

# The influence of storage on the farm and in dairy silos on milk quality for cheese production

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## Abstract

Refrigerated storage of good-quality milk from a single cow resulted in moderate deterioration of its quality, low level of bacterial growth (standard plate and psychrotroph counts), and low small losses of curd yield. When milk was collected from farm bulk milk tanks and from dairy silos, its quality deteriorated faster than that of single-cow milk, resulting in high bacteria counts and high loss of curd yield, most of which was already apparent for the farm bulk milk tank. Statistical analyses did not reveal any significant interaction between bacterial growth, milk composition, somatic cell count, and curd yield loss, indicating that other mechanisms such as enzymatic activity might be responsible. From the comparison between the high-quality milk from an uninfected cow's udder and the commingled milk on the farm and in the dairy silos, it appears that introduction of milk coming from infected udders might cause curd yield loss such as that noted in the present study.

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## 1. Introduction

Milk storage time comprises two distinct periods: in the farm bulk milk tank (BMT) and in the dairy silo. While milking practices and herd management are in the sole hands of the farmer, the storage times of milk in the BMT and in the silos are usually determined by the dairies. There is common agreement that milk quality deteriorates over time, and that holding the milk for a long time impairs its quality, mainly with respect to microbiological standards, but also with regard to other quality aspects, such as curd yield and suitability for manufacturing various dairy products (Barbano, Ma, & Santos, 2006; Barbano,

Rasmussen, & Lynch, 1991; Mara, Roupie, Duffy, & Kelly, 1998; Roupas, 2001). In bulk milk with high bacterial counts and high somatic cell count (SCC) the impairment of product quality is intensified (Barbano et al., 2006) and curd yield is lowered (Auldust & Hubble, 1998; Celestino, Iyer, & Roginski, 1996; Leitner, Krifucks, Merin, Lavi, & Silanikove, 2006; Marino, Considine, Sevi, McSweeney, & Kelly, 2005; Roupas, 2001).

Despite all the above considerations, very little research has addressed the changes in milk quality between its production on the farm and its processing in the dairy. In the present study the raw milk quality (SCC and bacteria counts) and its suitability for manufacturing cheese were studied in relation to three periods: storage of the milk from individual cow for a period similar to the storage period in the BMT; the storage period in the BMT; and the storage period in the dairy silo.

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## 2. Materials and methods

### 2.1. Study design

An Israeli-Holstein cow with SCC below  $25 \times 10^3$  cells mL<sup>-1</sup> and a gland free of bacteria as tested for three consecutive times according to accepted standards (Oliver, Gonzalez, Hogan, Jayarao, & Owens, 2004) was used to study the effects of storage on the highest quality milk that could be obtained. The cow was milked in the morning, into an individual sterile container, and a sample was taken to test udder status, the rest being used for storage testing.

In addition, fifteen Israeli-Holstein dairy farms were selected according to their history of milk SCC and bacterial counts. After stopping agitation, 2 L of raw milk from the upper opening of the BMT in each farm was collected in sterile bottles. The milk was sampled after the first milking that followed the emptying and cleaning of the tanks.

Finally, milk from 10 milk silos at a dairy plant was sampled. The samples were taken from the check valve into sterile bottles, after some milk had been drained, to ensure spout cleanliness.

### 2.2. Sample analysis

All milk samples, packed in ice, were transferred to the laboratory within 1 h and stored at 4 °C, with constant stirring. The milk was tested upon arrival and after 24 and 48 h, to simulate typical milk storage times in the BMT and in the dairy silo, respectively.

For every milk sample, SCC was tested with the Fossomatic 360 (Foss Electric, Hillerød, Denmark) and gross composition (protein, casein, fat and lactose contents) was determined with the Milkoscan FT6000 (Foss Electric). In addition, total protein and casein were determined according to the Gold Standard routine (Marshall, 1992) and ISO-IDF standard (ISO, 2002). Whey protein content was calculated by difference as: protein minus casein obtained from the Milkoscan. Proteose-peptones (p-p) were determined as previously described by Leitner et al. (2006). Titratable acidity (TA) was measured according to AFNOR (1986). The following bacterial counts in milk were evaluated according to accepted standards: standard plate count (SPC) (Marshall, 1992) and psychrotrophic (PSY) bacteria (IDF, 2004).

### 2.3. Clotting time and curd yield determination

Clotting time (Tc) was determined by the Optigraph instrument (Ysebaert, Frepillon, France). Samples (10 mL) were placed in the wells and equilibrated at 30 °C, which was ascertained by a green light signal from the instrument (~2–3 min). Fromase 15 TL (0.5 mL, Gist-Brocades nv, Delft, The Netherlands), diluted (~1:100) to achieve clotting within about 900 s, was then added simultaneously

to the wells. The instrument was set to measure Tc in seconds. Curd yield (Yc) was determined as described by Leitner, Merin, and Silanikove (2004). All samples were analyzed in triplicate.

### 2.4. Statistical analysis

The bacterial count and the SD for each count were transferred to their logarithmic form. The effects of storage duration on curd yield, Yc, and clotting time, Tc, were analyzed with a one-way ANOVA model in a randomized-block design, where the sample sources served as block:

$$\text{Model 1: } Y_{ij} = \mu + \beta_j + \alpha_i + e_{ij},$$

where  $Y$  is the dependent variable,  $\mu$  the overall mean,  $\beta_j$  represents the variance between samples (random effect),  $\alpha_i$  is the effect of the  $i$ th storage duration (fixed effect), and  $e_{ij}$  represents the random error.

Correlations were determined between both Yc or Tc and all the other variables, i.e., SCC, TA, fat, protein, lactose, casein, whey, p-p or somatic cells, and log bacteria counts (SPC and PSY).

Comparison between the composition of milk samples from dairy silos and from the BMT after 0, 24 and 48 h of storage was done by a two-way ANOVA model in a repeated-measure design where the examined effects were source of milk (Group) and storage duration (time):

$$\text{Model 2: } Y_{ijkl} = \mu + \alpha_i + e_{ij}^1 + \gamma_k + \alpha\gamma_{ik} + e_{ijkl}^2,$$

where  $Y$  is the dependent variable,  $\mu$  the overall mean,  $\alpha_i$  the effect of the  $i$ th group (fixed effect),  $e_{ij}^1$  represents the variance between samples within group (error 1),  $\gamma_k$  is the effect of the  $k$ th time (fixed effect);  $\alpha\gamma_{ik}$  is the interaction between group and time; and  $e_{ijkl}^2$  represents the random error (SAS Institute, 2000).

## 3. Results

### 3.1. Cold storage of milk from an individual cow

No significant differences were found among the three milk replicates and among the storage times, for any of the tested variables (Table 1). Though total bacteria count and that of psychrotrophs, which represent post-milking contamination, increased during the 48 h of storage, they remained below  $5 \times 10^3$  cfu mL<sup>-1</sup>. Curd yield decreased by 4% during the 48 h of storage. Because preliminary testing showed that there were no significant changes in SCC, fat, protein, casein and lactose during storage, these analyses were performed only upon arrival of the milk at the laboratory.

### 3.2. Cold storage of milk from farm bulk tanks

The milk from most of the sampled BMTs exhibited the typical composition of milk from Israeli herds; it

Table 1

Changes in characteristics of milk<sup>a</sup> from a bacteria-free quarters of a single cow (A single cow) and of milk from 15 farm bulk milk tanks (Bulk milk tank) at time of collection and after 24 and 48 h of storage

Variable time	Fat (%)	Protein (%)	Casein (%)	Whey proteins (%)	Protease-peptone (mg mL <sup>-1</sup> )	Titrateable acidity (mL NaOH 0.1 M)	Log standard plate count	Log psychrotrophs count	Clotting time (s)	Curd yield (g L <sup>-1</sup> )
<i>A single cow</i>										
0	2.85±0.4	3.16±0.1	2.42±0.1	0.61±0.1	336±67	6.5±0.4	1.7±0.5	1.3±0.4	1002±163	57.6±3.7
24	2.94±0.5	3.17±0.2	*	0.62±0.0	341±76	*	1.9±0.9	2.2±0.5	979±56	57.3±6.3
48	2.52±0.5	3.26±0.1	*	0.66±0.1	345±69	*	4.3±0.4	3.1±0.3	968±363	55.3±12.0
<i>Bulk milk tank</i>										
0	3.81±0.4	3.31±0.1	2.53±0.1	0.72±0.1	439±108	6.78±0.4	4.83±0.2	4.48±0.4	694±99	70.4±4.3
24	*	*	*	*	468±100	7.10±0.4	5.29±0.4	5.77±0.5	647±83	68.6±4.9
48	*	*	*	*	507±115	7.27±0.6	7.38±1.1	7.69±0.9	630±99	65.4±5.2

<sup>a</sup>Data entries denoted by an asterisk (\*) show that the value tested did not change significantly during storage.

Table 2

Statistical analysis of milk parameters from 15 farm tanks in relation to clotting time Tc, and curd yield Yc

	Clotting time (n = 15)		Curd yield (n = 13)	
	r	P[r]	R	P[r]
SCC	-0.242	0.4062	-0.357	0.2250
Fat	-0.040	0.8873	0.651	0.0159
Protein	0.192	0.4923	0.688	0.0093
Fat + protein	0.321	0.531	0.901	0.0003
Casein	0.697	0.0039	0.807	0.0009
Lactose	0.599	0.0183	0.726	0.0049
Whey proteins	-0.477	0.0721	-0.104	0.7359
Protease-peptone	-0.101	0.7191	0.190	0.5338
TA	-0.234	0.4020	0.111	0.7188
Log plate count	-0.031	0.9124	-0.423	0.1499
Log psychrotrophs	-0.106	0.7069	-0.454	0.1191

Table 3

Statistical analysis of milk parameters from 10 dairy silos in relation to clotting time Tc and curd yield Yc

	Clotting time (n = 10)		Curd yield (n = 10)	
	r	P[r]	R	P[r]
SCC	-0.082	0.8222	-0.420	0.2264
Fat	-0.185	0.6081	0.504	0.1373
Protein	-0.530	0.1148	0.639	0.0468
Casein	-0.079	0.8282	0.805	0.0049
Lactose	-0.217	0.5474	0.371	0.2913
Whey proteins	-0.673	0.0331	-0.091	0.8029
Protease-peptone	0.430	0.2152	0.112	0.7581
TA	0.109	0.7640	0.824	0.0033
Log plate count	0.151	0.6772	-0.411	0.2375
Log psychrotrophs	0.220	0.5417	-0.475	0.1655

qualified as Premium Grade, according to Israeli standards (Israel Dairy Board, 2005). The SCCs in 14 out of the 15 farms tested were  $\sim 230,000$  cells mL<sup>-1</sup>; in the remaining farm the SCC was  $\sim 1.6 \times 10^6$  cells mL<sup>-1</sup>, i.e., grade D, which is not fit for consumption. The levels of fat, casein and lactose and bacterial counts varied among the farms. The concentration of p-p, TA and bacterial counts (SPC and PSY) all increased during storage, whereas Yc and Tc decreased. However, no clear relationship with the values at time 0 was noted. Curd yield decreased constantly during storage, by about 2% in the 24 h stored samples and by about 7% further in the 48 h stored samples (Table 1).

Correlations between Tc or Yc and the results of analysis of freshly obtained milk are presented in Table 2. Tc was significantly correlated with level of lactose ( $P = 0.0183$ ) and casein ( $P = 0.0039$ ), almost significantly with level of whey proteins ( $P = 0.07$ ) but not significantly with protein level ( $P = 0.49$ ). Yc was significantly correlated with fat, protein, casein and lactose; and the best correlations were with (fat + protein) ( $P = 0.0003$ ) and (fat + casein; not shown) ( $P = 0.009$ ).

### 3.3. Cold storage of milk from dairy silos

For milk from the silos, Yc and Tc did not change significantly during storage. However, the absolute value of Yc was about 5% lower than that in the farm BMTs at time zero and about 2% higher than that in the BMTs after 48 h of storage. The only significant correlation of Tc was with the level of whey proteins (negative correlation,  $P = 0.0331$ ), whereas Yc was correlated significantly with levels of protein and casein but not with fat level (Table 3). Yc was also significantly correlated with TA ( $P = 0.0033$ ). The correlations between Yc and the analysis results differed significantly between the BMTs and the silos (significant interaction,  $P = 0.0241$ ). The results indicated that the major loss of curd (Yc) occurred at an early stage of storage in the BMTs and, to a lesser extent, in the silos (Fig. 1).

## 4. Discussion

The 4% reduction in curd yield from 0 to 48 h storage of milk from a bacteria-free udder indicates that casein

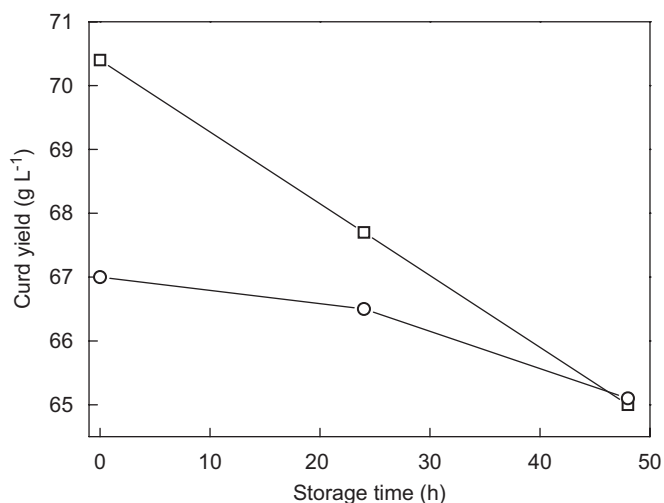


Fig. 1. Changes in curd yield according to origin of milk during cold storage. □, farms; ○, dairy silos.

continues to undergo proteolysis during storage, resulting in time-dependent impairment of curd formation, probably due to the activity of plasmin under cold storage (Crudden, Fox, & Kelly, 2005; Reimerdes & Herlitz, 1979), although the loss of Yc could be partially due to some reduction in the fat content of the milk due to some churning on the stirrer bar after 48 h storage.

Milk in the farm BMTs deteriorated faster than the milk coming from bacteria-free glands of individual cows. This was because, owing to the high prevalence of subclinical infections in western dairy herds, milk in the BMT, even of superior grade, includes milk coming from both subclinically infected and uninfected glands (Rainard, Ducelliez, & Poutrel, 1990). This is probably the reason for the high variability in curd yield from BMT Premium Grade milk, although its composition with respect to fat, protein, and even casein in all the BMT was similar. Our data indicate that SCC data can enable the prediction of the quality of milk with respect to curd yield only to a limited degree, which is even more limited if the milk was stored for several days before processing (Lindmark-Manssona, Branning, Alden, & Paulsson, 2006). Thus, our data suggest that entry of milk from subclinically infected udders into the BMT accounts for the difference in storage behaviour between the milk in the BMT and that from bacteria-free glands of the individual cows.

It is well documented that storage conditions influence milk quality, and past findings point to bacterial growth as a leading cause (Barbano et al., 2006; Roupas, 2001). In the present study, total bacteria counts in the BMT ranged from  $4 \times 10^3$  to  $7 \times 10^6$  per milliliter of milk, and the numbers of psychrotrophs varied over the same range. The presence of such high levels of psychrotrophs raises the question of their effect on milk quality during storage of the milk in the BMT. Despite the clear evidence that

storage in the BMT was associated with deterioration in milk quality and reduction in curd yield, according to the statistical analysis there was no correlation with the number of bacteria present in the BMT, except for the positive correlation between increased acidity and reduced clotting time. This strengthens the conclusions from other reports that, in addition to bacterial growth during storage, the health status of the herd also has a major influence on milk quality and curd yield (Barbano et al., 1991, 2006; Roupas, 2001).

Studies of milk from sheep, goats, and cows, on the individual gland level, have demonstrated that Yc was directly related to measures of subclinical infection, which reduced curd yield by 4% and 10% in sheep and goats, respectively (Leitner, Chaffer, et al., 2004; Leitner, Merin, et al., 2004), and by about 20% in cows (Leitner et al., 2006). Therefore, it is suggested that the subclinical mastitis status of the milked herd could also influence milk quality and curd yield.

However, the predictive correlations that worked reasonably well in this study on the individual level, and are consistent with previous results (Leitner, Chaffer, et al., 2004; Leitner, Merin, et al., 2004, and see Silanikove, Merin, & Leitner, 2006 for discussion of the physiological basis) lost a large part of their predictive value on the BMT level, and became almost useless on the silo level. Presumably, this loss of predictive power relates to the dilution of measures such as p-p, whey protein:casein ratio, and SCC in the transition from the individual level to the BMT and, to a larger extent, the silo levels. Nevertheless, the persistence of high variability in quality of milk with low SCC (Premium grade) strongly suggests that biochemical reactions initiated in subclinically infected mammary glands leave a marked trace that affects the quality and storage characteristics of the milk in the BMT. Our data, therefore, indicate the need for more thorough understanding of the biochemical reactions leading to the deterioration in quality of milk coming from sub-clinically-infected glands, and the need to develop sophisticated in-line techniques to enable the prediction of milk quality during milking either on the cow or on the farm level.

In summary, milk quality will deteriorate gradually during storage, most likely because of plasmin activity. Generally, curd yield loss appears to be faster and more pronounced in the dairy farms tanks and relatively moderate in the dairy silos. This probably relates to mixing of milk from subclinically infected udders (up to 50% in most Western dairy herds) with milk coming from bacteria-free glands, and to the dilution of this effect on the dairy plant silos level. The negative effect of bacterial growth during cold storage of milk in the farm tanks should be reconsidered in light of the basal milk quality (i.e., SCC count). In countries, such as Israel, where the average SCC in farm tanks is in the range of  $200,000 \text{ cells mL}^{-1}$ , this factor appears to be of redundant importance.

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