Milk Composition and Cheesemaking ability in Ewes: Influence of Major Milk Components and pH on Individual Cheese Yield at a Laboratory Scale in Ewes

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ABSTRACT

The relationships between the individual laboratory cheese yield and the composition of milk from four flocks of ewes in the north-west of Tunisia have been studied. A total of 3260 individual milk samples were taken and their physicochemical composition (fat, protein, useful matter, total solids and pH) and cheese yield at a laboratory scale were determined. Statistical analysis of field data confirmed that there were significant variations in cheese yield capacity with all environmental variation factors, the most notable being flock test date, days in milk and parity. The individual cheese yield was significantly influenced by initial pH of milk. Useful matter, (a combination of fat and protein contents) and total solids were the most relevant chemical variables related to variations in the laboratory cheese yield of the individual milks. Finally, the regression equations confirmed that cheese yield capacity was also subject to factors other than milk composition itself.

Keywords:

dairy ewes; individual laboratory

cheese yield; milk composition; pH

INTRODUCTION

Ewes' milk as a product high in fat and protein is mainly used in commercial or handmade quality cheeses and yogurts. Cheese yield has been studied for many years now, for obvious reasons, even though these studies are more frequent in dairy cattle than in dairy ewes and in small ruminants in general. Protein content of milk has received much attention from producers of milk and processors of dairy products. Increased recognition of the protein value has led processors to pay a premium for milk exceeding minimum protein percentages. To take advantage of such pricing schemes, producers have considered protein performance for culling decisions and breeding plans. Despite the attention and interest, less is known about protein than about fat in milk. The protein fractions do not contribute in the same way towards milk into cheese transformation. In fact, most of the casein, which represents around 80% of the protein contents, is incorporated in the cheese and the non-casein proteins are lost in the whey fraction (Othmane, 2000). The cheese-maker is then more interested in having high amounts of protein in the milk in order to maximise profits.

The use of rheological parameters of milk and cheese yield formulae has also mostly been studied with the aim of increasing cheese yield. For ewes' milk, some authors (Duranti and Casoli, 1991; Manfredini et al., 1992; Hurtaud et al., 1993; Delacroix-Buchet et al., 1994; Martini et al., 2004; Alvarenga et al., 2008) have studied the correlations among some physicochemical variables and renneting properties of milk such as clotting time, gel firming rate and gel strength. It was concluded that an increase in fat and protein contents in ovine milk, which contains more fat, protein and minerals than bovine and caprine milk, might result in reduced clotting time and increased curd firmness. There is, however, some variation between reports as to the incidence of renneting properties on cheese yield which had not been always checked in experimental manufacture, at least in dairy cattle (Banks and Muir, 1984; Remeuf et al., 1991; Colin et al., 1992).

Cheese is, of course, a product in which the protein and fat of the milk are concentrated, so it is clear that cheese yield is indeed related to casein and fat contents. However, cheese yield formulae are usually derived from regression equations established from practical experience carried out with bulk milk and no studies have been carried out on individual cheese yield. While such formulae are highly useful in practice (Van Boekel, 1993), it may be helpful to understand what is going on when cheese yield is directly estimated from small individual milk samples as collected within the milk recording scheme for dairy ewes.

The aim of this study was to investigate the relationship between the main physicochemical characteristics of the milk (fat, protein, useful matter and total solids contents, and pH) and its cheese ability, and to evaluate the effectiveness of these traits in improving individual cheese yield, which can be obtained at sheep

milk recording. Explaining the individual cheese yield variation from variation of the milk components allowed us to investigate the contribution of milk traits to have a great potential for high cheese yield, so that selection criteria can be established more accurately.

MATERIALS AND METHODS

Data collection

Four dairy flocks from the north-west of Tunisia, adhering to milk recording scheme, were included in this study conducted between April 2006 and July 2010. The individual milk samples were obtained at approximately monthly intervals following an alternative a.m.-p.m. recording scheme. All recorded flocks in the entire population are on the standard A4 plan of testing, and all ewes are milked twice daily. A total of 3260 data from 370 ewes were considered. A maximum of four monthly milk tests was carried out after weaning of lambs. The mean number of test days per lactation was 2.5 and each ewe averaged 3.6 lactations. Samples were prepared within a few hours for cheese-making in the laboratory and within two days for physicochemical analyses.

Milk analysis

Upon arrival at the laboratory, samples were analyzed after they were warmed to 40 °C and stirred. Contents of fat (F), protein (P) and total solids (TS) were determined by the mid infrared method using a Milko Scan 4000 (Integrated Milk Testing, Foss Electric). The instrument was calibrated against known sample standards according to the norm of the International Dairy Federation (IDF, 1964, 1996). The useful matter content (UM) was then defined as a combination of fat and protein contents, with weightings of 1 and 1.85, respectively (Barillet and Boichard, 1987): $UM = (F + 1.85 P)/2$.

Milk pH was measured at 20 °C with an Inglood electrode (Inglood Lot 405) just after arrival of milk samples at the laboratory. Measurements were also included in the statistical analysis of individual cheese yield, since pH value has a strong influence on cheese yield (Othmane et al., 1999) and renneting properties of milk (Pellegrini et al., 1997).

Cheese yield measurement

Test day ILCY (individual laboratory cheese yield), expressed in kg per 100 L of milk, was measured from individual milk samples (10 ml), compatible with milk recording in dairy sheep. ILCY was measured as described by Othmane (2000): preheated and homogenised milk samples were curdled at 37 °C for 1 h. In order to facilitate rennet distribution in the milk, the rennet was diluted 10-fold with bidistilled water before addition to the milk. ILCY was thus measured, the coagulum (cottage cheese + whey) being centrifuged for

a standard time of 15 min at 2 500 rpm after longitudinal cutting and the whey being removed after draining for 45 min with the test-tube face downward (the curd remained quiescent at the bottom of the tube). ILCY was defined as the weight of the centrifuge residue (curd obtained after expulsion of whey and draining in the open air) expressed in kg per 100 L of milk.

Statistical analysis

Data were gathered according to the different levels of the main environmental variables that were thought to affect milk yield and composition, as they have been using in the national dairy breed. The data were analyzed using a least squares method in a model (model ①) that included flock test date (112 levels), parity (6 levels), stage of lactation in months (5 levels) and birth type (2 levels) as fixed effects, and a random residual effect. Dependent variables were fat, protein, useful matter and total solids contents, milk yield and ILCY.

Relationships between physicochemical characteristics and cheese ability of milk were approached in three ways:

(1) Using the above-mentioned model, we calculated the phenotypic correlations between milk yield and composition traits and ILCY.

(2) The relative importance of pH and other chemical effects accounting for the variation of ILCY was estimated by comparing the values of the determination coefficient

(R^2) and the standard deviation of the residual error (SDE) (Pellegrini et al., 1997) for two other models including the following factors:

- Flock test date, parity, stage of lactation, birth type, and pH (model ②);
- Flock test date, parity, stage of lactation, birth type, pH and a chemical factor X as a covariate (model ③). The choice of chemical variables was in accordance with their phenotypic correlations with ILCY.

(3) A multiple regression analysis allowed us to establish the most relevant regression equations, which also brought additional information on the relationship milk composition-cheese ability to light.

The statistical analyses were based on least square techniques using GLM, VARCOMP and REG procedures by the Statistic Analysis System program SAS (1992).

RESULTS

Gross composition of milk

The average composition of bulk individual milk and laboratory cheese yield are summarised in table 1.

Table 1: Means and standard deviations of the test day physicochemical components and individual laboratory cheese yield (n = 3260)

Trait	Mean	Minimum	Maximum	Standard deviation
Milk yield (ml/d)	568	200	1488	181
Fat content (g/l)	68.5	20.4	145.7	19.5
Protein content (g/l)	59.3	30.4	93	8.0
Useful matter (g/l)	89.1	45.2	137.14	15.1
Total solids (g/l)	180.7	114.6	271.3	22.7
ILCY (kg/100 l)	33.87	10.17	49.95	6.1
PH	6.52	5.5	7.54	0.2

The mean values obtained for test day data for fat, protein and total solids were consistent with those previously reported for dairy ewes (Fuentes et al., 1998; Pellegrini et al., 1997; Othmane et al., 2002). As might be expected for milk rich in their main components, the fat, protein, useful matter and total solids contents of the ovine milk were substantially higher than those associated with bovine milk and tended to increase progressively throughout lactation (Table 3).

Cheese ability and effect of variation factors

The mean value for ILCY obtained from this study (33.87 kg/100 l) was within the range of those reported for real cheese yield in dairy ewes (Anifantakis and Kaminarides, 1983), although we are dealing with a different manufacturing process. Its behaviour was logically influenced by variation of the main components of individual milk as raw material.

Table 2 shows the statistical significance of the effects taken into account in model ① and table 3 shows the values of least-square means for each significant

effect. The individual laboratory cheese yield was significantly ($P < 0.01$) affected by FTD, lactation stage, parity and birth type. As expected, the FTD factor became the major variation factor for all variables. It accounted for 42% of the phenotypic variance in ILCY, indicating the importance of some effects specific to the day of test within each flock for the cheese - making process, as well as variation in milk composition. The ILCY was also highly influenced by days in milk. The lowest value was

obtained immediately after weaning (31.7 kg/100 L), which coincided with the maximum milk yield. It then increased up to the end of the normal lactation period, and this was mirrored by a considerable increase in milk composition (Table 3). The change in milk composition according to the stage of lactation was consistent with other studies carried out on individual ovine milk (Delacroix-Buchet et al., 1994; Fuertes et al., 1998; Atti et al., 2011).

Table 2: Analysis of variance: determination coefficient (R^2) and statistical significance of the fixed effects for physicochemical components and individual laboratory cheese yield measured on individual ewes' milk

Trait	R^2	Probabilities associated with effects			
		FTD	Parity	Birth type	Stage of lactation
Milk yield (ml/d)	50.4	***	***	***	***
Fat content (g/l)	49.6	***	***	***	***
Protein content (g/l)	47.8	***	***	NS	***
Useful matter (g/l)	54.4	***	***	*	***
Total solids (g/l)	51.1	***	***	***	***
PH	59.5	***	NS	NS	NS
ILCY (kg/100 l)	46.3	***	***	**	***

***: $P < 0.001$ **: $P < 0.01$ *: $P < 0.05$ NS: $P > 0.05$

FTD: flock test date

ILCY: individual laboratory cheese yield

Table 3: Least-square means for variables studied that vary significantly with factors in statistical model 1

	Milk yield ml/d	Fat content g/l	Protein content g/l	Useful matter g/l	Total solids g/l	ILCY ¹ kg/100 l
Days in milk						
1	910 ^a	59.4 ^d	54.8 ^d	80.4 ^d	169.2 ^d	31.7 ^c
2	642 ^b	69.7 ^c	59.7 ^c	90.0 ^c	181.8 ^c	33.2 ^b
3	455 ^c	77.4 ^b	63.0 ^b	97.0 ^b	190.8 ^b	34.7 ^a
4	348 ^d	84.1 ^a	65.8 ^a	102.9 ^a	199.3 ^a	35.0 ^a
5	355 ^d	82.5 ^{ab}	64.9 ^{ab}	101.3 ^a	198.7 ^a	34.5 ^a
Parity						
1	486 ^b	70.4 ^c	59.7 ^b	90.4 ^d	181.2 ^d	32.7 ^c
2	507 ^b	73.8 ^b	61.9 ^a	94.2 ^c	187.1 ^c	34.1 ^{ab}
3	538 ^b	75.7 ^b	62.2 ^a	95.4 ^b	189.5 ^b	34.2 ^a
4	638 ^a	74.9 ^b	61.8 ^a	94.6 ^{bc}	188.5 ^{bc}	33.7 ^b
5	592 ^a	75.0 ^b	61.8 ^a	94.6 ^{bc}	188.7 ^{bc}	33.8 ^b
> 5	492 ^b	77.9 ^a	62.4 ^a	96.7 ^a	192.8 ^a	34.3 ^a
Birth type						
Single	495 ^b	75.8 ^a	NS	94.9 ^a	189.2 ^a	34.1 ^a
Multiple	589 ^a	73.5 ^b	NS	93.7 ^b	186.7 ^b	33.6 ^b

^{a,b,c,d} Values with a different superscript letter in the same column were significantly different: $P < 0.05$.

¹ Individual laboratory cheese yield.

The ILCY was significantly ($P < 0.001$) influenced by parity but did not have a clear pattern. It rose with increasing parity for low ranking lactations (<4), especially between the first and second lactations. This was followed by a slight decrease in cheese yield capacity for the fourth and fifth lactations, although the highest cheese yield was obtained with the oldest ewes. The increase in ILCY was quantified by 5% when we compared ewes in first lactation and later parities (Table 3). Lambing type had a significant effect on milk yield, fat content and ILCY and a non significant effect on protein content and pH. This result agrees with others on Churra (Fuentes et al., 1998; El-Saied et al., 1999; Othmane et al., 2002) and Latxa (Gabiña et al., 1993) ewes for fat and protein contents. Ewes that gave birth to multiple lambs had a higher milk yield (589 ml/d) than ewes that gave birth to singletons (495 ml/d). The physiological explanation of this fact is that a high number of lambs correspond to a greater placenta area which results in higher mammary hormone level and a more developed udder (Delouis, 1981). However, milk from ewes with single lamb had higher cheese potential than that from ewes with multiple lambs. Differences between both categories reached 1.5% in favour of single birth ewes. This was due to milk richness in ewes that only had one lamb (Table 3).

Relationships between cheese ability and milk composition traits

Also fundamental to dairy selection programmes are the supposed relationships between the main milk components and the ILCY estimated from milk amounts compatible with that usually collected in the milk recording scheme in dairy ewes. We are therefore interested in finding out these relationships, especially since literature on this topic is very scarce. First, these relationships were investigated by computing the phenotypic correlations from the values of least square-means, after adjustment for the effects included in model ①.

Our estimates indicated that the ILCY was positively correlated to all chemical variables considered and correlations ranged between 0.40 and 0.58 (Table 4). The highest correlations were with fat content (0.53) and mainly with its combinations with protein content (0.56 for useful matter and 0.58 for total solids). The correlation of protein content with ILCY was 0.40. However, ILCY was low and inversely related to milk yield (-0.12) and milk pH (-0.14). So, as expected, less abundant and richer milk gave a higher laboratory cheese yield.

Table 4: Phenotypic correlations between variables studied

	Milk	Fat	Protein	Useful matter	Total Solids	ILCY ¹
Fat	-0.21 ***					
Protein	-0.26 ***	0.40 ***				
Useful matter	-0.26 ***	0.95 ***	0.66 ***			
Total Solids	-0.25 ***	0.94 ***	0.63 ***	0.98 ***		
ILCY ¹	-0.12 ***	0.53 ***	0.40 ***	0.56 ***	0.58 ***	
PH	-0.08 ***	0.01 NS	0.04 NS	0.02 NS	0.00 NS	-0.14 ***

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$ NS $P > 0.05$

¹ Individual laboratory cheese yield.

Analyses of variance including pH and the most relevant composition traits were then performed in order to

quantify the influence of the main chemical characteristics of milk on its cheese ability, independently of pH. This technique was inspired by that used by Pellegrini et al. (1997) to study relationships between physicochemical

composition and renneting properties of individual milk samples. Table 5 shows the values of R^2 and SDE for the different models relating to ILCY.

Table 5: Analyses of variance for individual laboratory cheese yield according to different models including the most relevant chemical variables

Model	Determination coefficient (R^2)	Standard deviation of residual error (SDE)
Model ①	46.3	4.23
Model ②	52.0	3.11
Model ③, X = fat content	65.5	2.63
Model ③, X = protein content	59.8	2.84
Model ③, X = useful matter	67.5	2.56
Model ③, X = total solids	68.5	2.52
Model ③, X = milk yield	52.8	3.08
Model ① $Y = \mu + F + e$; F: fixed effects mentioned in paragraph 4.2		
Model ② $Y = \mu + F + pH + e$		
Model ③ $Y = \mu + F + pH + b(X - \bar{X}) + e$		

The only factors having no part in milk characteristics (model ①) accounted for 46.3% of the variation in ILCY. The introduction of pH in the model as a covariable (model ②) increased the R^2 value by 12% and decreased the error by 26%, indicating the importance of pH for milk into cheese transformation. Similar results with milk coagulating properties were found by Manfredini et al. (1992) and Pellegrini et al. (1997) who reported that pH played an important role in explaining the variations of rennet clotting time and gel firming rate. On the other hand, when the most relevant milk components such as fat, protein, useful matter or total solids were also taken into account one by one, the model's efficiency was noticeably enhanced (model ③ versus models ① and ②). Independently of pH, the most determinant chemical variables undoubtedly remained the useful matter and

total solids contents. Their inclusion in the model of analysis increased the R^2 value by 30% and 32% (vs. Model ②), respectively. The associated errors decreased considerably in a similar way.

The analysis by multiple regressions enabled us to appreciate better the respective weights of the different variables on cheese yield capacity of the individual milk samples (Table 6). Equation [1] confirmed that fat and protein contents had an important influence on the individual laboratory cheese yield. Such a result was in concordance with those of Othmane (2000) on the churra dairy breed. In this way, the same author specifies also that the casein content does not enhance the individual cheese yield prediction in relation to that from protein content, since the casein explain, by itself, the essential of whole protein variations ($R^2 = 0.98$).

Table 6: Regression equations of individual laboratory cheese yield (ILCY) performed from the most relevant chemical variables

Equation number	Equation	R^2
1	$ILCY = 1.455 pH + 1.048 F + 0.659 P + 5.468$	0.33
2	$ILCY = 1.598 pH + 1.049 TS - 3.322$	0.34
3	$ILCY = 1.463 pH + 1.560 MU + 2.470$	0.31

R^2 : regression coefficient; TS: total solids; UM: useful matter; F: fat content; P: protein content.

Equations [2] and [3] indicated that useful matter (fat + 1.85 protein) gave a better explanation of the variations of

ILCY than total solids. This result could be explained by the fact that the total solids contain, apart from fat and

protein which go on to form part of the curd, other components which are directly transferred to the whey fraction such as lactose and serum protein contents. Furthermore, the regression equations of ILCY highlight the prominent influence of initial pH of milk on its cheese ability.

DISCUSSION

Our results were based on a relatively high field data set, so large sampling errors should not be expected. Moreover, at a given time all samples were analysed under the same controlled experimental conditions so that any possible bias arising from sample manipulation was reduced. Such advantages may strengthen our interpretations. Even though the mean value encountered for the individual laboratory cheese yield, like that of cottage cheese, falls within the range of those encountered in literature (Anifantakis and Kaminarides, 1983; Pirisi *et al.*, 1999; Othmane *et al.*, 2003), it tends to be slightly higher than industrial cheese yield. We think that this tendency is due to the very reduced milk amounts used and the forced draining of the cottage cheese. Draining seems more suitable during real manufacture; it lasts about 12 hours, depending on the type of cheese, whilst whey removal in the laboratory is accelerated by centrifugation and this can have a negative influence on adequate whey evacuation.

The negative correlation between ILCY and pH value indicated that, seemingly, more cheese yield is to be expected when milk pH is lower. This is true to a certain extent that probably corresponds to the range of pH from 6 to 6.7, as the most suitable range (maximum activity zone of proteases) for the cheese-making process (Delacroix-Buchet *et al.*, 1994; Ramet and Weber, 1980; Storry *et al.*, 1983). We must be careful here as these correlations do not usually indicate linear relationships. In fact, we checked, even in previous studies on the same breed, that the majority of milk samples that have not been coagulated one hour after renneting had a pH value over 7. Similar observations have also been reported by Delacroix-Buchet *et al.* (1994) for milk from Lacaune ewes. It is important to bear in mind that in the present study, from a technological point of view, milk transformation was carried out without any previous manipulation (pH adjustment, pasteurized and standardized milk, culture addition, etc.), which is usual in

the dairy industry.

With regard to the relationships among ILCY and the main milk components, the three focusing analyses were very consistent. They pointed out that useful matter and total solids are the most relevant for cheese yield prediction. This result is of great interest for the scientist and especially for the cheese-maker who tries to effectively monitor cheese yields and equably pay milk based on its cheese yield capacity.

The low R^2 (31 to 34%) associated to equations [1] to [3] and the strong influence of pH indicated the existence of other unidentified and/or non-identifiable factors, such as rennet strength, atmospheric conditions, manipulator effect etc., which control the efficacy of the transformation of milk into cheese. Consideration of such factors non-inherent in milk is particularly interesting when an accurate study of the effect of the milk composition itself on cheese-making process is required. Finally, it can be pointed out that in spite of its clear advantages, the ILCY estimation method also has its limitations and it is important to put it within its context (laboratory conditions, rennet strength, very reduced amount of individual milk, atmospheric conditions, etc.).

CONCLUSIONS

Estimation of cheese yield from very reduced individual milk amounts has been advocated for a better understanding of whether and how cheese ability can be influenced by milk composition. This study demonstrated that useful matter, as a combination of fat and protein contents, and total solids are well suited to cheese ability prediction, even if analyses are carried out independently to initial pH. The results indicated that pH value was prominent in explaining the variations of cheese ability of milk. Lastly, analysis by multiple regression confirmed that, in addition to milk characteristics, cheese yield capacity was subject to many other factors sometimes difficult to identify or over which the cheese plant has no control.

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