

Article

Influence of the Ratio of Sheep and Cow Milk on the Composition and Yield Efficiency of Lećevački Cheese

Siniša Matutinović ¹, Ante Rako ² , Milna Tudor Kalit ^{3,*}  and Samir Kalit ³ 

¹ Department of Production, Meggle Dairy d.o.o., Bihać, Grabeška 34, 77000 Bihać, Bosnia and Herzegovina; sinisa.matutinovic@gmail.com

² Department of Applied Sciences, Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia; Ante.Rako@krs.hr

³ Department of Dairy Science, University of Zagreb Faculty of Agriculture, Svetošimunska c. 25, 10000 Zagreb, Croatia; skalit@agr.hr

* Correspondence: mtudor@agr.hr; Tel.: +38-51-239-3647

Abstract: Lećevački cheese is a traditional Croatian hard cheese, which belongs to the group of hard Mediterranean cheeses produced from mixed milk (cow and sheep). The aim of this research was to determine the influence of different ratios and compositions of sheep milk on the composition and yield of Lećevački cheese. A total of 15 batches of Lećevački cheese were selected containing different ratios of sheep and cow milk from the regular production of a dairy plant. The ratio of sheep milk was as follows: up to 39%, from 40 to 44%, and from 45 to 50%. For each ratio, five batches were randomly selected. A higher ratio of sheep milk caused a noticeable increase in fat, protein, lactose, and total solids content, while the content of solids-not-fat significantly ($p < 0.05$) increased. A similar trend was found for casein content ($p < 0.1$). The highest ratio of sheep milk in mixed milk increased ($p < 0.05$) the protein content by almost 1%. However, the results showed that it is not reasonable to increase the sheep milk ratio in mixed milk above 44% (v/v) because it causes a higher ($p < 0.01$) moisture content in the cheese, as well as a lower fat content ($p < 0.01$) and fat recovery ($p = 0.07$) during the manufacturing of Lećevački cheese.

Keywords: mixed milk; cow milk; sheep milk; cheese composition; cheese yield; Lećevački cheese; Mediterranean



Citation: Matutinović, S.; Rako, A.; Tudor Kalit, M.; Kalit, S. Influence of the Ratio of Sheep and Cow Milk on the Composition and Yield Efficiency of Lećevački Cheese. *Fermentation* **2021**, *7*, 274. <https://doi.org/10.3390/fermentation7040274>

Academic Editor: Hiroshi Kitagaki

Received: 27 October 2021

Accepted: 17 November 2021

Published: 24 November 2021

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1. Introduction

Milk and dairy products produced from cows, sheep, goats, and buffalos are an important part of Mediterranean nutrition [1,2]. Moreover, the production of cheese from the mixed milk of different dairy animals is a common practice in Mediterranean countries [3]. There are many examples of cheeses produced from mixed milk, such as Teleme, Graviera, Kasser, Kopanisti, and Kefalotiri from Greece [4–6]; Casciotta d'Urbino, Murazzano, Fossa, Robiola di Roccaverano, and Castelmagno from Italy [7–10]; Cabrales, Mahon, Hispanico, and Iberico from Spain [11,12]; Halloumi from Cyprus [13]; Picante, cheeses with Appellation d'origine protégée, Amarelo, and Rabaçal from Portugal [14,15]; and Lećevački cheese from Croatia [16,17]. Park et al. [18] postulated that the composition of cow milk changes minimally over the year, taking into consideration that cows' lactation is not influenced by the season [19], which is not the case with sheep and goat milk. Therefore, it is difficult to maintain the standard quality of cheese produced from mixed milk [20].

The composition of the cheese produced from mixed milk strongly depends on different changeable milk parameters and on the composition of the milk that is used in its production, especially the protein and casein content [21]. Moreover, fat content and mineral composition, especially calcium, also influence the composition [18] and yield [22] of produced cheese. Cheese yield efficiency is the most important item in cheesemaking,

and its improvement is one of the most important tasks in the countries where milk is processed into cheese [23]. It can be defined as the weight of cheese in relation to the weight of milk used and can be presented as the value of efficiency of the recovery of fat and protein from milk into the cheese [24]. All formulas for the prediction of yield include the milk composition [25]. Cheese yield is lower if it is produced from milk with lower contents of fat, protein, and solids-not-fat [26]. Kalit et al. [27] reported that increasing the protein in milk by adding skim milk powder increases actual and adjusted cheese yield. Temizkan et al. [28] reported that the yield of cheese produced from sheep milk is higher than that produced from cow milk. Consequently, the yield of cheese produced from mixed milk with a higher ratio of sheep milk has to be higher. However, there has not been enough research to confirm that, especially considering the strong influence of season on the composition of sheep milk used in cheese production, which is typical for the Mediterranean area [29]. Different mixtures of sheep and cow milk drastically change the ratio of casein to fat as well as the average size of casein micelles and fat globules in mixed milk, which could also affect cheese yields.

Lećevečki cheese is a pastoral and traditional Croatian cheese that belongs to the group of hard Mediterranean cheeses. In contrast to the islands of the Adriatic sea and coastal regions where limited vegetation makes only sheep breeding possible, the environment of the Lećevečka region, located in the hinterlands of Dalmatia, is equally suitable for breeding sheep and cows. Such an environment dictated that in the past, this cheese was produced on family farms in the area of Lećevečka from full-fat sheep milk, cow milk, or mixed milk, depending on the season and herd composition (the number of sheep and cows that were possessed by farmers). Today, it is produced from mixed sheep and cow milk [16,17]. The composition of mixed milk depends on many different variabilities of milk, including the composition and physico-chemical properties of different milk types used in the production of cheese. The composition and yield of mixed milk cheese directly depend on the ratio of the mixture of milk, as well as the composition of the milk used in its production. The composition of the milk of the indigenous breed, whose milk is used for Lećevečki cheese making, is strongly affected by the flock, year, and season [29], so it is difficult to maintain a consistent quality of mixed milk cheeses. Therefore, the aim of this research was to determine the influence of different ratios and compositions of sheep milk on the composition and yield of Lećevečki cheese.

2. Materials and Methods

2.1. Sampling of Milk

For research purposes, 15 batches of Lećevečki cheese were selected containing different ratios of sheep and cow milk from the regular production from the Mils dairy plant in Split, Croatia. The ratio of sheep milk was as follows: up to 39%, from 40 to 44%, and from 45 to 50%. For each ratio, five batches were randomly selected. Bulk tank-mixed milk samples were taken after mixing sheep and cow milk and before processing into Lećevečki cheese. Batches were selected randomly, considering the season. Sheep and cow milk used in the production of Lećevečki cheese were collected from the Dalmatian hinterland between March and August. Sheep for milk production were bred semi-extensively, which included pasture during the vegetation and milking period. In addition, 150 g of wheat or barley were given to sheep during lactation. In contrast, the cows were bred intensively and fed with a concentrate according to their milk production. Similar to sheep, cows were outdoors during the vegetation period. Winter feeding included hay and some silage.

2.2. Milk Analysis

To determine the chemical composition, mixed milk samples were analyzed for the content of fat, protein, lactose, total solids, and total solids-not-fat with a mid-infrared spectrometry method using a MilkoScan FT-120 instrument [30]. The casein content of milk was detected using the direct method [31]. The value of pH was determined using the potentiometric method with a Schott CG 842 pH meter.

2.3. Making of Lečevački Cheese and Cheese Sampling

Following pasteurization, the sheep and cow milk were weighed and then mixed in a 600 L cheese vat. The process flow of the making of Lečevački cheese is presented in Table 1.

Table 1. Process flow of making of Lečevački cheese.

Step	Cheesemaking Parameters
Pasteurization of milk	Temperature: 74 ± 2 °C/30 s
Cooling of milk	Temperature: 30–32 °C
Addition of CaCl_2	Dosage: 0.02%
Addition of lysozyme	Dosage: 0.0025%
Addition of starter culture *	Dosage: 0.1%
Addition of rennet **	Dosage: 0.0016%
Coagulation	Duration: 30–40 min
Curd cutting	Size of wheat
Curd grain heating	Temperature: 41–42 °C
Drying of curd grains	Duration: 35 min
Cheese forming	Duration: 30 min
Pressing	Duration/pressure: 30 min/100 kPa Duration/pressure: 30 min/200 kPa Duration/pressure: 1 h/300 kPa
Salting (brine)	Salt concentration: 19–20 °Be Temperature: 14–16 °C pH value: 5.1 ± 0.1 Duration: 24 h \pm 2 h
Drying of the cheese	Temperature: 14–16 °C RAH: 70% Duration: 24 h
Ripening	Temperature: 14–16 °C RAH: 75–85% Duration: 90 days

* Homofermentative strains composed of *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactococcus lactis*;

** 100% chymosin. RAH = Relative air humidity.

During ripening, the cheese was kept on wooden shelves, turned upside-down, and washed every week. For analysis, 60 wheels of Lečevački cheese were produced from a total of 15 selected batches. Each batch of Lečevački cheese was weighed after 90 days of ripening.

2.4. Composition Analysis of the Cheese

The composition analysis of the cheese included the determination of fat content using the Van Gulik method [32], protein content using the block-digestion method [33], total solids content using the drying method [34], pH value using the potentiometric method using the Schott CG 842 instrument, lactic acid content using the titratable method [35], and salt content using the Volhard method [36].

2.5. Determination of Cheese Yield Efficiency

The cheese yield efficiency was determined by the weighing of the cheeses produced from the previously weighed mixed milk, and it was calculated as kg of milk used for the production of one kg of Lečevački cheese. Predictive cheese yield formulas were used according to the Van Slyke cheese yield equation [37]:

$$\text{Van Slyke Cheese Yield} = \frac{[(RF \times \% \text{ Fat in milk}) + (RC \times \text{Casein in milk})] \times RS}{(100 - \% \text{ Moisture of cheese})}$$

$$RF = \frac{(\% \text{ fat in cheese} \times \text{cheese wt})}{(\% \text{ fat in milk} \times \text{milk wt})}$$

$$RC = \frac{(\% \text{ casein in cheese} \times \text{cheese wt})}{(\% \text{ casein in milk} \times \text{milk wt})}$$

where *RF* is the fat recovered in cheese, *RC* is the casein recovered in cheese, and *RS* represents the other milk solids and added salt recovered in cheese. *RS* values for each cheese were calculated by substituting the *RF* and *RC* values into the following equation:

$$RS = \frac{RF \times \% \text{ Fat in milk}}{[(RF \times \% \text{ Fat in milk}) + (RC \times \% \text{ Casein in milk}) \times FDM]}$$

where *FDM* is fat in dry matter.

2.6. Statistical Analysis

Statistical analysis was done using the statistical program SAS (SAS Institute Inc., 2001, SAS/STAT User's Guide, Version 8.2., Cary, NC, USA). The influence of different ratios of sheep and cow milk on the chemical composition of cheese and cheese yield efficiency was studied by analysis of variance (PROC GLM). To determine the different mean values of the groups where statistical differences were found ($p < 0.05$), a Tukey–Kramer post hoc test was used. The obtained results were expressed as least square mean values \pm standard error (LSM \pm SE).

3. Results and Discussion

3.1. Mixed Milk Composition

The mixed milk composition used for the production of Lečevački cheese is presented in Table 2. A higher ratio of sheep milk in the mixed milk caused a noticeable, but not significant, increase in the content of fat, protein, lactose, and total solids, while the content of solids-not-fat significantly ($p < 0.05$) increased as a consequence of a higher content of sheep milk in the mixed milk. A similar trend was found for casein content (Table 2, $p < 0.1$). This is in accordance with Bonczar et al. [38], who found that the content of total solids, fat, and protein increased in mixed milk with an equal ratio of sheep and cow milk for the production of Bundz cheese. Similar results were obtained by Vyletelova-Klimešova et al. [39], who found that the addition of sheep milk to cow and goat milk increases the ratio of total solids, fat, protein, and casein in mixed milk. Mixing different ratios of sheep and goat milk for the production of Picante cheese influenced the content of fat and casein in the mixed milk [40]. The authors found that the content of casein was the lowest in the mixed milk with the lowest ratio of sheep milk. A similar effect was noticed when buffalo milk was mixed with cow milk for the production of Mozzarella cheese [21]. Authors found that the ratio of total solids, fat, and protein increased in mixed milk in comparison to cow milk. The pH value of the mixed milk was not changed as a consequence of different ratios of sheep and cow milk, which is in line with Bonczar et al. [38] and Vyletelova-Klimešova et al. [39].

Table 2. Influence of the ratio of sheep and cow milk on the composition of mixed milk used for making Lečevački cheese (least square mean \pm standard error).

Component	Ratio of Sheep Milk and Cow Milk (% v/v)			Level of Significance
	35–39 <i>n</i> = 5	40–44 <i>n</i> = 5	45–50 <i>n</i> = 5	
Fat (g/100 g)	4.43 \pm 0.18	4.80 \pm 0.20	4.79 \pm 0.22	NS
Protein (g/100 g)	4.06 \pm 0.09	4.26 \pm 0.10	4.37 \pm 0.12	NS
Lactose (g/100 g)	4.24 \pm 0.05	4.31 \pm 0.05	4.30 \pm 0.06	NS
Total solids (g/100 g)	13.68 \pm 0.25	14.26 \pm 0.27	14.36 \pm 0.30	NS
SNF (g/100 g)	9.16 ^b \pm 0.09	9.43 ^{ab} \pm 0.10	9.57 ^a \pm 0.11	$p < 0.05$
Casein (g/100 g)	3.20 ^b \pm 0.07	3.40 ^{ab} \pm 0.08	3.47 ^a \pm 0.08	$p < 0.1$
pH	6.65 \pm 0.37	6.61 \pm 0.37	6.64 \pm 0.42	NS

NS = Not significant; SNF = solids-not-fat. Means within the same row marked with different letters differ significantly.

3.2. Lećevečki Cheese Composition

The higher content of sheep milk in the mixed milk significantly ($p < 0.01$) decreased the ratio of the total solids and fat content of Lećevečki cheese (Table 3), while the ratio of moisture in total solids-not-fat, salt content, pH value, and lactic acid content were not changed as a consequence of increasing the ratio of sheep milk in the mixed milk. The highest ratio of sheep milk in the mixed milk (45–50% (v/v)) ($p < 0.05$) increased the protein content of Lećevečki cheese by almost 1% (Table 3). Freitas et al. [40] found that a higher ratio of sheep milk in mixed milk with goat milk causes a higher retention of moisture in hard cheese. This is a consequence of a noticeably higher casein content in mixed milk with a higher sheep milk content, which was the case in our study as well ($p < 0.1$). The higher capacity of water-binding of the casein matrix system of the cheese is a consequence of the higher casein content in the milk used for the production of this cheese. A similar result was found in the production of Bundz cheese when half of the cow milk was substituted with sheep milk [38]. Gobbetti et al. [7] also found that Fossa (pit) cheese produced from a mix of cow and sheep milk (70:30%) contained less moisture in comparison to Fossa (pit) cheese produced exclusively from cow milk. Authors postulated that lower total solids content in such cheese is a consequence of the lower fat content of the cheese, which was the case in our work as well. Aminifar et al. [41] found that moisture content in Lighvan cheese decreased when at least half of the cow milk was replaced with sheep milk. The same was found by Ponce de Leon-Gonzales et al. [42] in researching the influence of the mixing of sheep milk with cow milk on the composition of reduced fat Muenster-type cheese. They found that cheese produced from mixed milk has a lower moisture and higher protein content. Elgaml et al. [43] found that mixing cow and goat milk in different ratios (from 25% to 50%) changed the moisture content of Halloumi cheese. However, Niro et al. [44] did not find any influence of mixing of sheep milk (18%) with cow milk (82%) on the content of total solids of the cheese produced from mixed milk. The content of moisture in total solids-not-fat did not change significantly as a consequence of different ratios of sheep and cow milk.

Table 3. Influence of the ratio of sheep and cow milk on the composition of Lećevečki cheese (least square mean \pm standard error).

Component	Ratio of Sheep Milk and Cow Milk (% v/v)			Level of Significance
	35–39 $n = 5$	40–44 $n = 5$	45–50 $n = 5$	
Total solids (g/100 g)	61.02 ^a \pm 0.27	60.65 ^a \pm 0.30	59.22 ^b \pm 0.34	$p < 0.01$
Fat (g/100 g)	31.02 ^a \pm 0.42	30.88 ^a \pm 0.46	28.34 ^b \pm 0.47	$p < 0.01$
Protein (g/100 g)	24.34 ^b \pm 0.21	24.31 ^b \pm 0.23	25.2 ^a \pm 0.26	$p < 0.05$
MNFS (g/100 g)	56.51 \pm 0.43	56.93 \pm 0.46	56.91 \pm 0.50	NS
Salt (g/100 g)	1.41 \pm 0.04	1.40 \pm 0.05	1.39 \pm 0.05	NS
pH	5.09 \pm 0.03	5.04 \pm 0.03	5.08 \pm 0.03	NS
Lactic acid (%)	1.46 \pm 0.04	1.40 \pm 0.04	1.40 \pm 0.04	NS

NS = Not significant; MNFS = moisture in non-fat substance. Means within the same row marked with different letters differ significantly.

The fat content of Lećevečki cheese produced from mixed milk with the highest ratio of sheep milk (45–50%) was significantly ($p < 0.01$) lower, which is in accordance with Gobbetti et al. [7]. The authors found that Fossa hard cheese produced from mixed milk (30% sheep and 70% cow milk) had less fat content (32.7%) in comparison to Fossa cheese produced exclusively from cow milk. Fossa cheese produced from pure cow milk had a fat content of 38.8%. This could be a consequence of the fact that the fat globules of cow milk are larger than those of sheep milk. Larger fat globules are better entrapped within the cheese casein matrix in comparison to smaller fat globules. Bonczar et al. [38] determined that cheese produced from mixed sheep and cow milk (50:50%) contained less fat in comparison to cheese produced only from cow milk, but more fat than cheese produced only from sheep milk. Similar to the result of this research, Freitas et al. [40] found that cheese produced from mixed milk with a higher ratio of sheep milk in comparison to goat milk contained less

fat. The fat and protein of milk represent the greatest part of the total solids of the cheese produced from that milk, so the higher content of protein in mixed milk could cause the cheese to have a lower fat content as was the case in our research (Table 3), especially taking into consideration that the higher sheep milk ratio in the mixed milk caused a lowering of the total solids in Lečevački cheese. In contrast, Ponce de Leon-Gonzales et al. [42] did not find any differences in the fat content of Muenster cheese when 20% of cow milk was replaced with sheep milk, nor did Niro et al. [44] in Caciocavallo cheese produced with 18% sheep milk and 82% cow milk. Moreover, Freitas and Malcata [14] made cheese from mixed goat and sheep milk in different ratios (20:80% and 40:60%) and did not find any differences in the fat content of these cheeses, while Halloumi cheese produced from mixed goat and cow milk with 25% and 50% of goat milk had a higher fat content [43]. In contrast to our research, Vyletelova-Klimešova et al. [39] found that cheese produced from mixed milk (cow, sheep, and goat milk) contained more fat.

This investigation showed that mixed milk with 40–44% of sheep milk did not influence the protein content of Lečevački cheese in comparison to the ratio of 35–39%, which is in accordance with Niro et al. [44], who found that replacing 18% of cow milk with sheep milk did not change the protein content of Caciocavallo cheese. However, further increasing the ratio of sheep milk in the mixed milk (45–50%) caused a significant ($p < 0.05$) increase in the protein content of Lečevački cheese. Gobbetti et al. [7], as well as Ponce de Leon Gonzales et al. [42], concluded that hard cheese produced with mixed milk with a higher ratio of sheep milk contains a higher level of protein, which is a result of the fact that sheep milk has a higher protein content in comparison to cow milk. The obtained results are in line with those previously reported [38,39,43]. In accordance with our research, Fenelon et al. [45] and Madsen and Ardo [46] reported that a decrease of fat in cheese causes moisture and proteins to increase.

Our investigation showed that different ratios of sheep and cow milk did not significantly influence the salt content of the cheese (Table 3), which is in line with the results of previous reports [6,28,42,47]. In contrast, Aminifar et al. [41] reported that the type of milk used in the production of Linghvan cheese, which ripened in 12% brine for three months, influenced the salt content of the cheese. They reported that cheese produced from sheep milk contained less salt than cheese produced from mixed milk (50:50%), or from pure cow milk. Moreover, Elgaml et al. [43] found that different ratios of goat and cow milk (25:75% and 50:50%) influenced the salt content of the cheese, which they connected with the different moisture content in these cheeses. Cheese with a higher moisture content absorbs more salt by diffusion [48]. In spite of the fact that a significantly ($p < 0.01$) different content of moisture was found among Lečevački cheeses produced with different ratios of sheep and cow milk, these differences did not influence the intensity of the absorption of salt during the brining of Lečevački cheese in this research.

This research showed that different ratios of sheep and cow milk did not have an influence on the pH value of Lečevački cheese (Table 3). Regardless of the type of milk used for cheesemaking, three main factors directly influence the pH value of cheese: the fermentation of lactose, accumulation of amino acids during ripening of the cheese [49], and fat content of the cheese [45,50]. No differences were found in the concentration of lactic acid as an indicator of the fermentation of lactose among the cheeses produced with different ratios of sheep and cow milk (Table 3). Moreover, there were no differences in the concentration of amino acids, expressed as the ratio of trichloroacetic acid-soluble nitrogen to the total nitrogen, of Lečevački cheese made from the different ratios of sheep and cow milk (data are not published). Similar results were obtained by Ponce de Leon-Gonzales et al. [42], Bonczar et al. [38], and Aminifar et al. [41], who also did not find any differences in the pH value of the cheeses produced with different ratios of sheep and cow milk. Moreover, Freitas et al. [40] did not find any differences in pH value as a consequence of mixing sheep and goat milk. However, Niro et al. [44] reported that the addition of sheep milk to cow milk (18:82%) decreases pH value as a consequence of the higher production of lactic acid in the manufacturing of pasta filata cheeses. When the ratio of goat milk is

increased in a mixture with cow milk (from 25% to 50%), the pH value decreases [43]. Some authors reported that full-fat cheeses have a higher pH value in comparison to corresponding reduced fat cheese [45]. In spite of significant ($p < 0.01$) differences in the fat content of Lećevečki cheese produced from the different ratios of sheep and cow milk, these differences were not sufficient to influence the pH value of the Lećevečki cheese in our experiment.

3.3. Cheese Yield Efficiency

A higher ratio of sheep milk in mixed milk caused a noticeable tendency ($p = 0.11$) to increase actual yield (Table 4). There could be two reasons for this trend: a higher SNF ($p < 0.05$) and a higher casein ($p < 0.1$) content of the mixed milk with a higher ratio of sheep milk (Table 2). This was the reason for less mixed milk with a higher ratio of sheep milk being required for the making of one kg of Lećevečki cheese. Additionally, Lećevečki cheese produced with a higher ratio of sheep milk had a higher moisture content (Table 3). It is well known that cheese that retains more moisture during its production has a higher cheese yield [51]. On average, from 100 kg of mixed milk, 13.54 kg of Lećevečki cheese was made. Zedan et al. [21] reported that the cheese yield of fresh Mozzarella produced from mixed buffalo and cow milk is higher (11.66 kg/100 kg of milk) in comparison to the cheese produced from pure cow milk (10.66 kg/100 kg of milk). They explained that the differences are due to higher total solids, which means a higher fat and protein content of buffalo milk in comparison to cow milk. Authors cited El-Zoughby (1998), who reported that cheese yields of Mozzarella produced from different types of milk vary greatly. For buffalo milk it was 11.62 kg/100 kg of milk, for cow milk it was 8.95 kg/100 kg of milk, for goat milk it was 7.93 kg/100 kg of milk, for mixed buffalo and cow milk it was 10.50 kg/100 kg of milk, for mixed buffalo and goat milk it was 10.35 kg/100 kg of milk, for mixed cow and goat milk it was 8.37 kg/100 kg of milk, and for mixed buffalo, cow, and goat milk it was 9.15 kg/100 kg of milk. Similar results were obtained by Economides et al. [13], who found there to be a higher yield of Halloumi cheese produced from mixed sheep and goat milk in comparison to the cheese produced from cow milk. Temizkan et al. [28] determined that actual and adjusted yields were higher for the Kashar cheese produced from sheep milk (13.77 kg/100 kg of milk and 12.31 kg/100 kg of milk, respectively) in comparison to Kashar cheese produced from cow milk (8.04 kg/100 kg of milk and 7.53 kg/100 kg of milk, respectively). Wendorff [52] reported that the cheese yield of cheese produced from sheep milk varies between 16.1 kg for Manchego cheese and 21.9 kg for blue cheese, while Kalit [24] reported that the actual yield of semi-hard Tounj cheese produced from raw cow milk was 11.23 kg/100 kg of milk. Merćep et al. [53] reported that the actual yield of semi-hard cow Trappist cheese varies between 9.25 kg and 9.49 kg/100 kg of milk. Tuta [26] determined that the actual cheese yield of semi-hard Trappist cheese varies between 8.78 and 9.53 kg/100 kg of milk, whereas the yield of PDO Paški cheese, which is produced exclusively from the milk of the Croatian Paška sheep breed, varies between 18.92 and 19.60 kg/100 kg of milk, depending on the type of rennet used [54]. Finally, Faccia et al. [55] found that the yield of the Fior di Latte cheese produced from mixed sheep and goat milk is higher than the corresponding cheese produced from cow milk. Moreover, from Table 4 it can be seen that Van Slyke cheese yield increased as a consequence of increasing the ratio of sheep milk up to 44%, while further increasing the ratio of sheep milk in mixed milk did not cause a further increase of the Van Slyke cheese yield. This could be associated with the trend of the decreasing recovery of fat, which dropped significantly ($p = 0.07$) from 0.76 (35–39% of sheep milk) to 0.66 in mixed milk with 45–50% of sheep milk (Table 4). Casein recovery was stable regardless of the ratio of sheep milk in mixed milk. Milani and Wendorff [56] determined that fat recovery varied from 0.83 to 0.84, while casein recovery varied from 0.94 to 0.96 in the making of hard sheep cheeses, while fat and protein recovery in the production of cow semi-hard Tounj cheese were 0.85 and 0.77, respectively [24]. Our results are noticeably lower than those obtained by other authors, probably due to the fact that the fat globules of cow milk are larger in diameter than those

of sheep milk [57], which could be the reason that these fat globules were not efficiently retained in the casein matrix of the mixed milk with a higher sheep milk ratio (due to the different casein composition of cow and sheep milk). The other reason could be the fact that the Lećevečki cheese for this research was produced in the regular production from the Mils dairy plant in Split (Croatia) and not in fully controlled experimental conditions.

Table 4. Influence of the ratio of sheep and cow milk on the yield efficiency of Lećevečki cheese (least square mean \pm standard error).

Component	Ratio of Sheep Milk and Cow Milk (% v/v)			Level of Significance
	35–39 <i>n</i> = 5	40–44 <i>n</i> = 5	45–50 <i>n</i> = 5	
Actual yield *	7.48 \pm 0.23	7.38 \pm 0.26	7.25 \pm 0.29	<i>p</i> = 0.11
Van Slyke cheese yield	10.63 \pm 0.33	10.96 \pm 0.35	10.74 \pm 0.37	NS
Fat recovery	0.76 \pm 0.03	0.74 \pm 0.04	0.66 \pm 0.05	<i>p</i> = 0.07
Casein recovery	0.82 \pm 0.04	0.83 \pm 0.05	0.82 \pm 0.05	NS

NS = Not significant. * kg of milk required for the production of 1 kg of cheese.

4. Conclusions

In spite of the fact that different mixtures of sheep and cow milk could drastically change mixed milk composition, these changes only have a slight influence on hard cheese composition such as in the case of Lećevečki cheese. This could allow cheesemakers to choose from a wide range of mixtures of sheep and cow milk depending on the season due to the fact that the quantity of sheep milk produced in Mediterranean areas such as the coast of Croatia is strongly dependent on the season. However, our results showed that it is not reasonable to increase the sheep milk ratio in mixed milk above 44% (v/v) because it causes higher moisture content in the cheese, as well as lower fat content and fat recovery during the manufacturing of Lećevečki cheese.

Author Contributions: Conceptualization and methodology, S.K.; investigation, S.M.; formal analysis, S.M. and M.T.K.; data curation, A.R.; writing—original draft preparation, S.M. and S.K.; writing—review and editing, M.T.K. and A.R.; supervision, S.K., A.R. and M.T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the Split-Dalmatia County, Agriculture Development Program, Agreement Number 402-01/11-05/31.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tornambe, G.; Di Grigoli, A.; Alicata, M.L.; De Pasquale, C.; Bonnano, A. Comparing quality characteristics of “Caciocavallo Palermitano” cheese from traditional and intensive production systems. In Proceedings of the 15th Meeting of the FAO-CIHEAM Mountain Pastures Network, Les Diablerets, Vaud, Switzerland, 7–9 October 2009; pp. 153–156.
2. Selvaggi, M.; Laudadio, V.; Dario, C.; Tufarelli, V. Investigating the genetic polymorphism of sheep milk proteins: A useful tool for dairy production. *J. Sci. Food Agric.* **2014**, *94*, 3090–3099. [[CrossRef](#)] [[PubMed](#)]
3. Hayaloglu, A.; Ozer, B. Cheeses of Turkey: 2. Varieties ripened under brine. *Dairy Sci. Technol.* **2008**, *88*, 225–244. [[CrossRef](#)]
4. Alichanidis, E.; Polychroniadou, A. Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: A review. *Dairy Sci. Technol.* **2008**, *88*, 495–510. [[CrossRef](#)]
5. Nega, A.; Moatsou, G. Proteolysis and related enzymatic activities in ten Greek cheese varieties. *Dairy Sci. Technol.* **2012**, *92*, 57–73. [[CrossRef](#)]
6. Pappa, E.C.; Kandarakis, I.; Zefridis, G.K.; Antifantakis, E.M.; Robertson, J.A.; Mills, E.N.C. Teleme white-brined cheese from sheep or goat milk. *Int. Dairy J.* **2012**, *24*, 83–86.

7. Gobetti, M.; Folkertsma, B.; Fox, P.F.; Corsetti, A.; Smacchi, E.; De Angelis, M.; Rossi, J.; Kilcawley, K.; Cortini, M. Microbiology and biochemistry of Fossa (pit) cheese. *Int. Dairy J.* **1999**, *9*, 763–773. [\[CrossRef\]](#)
8. Gobetti, M. Extra-hard varieties. In *Cheese: Chemistry, Physics and Microbiology*, 3rd ed.; Fox, P.F., McSweeney, P.L.C., Cogan, T.M., Guinee, T.P., Eds.; Elsevier Academic Press: London, UK, 2004; Volume 2, pp. 51–70.
9. Pirisi, A.; Comunian, R.; Urgeghe, P.P.; Scintu, M.F. Sheep's and goat's dairy products in Italy: Technological, chemical, microbiological, and sensory aspects. *Small Rumin. Res.* **2011**, *101*, 102–112. [\[CrossRef\]](#)
10. Bertolino, M.; Dolci, P.; Giordano, M.; Rolla, L.; Zeppa, G. Evolution of chemico-physical characteristics during manufacture and ripening of Castelmano PDO cheese in wintertime. *Food Chem.* **2011**, *129*, 1001–1011. [\[CrossRef\]](#)
11. Medina, M.; Nuñez, M. Cheeses made from ewe's and goat's milk. In *Cheese: Chemistry, Physics and Microbiology*, 3rd ed.; Fox, P.F., McSweeney, P.L.C., Cogan, T.M., Guinee, T.P., Eds.; Elsevier Academic Press: London, UK, 2004; Volume 2, pp. 279–299.
12. Martinez, S.; Franco, I.; Carballo, J. Spanish goat and sheep milk cheeses. *Small Rumin. Res.* **2011**, *101*, 41–54. [\[CrossRef\]](#)
13. Economides, S.; Georgiades, E.; Mavrogenis, A.P. The effect of different milks on the yield and chemical composition of Halloumi cheese. *Tech. Bull. Agric. Res. Inst.* **1987**, *90*, 2–7.
14. Freitas, A.C.; Malcata, F.X. Influence of milk type, coagulant, salting procedure and ripening time on the final characteristics of Picante cheese. *Int. Dairy J.* **1996**, *6*, 1099–1116. [\[CrossRef\]](#)
15. Freitas, A.C.; Malcata, F.X. Microbiology and biochemistry of cheeses with Appellation d'Origine Protégée and manufactured in the Iberian peninsula from ovine and caprine milks. *J. Dairy Sci.* **2000**, *83*, 584–602. [\[CrossRef\]](#)
16. Bubić, M. Contribution to the Knowledge of Quality and Production of Lećevački Cheese. Master's Thesis, Faculty of Veterinary, University of Zagreb, Zagreb, Croatia, 1981.
17. Matutinović, S.; Rako, A.; Kalit, S.; Havranek, J. The importance of traditional cheeses with a special focus on Lećevački cheese. *Mljekarstvo* **2007**, *57*, 49–65.
18. Park, Y.W.; Juarez, M.; Ramos, M.; Haenlein, G.F.W. Physico-chemical characteristics of goat and sheep milk. *Small Rumin. Res.* **2007**, *68*, 88–113. [\[CrossRef\]](#)
19. Teter, A.; Kędzierska-Matysek, M.; Barłowska, J.; Król, J.; Brodziak, A. Nutritional value and coagulation properties of milk from local cow breeds, including the selected macro- and micronutrients and trace elements. *Mljekarstvo* **2020**, *70*, 210–220. [\[CrossRef\]](#)
20. Campos, G.; Robles, L.; Alonso, R.; Nuñez, M.; Picon, A. Microbial dynamics during the ripening of a mixed cow and goat milk cheese manufactured using frozen goat milk curd. *J. Dairy Sci.* **2011**, *94*, 4766–4776. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Zedan, I.A.; Abou-Shaloue, Z.; Zaky, S.M. Quality evaluation of Mozzarella cheese from different milk types. *Alex. Sci. Exch. J.* **2014**, *35*, 162–177.
22. Fox, P.F.; Cogan, T.M. Factors that affect the quality of cheese. In *Cheese: Chemistry, Physics and Microbiology*, 3rd ed.; Fox, P.F., McSweeney, P.L.C., Cogan, T.M., Guinee, T.P., Eds.; Elsevier Academic Press: London, UK, 2004; Volume 1, pp. 583–608.
23. Pretto, D.; De Marchi, M.; Penasa, M.; Cassandro, M. Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. *J. Dairy Res.* **2013**, *80*, 1–5. [\[CrossRef\]](#)
24. Kalit, S. Biochemical Changed of Tounj Cheese during Ripening. Ph.D. Thesis, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, 2003.
25. Katz, G.; Merin, U.; Bezman, D.; Lavie, S.; Lembersky-Kuzin, L.; Leitner, G. Real-time evaluation of individual cow milk for higher cheese-milk quality with increased cheese yield. *J. Dairy Sci.* **2015**, *99*, 4178–4187. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Tuta, N. Production of Trappist-Style Semi-Hard Cheese. Bachelor's Thesis, Department for Chemistry and Technology, University Department for Professional Studies, University of Split, Split, Croatia, 2011.
27. Kalit, S.; Tudor Kalit, M.; Dolenčić Špehar, I.; Salajpal, K.; Samaržija, D.; Anušić, J.; Rako, A. The Influence of Milk Standardization on Chemical Composition, Fat and Protein Recovery, Yield and Sensory Properties of Croatian PGI Lički Škripavac Cheese. *Foods* **2021**, *10*, 690. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Temizkan, R.; Yasar, K.; Hayaloglu, A.A. Changes during ripening in chemical composition, proteolysis, volatile composition and texture in Kashar cheese made using raw bovine, ovine or caprine milk. *Int. J. Food Sci. Technol.* **2014**, *49*, 2643–2649. [\[CrossRef\]](#)
29. Matutinović, S.; Kalit, S.; Salajpal, K.; Vrdoljak, J. Effects of flock, year and season on the quality of milk from an indigenous breed in the sub-Mediterranean area. *Small Rumin. Res.* **2011**, *100*, 159–163. [\[CrossRef\]](#)
30. International Organization for Standards. ISO 9622. *Milk and Liquid Milk Products—Guidelines for the Application on Mid-Infrared Spectrometry*; International Organization for Standards: Geneva, Switzerland, 2013.
31. International Organization for Standards. ISO 17997-2. *Milk—Determination of Casein-Nitrogen Content—Part 1: Direct Method*; International Organization for Standards: Geneva, Switzerland, 2013.
32. International Organization for Standards. ISO 3433. *Cheese—Determination of Fat Content—Van Gulik Method*; International Organization for Standards: Geneva, Switzerland, 2008.
33. International Organization for Standards. ISO 8968-2. *Milk—Determination of Nitrogen Content—Part 2: Block-digestion method (Macro method)*; International Organization for Standards: Geneva, Switzerland, 2001.
34. International Organization for Standards. ISO 5534. *Cheese and Processed Cheese—Determination of the Total Solids Content (Reference Method)*; International Organization for Standards: Geneva, Switzerland, 2004.
35. Association of Official Analytical Chemists (AOAC). *Acidity of Milk, Titrimetric Method. Official Method*; Association of Official Analytical Chemists: Washington, DC, USA, 2000.

36. Association of Official Analytical Chemists (AOAC). *Chloride (Total) in Cheese Volhard Method. Official Method*; Association of Official Analytical Chemists: Washington, DC, USA, 2006.
37. Wendorff, W.L.; Kalit, S. Processing of sheep milk. In *Handbook of Milk of Non-Bovine Mammals*, 2nd ed.; Park, W.Y., Haenlein, G.F.W., Wendorf, W.L., Eds.; Wiley Blackwell: West Sussex, UK, 2017; pp. 222–260.
38. Bonczar, G.; Regula-Sardat, A.; Pustkowiak, H.; Zebrovská, A. Effect of mixing of ewe's and cow's milk on Bundz cheese properties. *Zywność Nauka Technol. Jakość* **2009**, *16*, 96–106.
39. Vyletelova-Klimešova, M.; Hanuš, O.; Horaček, J.; Vorlova, L.; Nemečková, I.; Nejeschlebova, L.; Kopecky, J. Characteristic and quality and food safety of regional cheese produced from mixed milk. *Acta Univ. Agric. Silvica* **2014**, *62*, 1171–1182. [[CrossRef](#)]
40. Freitas, A.C.; Fresno, J.M.; Prieto, B.; Malcata, F.X.; Carballo, J. Effects of ripening time and combination of ovine and caprine milks on proteolysis of Picante cheese. *Food Chem.* **1997**, *60*, 219–229. [[CrossRef](#)]
41. Aminifar, M.; Hamed, M.; Emamdjomeh, Z.; Mehdinia, A. The effect of ovine and bovine milk on the textural properties of Lingshan cheese during ripening. *Int. J. Dairy Technol.* **2013**, *66*, 45–53. [[CrossRef](#)]
42. Ponce de Leon-Gonzalez, L.; Wendorf, W.L.; Ingham, B.H.; Thomas, D.L.; Jeaggi, J.J.; Houck, K.B. Influence of ovine milk in mixture with bovine milk on the quality of reduced fat Muenster-type cheese. *J. Dairy Sci.* **2002**, *85*, 36–42. [[CrossRef](#)]
43. Elgaml, N.B.; Moussa, M.A.M.; Saleh, A.E. Comparison of the properties of Halloumi cheese made from goat milk, cow milk and their mixture. *J. Sustain. Agric. Sci.* **2017**, *43*, 77–87. [[CrossRef](#)]
44. Niro, S.; Fratianni, A.; Tremonte, P.; Sorrentino, E.; Tipaldi, L.; Panfili, G.; Coppola, R. Innovative Caciocavallo cheeses made from a mixture of cow milk with ewe or goat milk. *J. Dairy Sci.* **2014**, *97*, 1296–1304. [[CrossRef](#)]
45. Fenelon, M.A.; Guinee, T.P. Primary proteolysis and textural changes during ripening in Cheddar cheeses manufactured to different fat contents. *Int. Dairy J.* **2000**, *10*, 151–158. [[CrossRef](#)]
46. Madsen, J.S.; Ardo, Y. Exploratory study of proteolysis, rheology and sensory properties of Danbo cheese with different fat contents. *Int. Dairy J.* **2001**, *11*, 423–431. [[CrossRef](#)]
47. Freitas, A.C.; Sousa, M.I.; Malcata, F.X. Effect of ripening time and the combination of ewe and goat milk on the microflora of Picante cheese. *Ital. J. Anim. Sci.* **1995**, *7*, 361–377.
48. Scott, R. *Cheesemaking Practice*; Robinson, R.K., Wilbey, R.A., Eds.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 1998; ISBN 9781461558194.
49. Fox, P.F. Cheese. An overview. In *Cheese: Chemistry, Physics and Microbiology*, 3rd ed.; Fox, P.F., McSweeney, P.L.C., Cogan, T.M., Guinee, T.P., Eds.; Elsevier Academic Press: London, UK, 2004; Volume 2, pp. 1–18.
50. Van Hekken, D.L.; Park, Y.W.; Tunick, M.H. Effect of reducing fat content on the proteolytic and rheological properties of Cheddar-like caprine milk cheese. *Small Rumin. Res.* **2013**, *110*, 46–51. [[CrossRef](#)]
51. Vacca, G.M.; Stocco, G.; Dettori, M.L.; Summer, A.; Cipolat-Gotet, C.; Bittante, G.; Pazzola, M. Cheese yield, cheesemaking efficiency, and daily production of 6 breeds of goats. *J. Dairy Sci.* **2018**, *101*, 7817–7832. [[CrossRef](#)]
52. Wendorff, W.L. Milk composition and cheese yield. In *Proceedings of the 8th Great Lakes Dairy Sheep Symposium*, Ithaca, NY, USA, 7–9 November 2002; pp. 104–117.
53. Merčep, A.; Kirin, S.; Zdolec, N.; Cvrtila Fleck, Ž.; Filipović, I.; Njari, B.; Mitak, M.; Kozačinski, L. Quality of trapist cheese from Croatian dairy plant. *Mljekarstvo* **2010**, *60*, 288–298.
54. Oštarić, F.; Antunac, N.; Prpić, Z.; Mikulec, N. Influence of rennet on the quality of Paški cheese. *Mljekarstvo* **2015**, *65*, 101–110.
55. Faccia, M.; Trani, A.; Gambacorta, G.; Loizzo, P.; Cassone, A.; Caponio, F. Production technology and characterization of Fior di latte cheeses made from sheep and goats milks. *J. Dairy Sci.* **2015**, *98*, 402–410. [[CrossRef](#)]
56. Milani, F.X.; Wendorff, W.L. Goat and sheep milk products in the United States. *Small Rumin. Res.* **2011**, *101*, 134–139. [[CrossRef](#)]
57. Havranek, J.; Kalit, S.; Antunac, N.; Samaržija, D. *Cheesemaking*; Croatian Dairy Union: Zagreb, Croatia, 2014; ISBN 9789537472085.