

Analytical Methods

Studies on a fast method for determining the yield in the production of Argentinean sheep cheeses

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Abstract

A modified previously developed method was used to predict cheese yield from a small volume of sheep milk. Bulk milk samples were collected from a herd of Pampinta sheep throughout lactation. Yields predicted with this technique, even though higher, were well correlated with yields measured from actual cheesemaking, employing the same milk batches. Correction of predicted yields with a formula resulted in values very close to the actual yields. Predicted yield noticeably increased throughout lactation. Chemical acidification of milk markedly reduced predicted yield, while storage (at constant pH), either at 4 °C for one day or at –18 °C for up to 2 months, had no visible effects on it. Milks collected the same day from individual animals showed wide variations in predicted yield. This was true for the beginning, middle and the end of lactation, the dispersion being slightly lower in the middle.

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1. Introduction

Although Argentina is a big producer of cows milk, the dairy sheep industry has not received much attention, until recently. For many years this industry was mainly artisanal, and data concerning homemade cheeses were very difficult to acquire. In 2002, 56 milk farms with an average flock size of 150 individuals were reported (McCormick & Lynch, 2003). During the 2001–2002 period, 553,000 l of milk were processed, with a total cheese production of 75,300 kg. Even though low, this production was significantly higher than that of the 1996–1997 period, which accounted for only 39,000 kg of cheese (McCormick & Lynch, 2003). Nowadays, sheep cheese production is reach-

ing industrial scale, and several government institutions help cheese manufacturers so as to improve the quality of milk and cheeses. Most sheep cheeses produced in Argentina fall into the semi-hard cheese category, within which they are given many different names (McCormick & Lynch, 2003). At present there are about 74,580 sheep for milk production in Argentina, but only 3200 belong to registered dairy industries (McCormick, Borra, Peña, & Lynch, 2006). The future of sheep cheese production could be interesting if adequate policies are developed and commercialization problems are solved.

Cheese yield, defined as the weight of cheese obtained from a given weight of milk, is considered a major factor affecting efficiency and profitability of cheese manufacture (Emmons, 1993). It has been stated that 1% loss in cheese yield is intolerable to cheese makers from an economic point of view (Lacroix, Verret, & Emmons, 1991). Factors affecting cheese yield are associated with milk (gross composition, content of free fatty acids, amount and genetic variants of caseins, somatic cell counts, microbiological

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quality, presence of antimicrobial agents) (Skeie, 2007) and technology (pasteurization, coagulation, curd firmness at cutting and other manufacturing parameters) (Fenelon & Guinee, 1999). Among the factors mentioned above, cheese yield is essentially dependent on milk composition, particularly fat and protein (Brito, Niklitschek, Molina, & Molina, 2002; Guo, Park, Dixon, Gilmore, & Kindstedt, 2004; Lawrence, 1991). Given that sheep milk has a higher protein and fat content than have cow or goat milk, cheese yield is a decisive economic parameter for cheese manufacture (Assenat, 1985).

Numerous predictive formulae for cheese yield have been developed for monitoring cheesemaking operation and evaluating efficiency (Emmons, Ernstrom, Lacroix, & Verret, 1990; Fenelon & Guinee, 1999; Van Slyke & Price, 1949), grouped mainly in two categories (Emmons et al., 1990). The first type is based on final composition of cheeses; however, this is not applicable in Argentina, because there are no standard ranges of cheese composition officially available for sheep cheeses. The second category is derived from actual cheese and milk composition, thus being more practical and potentially applicable to Argentinean sheep cheeses. Even when these latter predictive formulae were extensively developed and proved to be relatively accurate (Zeng, Soryal, Fekadu, Bah, & Popham, 2007), their application required the determination of (at least) fat, total protein and total solids content in every milk sample. These analyses are easily performed using infrared milk analyzers, which are very expensive but not available in small sheep cheese plants.

On the other hand, in order to characterize the cheesemaking potential of milk, Othmane, Carriedo, de la Fuente Crespo, & San Primitivo (2002) developed a fast, inexpensive and reliable cheese micro-manufacturing method, which used small quantities of milk and showed reasonably acceptable performance. Moreover, this method has the advantage of being sensitive to the influence of parameters that are not taken into account by the previously mentioned formulae for cheese yield, such as changes produced during the acidification or freezing of the milk. Finally, the method allows the processing of large numbers of milk samples collected from individual animals. Hence, the method could be of great interest as a selection criterion in dairy sheep, since milk collected from different breeds and even individuals, showed high genetic variations in β -lactoglobulin and casein fractions, which have proved to exert a high influence on clotting speed, curd firmness and cheese yield (Amigo, Recio, & Ramos, 2000).

The objective of this study was to compare the cheese yield, calculated by using a modification of the technique developed by Othmane et al. (2002), with the actual cheese yield obtained from the same milks by two different cheesemaking protocols. The experiments were carried out for bulk milk of the same sheep breed throughout lactation. In addition, the influences of storage at refrigeration and freezing temperatures and the previous acidification of milk

on cheese yield were studied. Finally, the variations among individuals at three different stages of lactation were examined for each animal within the herd.

2. Materials and methods

2.1. Milk samples

Sheep milk used in this study was from the Pampinta breed herd at the Estación Experimental Anguil from Instituto Nacional de Tecnología Agropecuaria (La Pampa, Argentina). The Pampinta sheep breed is an Argentinean typical breed, developed by backcrossing local Corriedale ewes with imported East Friesian (Ostfriesisches Milchshaf) milk-type rams (Medrano, 1975), and possesses genetic traits of high milk (Suarez et al., 1996), meat and wool production.

Eight batches (of 80 l) of milk were collected from the bulk milk of the Pampinta ewe breed throughout the lactation from September (spring) to May (autumn). These milks were analyzed for moisture (International Dairy Federation, 1987), fat (International Dairy Federation, 1981), and protein (International Dairy Federation, 1993), and were used to make cheeses using two different procedures.

Overall, 76 animals were used to collect bulk milk. In addition, samples of 100 ml of milk were collected from each individual animal at the beginning (30 days), middle (108 days) and the end (213 days) of lactation. Cheese yield was evaluated for both bulk and individual milk samples.

2.2. Cheesemaking

Taking into account that, in Argentina, a very small production scale is very common, cheeses were manufactured from each stage of lactation, using 80 l of milk. Milk was batch-pasteurized at 65 °C for 20 min, cooled to 37 °C and treated with CaCl_2 to a final concentration of 0.014% (w/v). Milk was then divided into two portions of 40 l each for the manufacture of two types of semi-hard cheeses.

One cheese (S) was made using a direct vat starter (DVS) of *Streptococcus thermophilus* (Chr. Hansen™, Argentina) in a dose high enough to achieve 10^6 CFU/ml in cheese milk. MAXIREN™ 150, 100% chymosin, rennet strength 150,000 IMCU/ml (Gist-brocades, France) was added at a final concentration of 0.014 g/l for milk coagulation. When the curd formation was complete, it was cut to the size of a corn grain using a cheese knife. A curd washing step was carried out by replacement of 10% of the whey with hot water (60 °C), and the mix was cooked at 43 °C.

The second type of cheese (L) was manufactured using a mix of DVS cultures of *Streptococcus thermophilus* (60%), *Lactobacillus helveticus* (20%) and *L. bulgaricus* (20%) (Chr. Hansen™, Argentina), in a dose sufficient to reach a total starter bacterial count of 10^6 CFU/ml in cheese milk, and cooked at higher temperature (47 °C) without curd washing, with other parameters under the same conditions as for S cheeses manufacture.

Cheeses of approximately 0.7 kg were obtained, pressed for 18 h (0.2–0.3 kg/cm²) and brined at 12 °C for 8 h (20% w/v, pH 5.4). Actual yields of the cheeses were expressed as kg of cheese (weighed just before salting) per 100 kg of sheep milk. Cheese moisture was determined in all samples after three days of manufacture (International Dairy Federation, 1982). Ripening was carried out at 12 °C and 80% relative humidity for 180 days.

The specific manufacture parameters were specifically selected for each cheese variety, in order to obtain two semi-hard cheeses with slightly different moisture contents. One variety (S) was expected to have higher moisture, soft taste and shorter time of ripening (40–45 days), and the other one (L) lesser moisture and an optimal period of ripening considerably longer (90–180 days), during which taste and flavour would better develop. This scheme would allow the marketing of sheep cheeses at any time of the year, from a manufacturing season which runs for six months, similar to that found in Spain for Idiazabal cheese production (Mendia, Ibañez, Torre, & Barcina, 2000).

2.3. Yield prediction assay (YPA)

2.3.1. General

A fast methodology, developed by Othmane et al. (2002) to predict cheese yield from a small volume of milk, was modified and successfully applied to study milks from a regional ewes' herd throughout lactation. As with that of Othmane et al. (2002), our method involves coagulation of milk at 37 °C in small tubes, cutting of the curd, separation of whey by centrifugation of the tubes for 15 min, further whey drainage for 45 min and weighing of the residues of centrifugation. The modifications introduced to the original scheme included the volume of milk samples, the tubes capacity and the pattern of curd cutting.

Fig. 1 shows five different experimental schemes tested in order to select the best combination of coagulant dose and method of curd cutting. All of the schemes were simultaneously applied to several bulk milk samples in six replicates in preliminary experiments. Media and standard deviations (data not shown) were contrasted among schemes, and the one with the lowest coefficient of variation was finally selected (bold-marked in Fig. 1).

2.3.2. Rennet preparation

MAXIREN 150 (100% chymosin, rennet strength 150,000 IMCU/ml) was used. To avoid activity losses, commercial rennet powder was diluted 100-fold in double-distilled water just before addition to milk. The standard dose of coagulant was of 0.014 g/l and was the same as that utilized for cheesemaking.

2.3.3. Coagulation

Twenty-five millilitres of milk sample were exactly weighed in previously weighed plastic tubes with conical base (longitude 85 mm, diameter 22 mm, volume 30 cm³), and warmed to 37 °C. According to the coagulant dose

and cutting scheme selected, 35 µl of the 100-fold diluted rennet were added to milk and mixed by rapidly inverting the tubes several times immediately after rennet addition. Coagulation was performed at 37 °C in a water bath for 90 min. The gel obtained was vertically cut all along the tube length, during this period, by using a knife adapted to the tubes diameter. Three successive cutting steps were made at 30, 60 and 90 min after rennet addition. The first was by two parallel cuts and one perpendicular cut, while the others were made in the form of a cross. Ten minutes after the third cut, the tubes were centrifuged at 1000g for 15 min, and the whey was drained. The tubes were opened and positioned, facing downwards, for 45 min to further drain the whey. The weights of the residues remaining at the bottom of the tubes were divided by the mass of milk utilized to estimate the cheese yields. These yields were compared with those obtained in cheeses manufactured with 80 l of milk, which were also determined as % (w/w).

2.3.4. Moisture determination

Total solids and moisture were determined during the YPA, immediately after centrifugation and weighing, respectively, using the same method as applied to determine cheese moisture (International Dairy Federation, 1982).

2.4. Influence of milk acidification and low-temperature milk storage on cheese yield

The effect of the acidification on the cheese yield was studied using bulk milk which was chemically acidified by incubation with 0.1 and 0.2% of gluconic acid δ -lactone at 37 °C for 75 min. After incubation, samples were evaluated for the YPA.

The influence of low-temperature milk storage on cheese yield was estimated from three bulk milk samples corresponding to different stages of lactation. Experiments were performed by dividing each sample into five portions which were separately evaluated by the YPA. Fresh milk was used as a control and another portion after preservation at 4 °C for 24 h. The remaining three portions were stored at –18 °C during 7, 30 and 60 days, respectively, prior to their evaluation.

2.5. Data analysis

Bulk milk samples were assayed in six replicates. Cheese yields, estimated in experiments, using either chemically acidified or frozen milks by the YPA, were analyzed with the one-way ANOVA procedure of Statgraphics™ Plus software (v 3.0, Statistical Graphics Corp.). Individual milk samples in duplicates were analyzed.

Cheese yields estimated from bulk milks by the YPA were corrected with the use of a formula developed by Maubois and Mocquot (1967), which calculates cheese yield variation caused by cheese moisture variation, as follows:

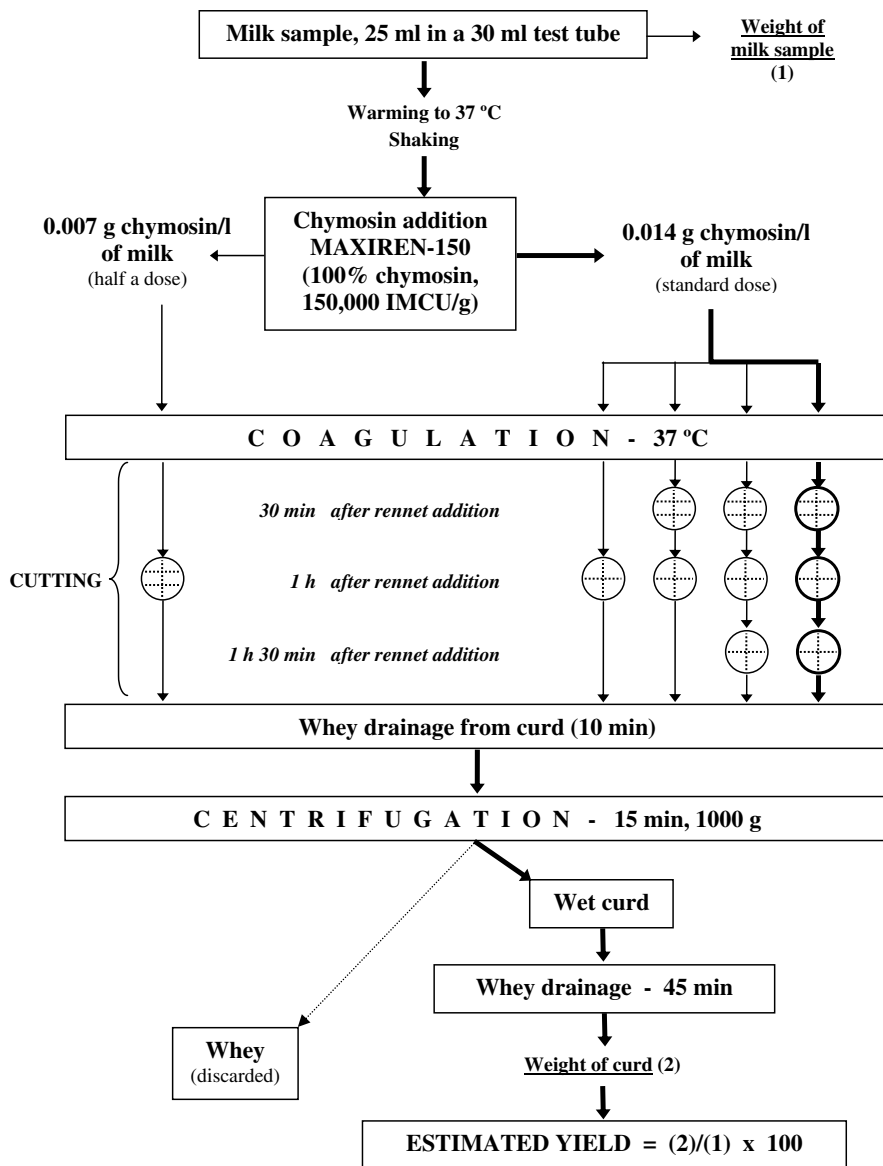


Fig. 1. Flow chart indicating different alternatives tested in order to determine the best conditions for the yield prediction assay (YPA). The alternative chosen and subsequently used in this study is shown in bold characters. Adapted from Othmane et al. (2002). ⊕ and ⊗: cutting directions shown in a cross section of the test tube.

$$\eta_t = \eta_{ch}[(M_{ch} - TS_{sypa}) / (M_{rypa} - TS_{sypa})],$$

where η_t is the transformed yield (%), η_{ch} is the predicted yield (%), M_{ch} is the cheese moisture (%), M_{rypa} is the moisture of centrifugation residues of YPA (%) and TS_{sypa} is the total solids of centrifugation supernatants of YPA (%).

Both original and transformed yields were correlated with actual cheese yields through simple linear regression.

3. Results and discussion

3.1. Comparison between yields from YPA and those from actual pilot plant cheesemaking

Table 1 shows the composition of the bulk milk samples and the yields obtained for S and L cheesemaking, at each

stage of lactation studied. The amount of cheese obtained per vat was highly variable, depending mainly on the total solids content of the milk, which changed noticeably throughout the lactation period.

Yields predicted according to YPA were perceptibly higher than those obtained from actual cheesemaking. This is not surprising taking into account the obvious differences between both processes, especially in whey draining. This step in actual cheesemaking takes one day, which is facilitated by the acidification of starter microflora, cooking and agitation. On the other hand, in the YPA this process was quick and forced by centrifugation. However, yields predicted with this assay, even though higher, showed a good linear correlation with respect to the actual yields (Fig. 2). Values of R^2 obtained from linear regression were 0.928 and 0.957 for S and L cheeses, respectively.

Table 1
Main composition of milk, S and L yields from actual pilot plant cheesemaking and moisture during the lactation period

	Days of lactation							
	30	60	86	108	185	213	241	276
Total solids (%)	16.0 ± 0.4	16.9 ± 0.3	17.3 ± 0.6	17.7 ± 0.5	19.4 ± 0.5	21.1 ± 0.4	19.9 ± 0.3	25.1 ± 0.8
Fat (%)	5.45 ± 0.21	6.30 ± 0.16	6.30 ± 0.29	6.85 ± 0.23	8.20 ± 0.35	9.20 ± 0.31	7.80 ± 0.28	11.20 ± 0.44
Total protein (%)	5.47 ± 0.21	5.56 ± 0.23	5.28 ± 0.36	5.13 ± 0.24	6.02 ± 0.06	6.62 ± 0.51	6.68 ± 0.31	8.39 ± 0.26
Casein (%)	4.00 ± 0.16	4.14 ± 0.31	3.76 ± 0.01	3.80 ± 0.12	4.60 ± 0.11	4.84 ± 0.51	5.09 ± 0.42	6.73 ± 0.15
SN-4.6 (%)	1.47 ± 0.12	1.42 ± 0.11	1.52 ± 0.07	1.33 ± 0.19	1.42 ± 0.15	1.78 ± 0.10	1.59 ± 0.16	1.66 ± 0.07
L cheese yield (% w/w)	16.3 ± 0.7	16.7 ± 0.5	17.8 ± 0.5	18.7 ± 0.8	21.4 ± 0.2	23.6 ± 0.6	21.9 ± 0.7	28.2 ± 0.9
S cheese yield (% w/w)	19.7 ± 0.5	19.6 ± 0.4	17.8 ± 0.8	19.0 ± 0.5	21.9 ± 0.1	25.8 ± 0.4	23.5 ± 0.6	29.2 ± 0.4
L cheese moisture (%)	44.5 ± 1.1	43.1 ± 1.9	41.3 ± 1.1	39.8 ± 0.6	40.5 ± 0.8	45.0 ± 2.0	42.4 ± 0.7	45.0 ± 1.0
S cheese moisture (%)	44.5 ± 0.7	44.9 ± 0.8	42.2 ± 1.3	42.7 ± 1.1	41.3 ± 1.6	44.3 ± 0.9	43.5 ± 0.8	42.5 ± 0.8

Means of three replicates. SN-4.6: Soluble nitrogen at pH 4.6 expressed as % of protein.

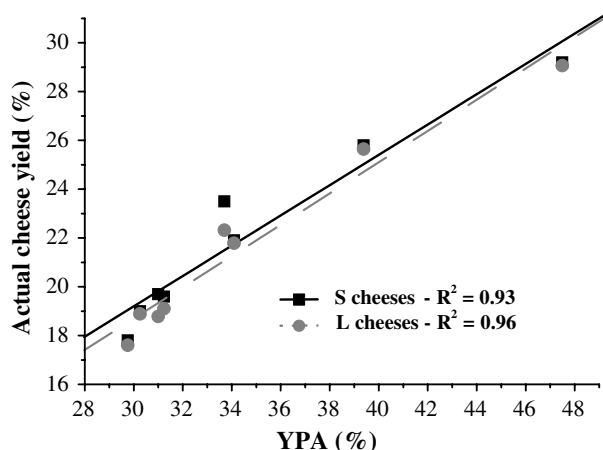


Fig. 2. Actual yields obtained in S and L pilot scale cheesemaking vs. yields estimated from 25 ml of the respective milks by the yield prediction assay (YPA).

Values of estimated yield according to YPA were transformed using the formula of Maubois and Mocquot (1967). Resultant yield values were almost equal to those from actual pilot plant cheesemaking. Therefore, the slopes obtained for Fig. 2 changed to values close to one, while the linear correlation was not negatively affected. Consequently, the proportionality between the actual and YPA yields would change depending on the cheese moisture. Nevertheless, our method *per se* would be useful to predict yields when manufacture protocols for semi-hard cheeses are used and the moisture is within the ranges established for these cheeses (approximately 38–45%).

The Van Slyke formula was specifically established to predict yield for hard cheese (Van Slyke & Price, 1949), such as Cheddar, and casein content of the milk has to be known. Zeng et al. (2007) developed and successfully applied predictive formulae for calculating the yield of soft cheeses from goat milk, but coefficients of predictive formulae were relatively low and need further validation for semi-hard and hard cheeses. On the other hand, the YPA utilized in this work has proved to be a useful technique for predicting yield of semi-hard sheep cheeses, without the need of determining total solids, fat, total protein or casein contents of milk.

3.2. Influence of acidification of milk on cheese yield

Table 2 shows the pH and yields estimated by the YPA of milk acidified with gluconic acid δ -lactone. Milk used was from the middle of lactation (108 days). As shown in Table 2, acidification significantly reduced cheese yield. Treatment with gluconic acid δ -lactone 0.1% reduced the pH of milk by 0.2, and yield by 14%. Double doses of gluconic acid δ -lactone lowered pH of the milk by 0.4, and subsequently decreased cheese yield, which was comparatively lower than the yield when 0.1% δ -lactone was used.

3.3. Effect of low-temperature milk storage on cheese yield

As a consequence of the high variation in the solid content and predicted yields among milks from the middle to the end of the lactation period, the three experiments were considered as independent assays. Given that we found a negative effect of milk acidification on cheese yield, it is important to observe that pH of milks remained constant throughout the low-temperature storage, and then it could not be considered a variable influencing the yield. As shown in Table 3, while values corresponding to fresh, refrigerated (4 °C) and frozen (−18 °C) milks were statistically different within the same experiment, a clear influence of the temperature and storage time was not evident. As a result, we concluded that the observed differences can be

Table 2
Effect of milk acidification on the yield estimated by the yield prediction assay (YPA)

	Milk treatment		
	Without acidification	0.1% GA δ -lactone ^d	0.2% GA δ -lactone ^d
pH	6.55 ± 0.04 ^a	6.31 ± 0.02 ^b	6.17 ± 0.04 ^c
Estimated yield (% w/w)	29.57 ± 0.66 ^a	25.45 ± 0.33 ^b	23.78 ± 0.34 ^c

Milk of 108 days of lactation. Means of 6 replicates. GA δ -lactone: gluconic acid δ -lactone.

^{a-c}Values in the same row with different superscripts differ ($P < 0.05$).

^d Treatment with GA δ -lactone was carried out at 37 °C for 75 min.

Table 3
Effect of low-temperature milk storage on yield estimated by the yield prediction assay (YPA)

Days of lactation	Storage treatment				
	Fresh milk	4 °C for 24 h	−18 °C for 7 d	−18 °C for 30 d	−18 °C for 60 d
185	34.1 ± 0.4 ^b	33.5 ± 0.5 ^{b,c}	32.9 ± 0.9 ^c	33.8 ± 0.5 ^b	35.6 ± 0.2 ^a
213	39.4 ± 0.9 ^a	39.1 ± 0.7 ^a	37.1 ± 0.7 ^b	37.7 ± 0.7 ^b	n.d.
241	33.7 ± 0.3 ^a	n.d.	33.0 ± 0.4 ^b	33.3 ± 0.2 ^b	n.d.

Rows correspond to three different experiments using milk from different stages of lactation. Means of six replicates.

^{a-c}Values in the same row with different superscripts differ ($P < 0.05$). n.d.: not determined.

better explained by the experimental error of the method than by the influence of the studied variables.

Data on the effects of freezing of sheep milk on cheese yield are scarce. However, results from YPA were in agreement with those from Zhang, Mustafa, Ng-Kwai-Hang, & Zhao (2006), who found that cheese yield was not affected by freezing of sheep milk, either at −15 °C or −25 °C, for up to 2 months.

3.4. Variation in cheese yield among milks from different animals throughout the lactation

It is noteworthy that, for this experiment, a proportion ranging from 15% to 20% of the milk samples from individual ewes of the herd, which had pH values rather higher than the rest, could not be analyzed, because milk did not coagulate at the moment of the first curd cut (30 min). That might have been caused by sub-clinical mastitis, although this was not confirmed. However, the results were analyzed without including those individual samples.

Fig. 3 shows a markedly high dispersion among yields, estimated by the YPA, for milks from individual ewes of the herd, with coefficients of variation of 15.1%, 10.6% and 12.6% at 30, 108 and 213 days of lactation, respectively. According to its smaller variability, milk of mid-lactation appeared to be more stabilized than the beginning or the end of lactation milks in terms of cheese yield.

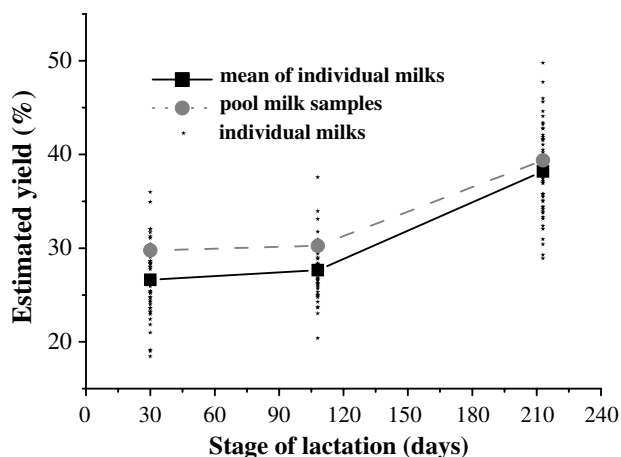


Fig. 3. Cheese yields estimated by the yield prediction assay (YPA) for milks from individual ewes of the herd at three stages (30, 108 and 213 days) of lactation.

With respect to the yield average, there were increases throughout lactation, the mean value for late lactation (38.2%) being significantly higher ($P < 0.01$) than those for the early or mid lactation (26.6% and 27.7%, respectively). In every case, these values were slightly lower (less than one standard deviation) than those estimated for bulk milk. One possible reason for these small differences could be the contribution of the individual milk samples which were not considered in this analysis.

4. Conclusions

Cheese yields, estimated from sheep milk throughout an entire lactation by the yield prediction assay, showed a good linear correlation with those obtained in actual cheesemaking, for two cheese varieties. Yields determined by the yield prediction assay were lower than that for actual cheesemaking, but a further transformation with a formula that takes into account cheese moisture made them almost equal. Yields increased with lactation as the total solids content of milk did, particularly toward the end. Acidification of milk had a strong negative effect on cheese yield. However, refrigeration or frozen storage of milk for up to 60 days did not appreciably affect yield. These observations are of great importance for sheep cheese producers who process small volumes of milk and frequently collect and store milk from several days or even weeks before making cheese. The economic significance of cheese yield is especially great for sheep cheeses, and the present study showed that yield may not appreciably change by cold storage. Finally, our method revealed great differences among yields predicted from individual ewe's milks. Even though high, the relative lower variability in yield for mid lactation would indicate less variability of milk properties among animals during this period. Since that variation originates mainly from the genetic ewe-to-ewe divergences, a further study of the genetic variability among the ewes utilized in this work would complement our results and permit improved selections and crosses of ewes, on the basis of their milk coagulation properties.

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