation of FFA in raw-milk cheeses at the end of ripening indicates a higher intensity of lipolysis in these cheeses. However, there were no significant differences in the sensory evaluation of raw and pasteurized milk cheeses. Therefore, pasteurization of the milk does not entail any significant changes to Valdeón cheese, and provides hygienic and sanitary guarantees and greater technological control of the process.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/dairy4010016/s1, Figure S1: Evolution of free fatty acid concentrations (means ± standard deviation) in Valdeón cheeses made with raw and pasteurized milk during the ripening process. Significant differences are indicated as *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, Figure S2: Evolution of free fatty acid concentrations (mean ± standard deviation) during the ripening of Valdeón cheese made with pasteurized milk at different seasons of the year, Figure S3: Sensory scores: (A) aroma, (B) taste of Valdeón cheese made from pasteurized or raw milk, after 120 days of ripening.

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