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GENETIC AND PHENOTYPIC VARIABILITY OF MILK COAGULATION PROPERTIES, CHEESE YIELD, NUTRIENTS RECOVERIES AND CHEESE SENSORY PROPERTIES ASSESSED ON INDIVIDUAL MILK OF BROWN SWISS COWS

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RIASSUNTO

L'attitudine del latte alla caseificazione rappresenta un argomento che desta molto interesse per l'aumento della quota prodotta della materia prima destinata alla produzione di formaggio. Negli ultimi anni la ricerca scientifica si è occupata soprattutto della determinazione ed identificazione delle proprietà di coagulazione del latte atte ad essere impiegate come fattore di valutazione e, di riflesso, come possibile carattere obiettivo di selezione nelle vacche da latte. Ad oggi, la relazione tra le proprietà di coagulazione del latte e la resa casearia non è del tutto chiara. La resa in formaggio rappresenta l'indice che definisce l'efficienza del processo di caseificazione ed è per questo utilizzato come strumento di controllo economico nei caseifici. Non sono stati ancora proposti degli studi che vadano a porre l'attenzione su fenotipi legati alla resa casearia ed alla qualità del formaggio prodotto dal latte individuale di specie bovina. Tali caratteristiche variano in funzione di una serie di fattori sia di natura ambientale che genetica.

Con la presente tesi sono stati presi in considerazione i caratteri che definiscono l'attitudine casearia del latte individuale di vacche di razza Brown Swiss. In particolare, l'indagine scientifica ha riguardato le proprietà di coagulazione del latte, la resa casearia e le perdite nel siero dei componenti del latte ed, infine, la qualità del formaggio tramite la analisi fisico-chimica e sensoriale.

La valutazione delle proprietà di coagulazione del latte ha previsto l'utilizzo di due strumenti che presentano tecnologie di funzionamento differenti (meccanico ed ottico). Il confronto degli stessi caratteri (RCT, k_{20}, a_{30}, a_{45}) ottenuti con i due strumenti ha sottolineato differenze sia da un punto di vista fenotipico che da un punto di vista genetico, soprattutto per i campioni di latte coagulanti dopo 30 minuti dall'inizio

dell'analisi. Il tempo di coagulazione (RCT) è stato il parametro in cui sono state riscontrate minori differenze tra i risultati ottenuti dai due differenti strumenti. L'aumento della durata dell'analisi a 90 minuti ha permesso di: ottenere l'RCT per tutti i campioni analizzati, stimare un nuovo parametro di consistenza del coagulo a 45 minuti dall'inizio dell'analisi (a_{45}), determinare l'ereditabilità e le correlazioni genetiche con i caratteri qualitativi del latte per il k_{20} ed anche per l'a_{45}. I risultati ottenuti suggeriscono l'eventuale utilizzo dello strumento ottico per la valutazione delle primissime fasi del processo di coagulazione dove i cambiamenti chimico-fisici del latte non sono visibili.

La resa casearia è stata determinata mettendo a punto una procedura di micro caseificazione utilizzando 1500 ml di latte per campione. I risultati ottenuti hanno evidenziato un'elevata qualità del latte di razza bruna con una resa media a fresco del 15% circa. È stato possibile stimare il recupero nella cagliata dei componenti del latte: questi caratteri non sono risultati costanti ma è stata osservata una certa variabilità sulla base dei fattori presi in considerazione nel presente studio (stadio di lattazione, ordine di parto, produzione di latte). È stato osservato che la resa non è influenzata solamente dalla materia utile del latte ma anche dall'acqua. Da un punto di vista genetico, è stata stimata per la prima volta nel latte bovino, l'ereditabilità della resa casearia (della cagliata, della sostanza secca e dell'acqua) e del recupero di nutrienti nella cagliata (proteina, grasso, sostanza secca ed energia). I risultati ottenuti hanno evidenziato la presenza di una rilevante componente genetico additiva degli animali, potenzialmente sfruttabile per finalità selettive.

Infine, sono state valutate le caratteristiche qualitative ed organolettiche dei formaggi prodotti a livello individuale. Dallo studio delle potenziali fonti di variazione è emerso che lo stadio di lattazione risulta essere un fattore altamente significativo. Tale

effetto influenza i cambiamenti di composizione del latte durante la fase produttiva della vacca, mentre l'ordine di parto non ha evidenziato alcun legame importante con i caratteri analizzati. Alcuni parametri, soprattutto quelli relativi alla *texture*, sembrano legati alla resa casearia del latte. La raccolta di questi caratteri, a livello individuale, permetterà anche la stima dei parametri genetici.

ABSTRACT

Milk cheese-making ability have received great interest from the dairy industry in the worldwide increasing of the amount of milk used for cheese production. In recent years, scientific research was mainly occupied to identify and study phenotypic and genetic variability of milk coagulation properties. The relationship between these traits and cheese yield is not entirely clear. Cheese yield, milk nutrients recoveries in the curd and whey losses represent indices that defines the efficiency of the cheese-making process and are used as tools for economic control in the dairies. To our knowledge, any studies have not yet been proposed on the assessing of yield and quality traits of individual cheese variability using bovine milk. These traits are influenced by environmental and genetic factors.

In the present thesis quality cheese-making traits of milk from individual cows of the Brown Swiss were assessed. In particular, this study has focused on the milk coagulation properties, the cheese yield, the nutrients recoveries in the curd and, finally, the quality (chemical components, physical traits and sensory properties) of cheese.

Milk coagulation properties were compared through a traditional mechanical device and a near-infrared optical device. This comparison of MCP traits (RCT, k_{20}, a_{30}, a_{45}) has emphasized phenotypic and genetic differences between measures obtained by the two devices, especially for samples coagulating after 30 minutes (NC samples) from start analysis. Rennet coagulation time (RCT) was the trait presenting less differences when assesses by a different instrument. Extending the analysis the analysis by either instruments allowed to: obtain RCT for all samples analysed, estimate a new curd firmness trait (a45; the width of the resulting graph after 45 min from the rennet addition), estimate the heritability of k_{20} and a_{45} and genetic correlations with the milk

production and qualitative traits. The results obtained suggested the use of the optical instrument for the assessment of the first phase of coagulation process where the chemical-physical changes of the milk are not visible.

Cheese yield was estimated by developing an individual model-cheese production procedure 1500 ml of milk per sample. The described model cheese-producing procedure and the obtained results provide new insight into variation and relationships among different cheese yield (curd, dry matter and water) and recovery (protein, fat, dry matter and energy) traits at the individual level.

The results showed high milk quality of milk Brown Swiss breed presenting on average a cheese yield of 15%. Measures of nutrient recoveries (protein, fat, total solids and energy) were computed exhibiting a great variability. It has been observed that the yield is not only influenced by the milk dry matter but also by the milk water. From a genetic point of view, heritability has been estimated for cheese yield (of the curd, the dry matter and water) and the recovery of nutrients in the curd (protein, fat, dry matter and energy) and the results have shown a certain importance of genetic factors on the variability of these traits. Clearly, additional research on this topic is warranted, especially in terms of assessing the genetic background of these traits and the methods for their indirect prediction.

Finally, it was evaluated the qualitative traits and sensory properties of cheeses produced at the individual level (from each cow). The results showed a great variability of these traits at individual level. From the variation factors considered in this study, stage of lactation appeared to be important reflecting the changes in milk composition, while order of parity did not show any significant relationship with the analysed traits. cheese composition and few sensory properties (related to the cheese texture), were

influenced by the cheese yield of milk. Collection of these data at the individual level will also allow to estimate genetic parameters of these traits.

GENERAL INTRODUCTION

Technological quality of milk is increasingly becoming a topic of global world interest because of the growing cheese production and consumption (International Dairy Federation, 2011). The quote of milk delivered to cheese production has increased by 10% in the European Union and North America, who remain the largest producers in the world with more than 50% in the case of the EU and a little less in the case of the North American continent. This increased use of milk for cheese production has also been reported in other European countries, in Oceania and Latin America.

Under this scenario, the genetic improvement of milk technological traits is essential to address social demands and this requires the definition of new phenotypes for new breeding goals in dairy cattle. However, before implementing these new phenotypes in breeding programs it is important to get knowledge on the phenotypic (i.e., distributions, potential sources of variations etc.) and genetic variation of them. Unfortunately, only a few studies have attempted to quantify the genetic and phenotypic variation of cheese-making properties and this is basically due to the difficulty of assessing such a traits at the individual level. Besides this, the relationships between these cheese-making properties and traits currently included in breeding programs have not been fully elucidated. On the basis of these consideration, three main features related to the “so-called” cheese-making properties will be introduced thereafter, specifically: the milk coagulation properties, the individual cheese yield and sensory properties of cheese.

MILK COAGULATION PROPERTIES

Assessment of milk coagulation properties (**MCP**) plays an important role in determining the technological quality of milk (Annibaldi et al., 1977). Coagulation ability is evaluated by few but fundamental aspects related to technological quality of milk such as reactivity to the rennet, curd-firming capacity, curd firmness, permeability and contractility of curd, and curd syneresis.

Several technologies (mechanical, optical, thermal, ultrasonic, and vibrational) can be used to study MCP (Laporte et al., 1998; O'Callaghan et al., 2002; Klandar et al., 2007). The most common approach, both at the research and industry levels, is to record the viscosity of milk by analysis at fixed temperature after the addition of rennet (Bittante, 2011). Conventionally, three single-point MCP traits are carried out using lactodynamograph (mechanical renneting meters) that records curd firmness over time (CF_t; Bittante et al., 2012) and produces firmness/time graphs outputs: rennet coagulation time (RCT, min) that is the interval time between the addition of rennet and the start of coagulum formation, curd-firming time (k_{20}, min) obtained by measuring the difference in time between RCT and the achievement of 20 mm of curd firmness, and curd firmness (a_{30}, mm) that is defined by the width of the graph 30 minutes after rennet addition (Annibaldi et al., 1977; McMahon and Brown, 1982).

Despite the large number of studies regarding factors involved on MCP, the results are sometimes difficult to compare because of the large variability among different analysis (i.e., concentration and type of rennet, temperature, instruments ect.). In general, factors affecting MCP can be classified as follows (Bittante et al., 2012): instrument type and setup (that include temperature, concentration and enzyme activity of rennet); repeatability and reproducibility of the method; pre-treatment of milk

samples (interval time from sampling, the use of preservatives, storage conditions, standardization of milk); milk quality which is the general target of analysis.

Few studies have determined repeatability and reproducibility of results obtained by mechanical instruments underlining low values for both parameters, especially in the case of k_{20} (Caroli et al., 1990; Dal Zotto et al., 2008; Bittante e t al., 2011).

Little is known about the comparison of MCP traits assessed by different device. Optical instruments (infrared analysis) have used to assess milk coagulation, curd firming and syneresis (Payne et al., 1993; Fagan et al., 2007; Mateo et al., 2009) and to estimate the prediction of MCP, using the phenotypical measures recorded by mechanical lactodynamograph. In this case, mid-infrared spectra (MIRS) of raw untreated milk have been used to calculate MCP, after appropriate instrument calibration (Dal Zotto et al., 2008; De Marchi et al., 2009). The correlations between traditionally estimated MCP measures and MIRS predictions of such values are medium to high (Dal Zotto et al., 2008; De Marchi et al., 2009). Therefore, MIRS analysis cannot replace mechanical measures, but MIRS can be used at population level for genetic purposes (Cecchinato et al., 2009). Infrared instrument have been used also to simulate the mechanism of the pendula submerged in oscillating milk samples (mechanical technique) proposing detectors that record absorbance at a single near-infrared (NIR) wavelength in a still sample during coagulation (Kübarsepp et al., 2005). However few comparisons between instruments presenting different measure technology have been proposed, and all were based on a small number of samples and/or were conducted under different analytical conditions (Panari et al., 2002; Kübarsepp et al., 2005; Pretto et al., 2011).

Another aspect that has to be considered is the time-testing of MCP analysis. Coagulation could be not noted during the 30-min test interval resulting in milk samples

on which RCT, k_{20} and a_{30} are not estimated. Milk samples presenting this characteristics is termed noncoagulating (NC; Ikonen et al., 2004; Tyrisevä et al., 2004). The presence of these types of milk is growing concern because of the dissemination of the Holstein-Friesian breed worldwide, as these cows are known to yield both late-coagulating (LC) and NC milk (De Marchi et al., 2007). The presence of NC milk samples determinates statistical problems in terms of correct evaluation of data from coagulating samples (Cecchinato and Carnier, 2011; Cecchinato et al., 2011). Few studies, presented the extending of analysis to 45 minutes (Mariani et al., 1997; Cecchi et al., 2002) or 60 minutes (O'Brien et al., 2002; Auldist at al., 2004), measuring at different intervals time the curd firmness.

Exploitable additive genetic variation exists for MCP (Ikonen et al., 1997 and 1999; Cassandro et al., 2008). Several studies (about 20 reports) have reported estimates of heritability for MCP, using measurements provided by mechanical instruments (Ikonen et al., 2004; Tyrisevä et al., 2004; Cassandro et al., 2008). As in the case of phenotypic purposes, also for genetic studies, differences in analytical conditions made not easily the comparison and the interpretation. Only Vallas et al. (2010) presented genetic results obtained from MCP measured using an optical instrument. To our knowledge, comparison of genetic parameters estimated from MCP obtained by using mechanical and instruments presenting another technology have not been carried out.

Several studies investigated variation and genetic aspects of MCP in dairy cattle populations (Ikonen et al., 1999; Cassandro et al., 2007; Vallas et al., 2010). In those studies, records of NC milk were not included in the statistical analysis because of unavailable information on RCT and inability of the linear model to handle properly NC milk records. Alternatively, a different trait definition (i.e., occurrence of milk coagulation at a given time), involving categorization on the binary scale, was used

(Tyrisevä, et al., 2004), albeit this approach suffers from a severe information loss (Kotsiantis and Kanellopoulos, 2006). Both approaches involve a rough overcome of data peculiarities. In the first case, milk samples with very unsatisfactory MCP are omitted from the analysis, biasing the estimation of location and dispersion parameters of RCT. In the second case, milk samples exhibiting different time of curd formation after rennet addition (e.g., after 7 or 25 min) are treated alike and continuous variation of RCT for coagulated samples is inappropriately neglected.

CHEESE YIELD

Generally cheese-making can be considered a dehydration process where milk components are concentrated, particularly fat and protein contents which are considered factors influencing efficiency and profitability of the process and determining differences in the resulting cheese yield (**CY**; Emmons, 1993). Measurements of CY are used to: determine systems for milk payment, assess the effectiveness of processing modifications, and evaluate effectiveness of the introduction of new possible ingredients in cheese manufacture (Banks, 2007).

The classical definition of CY is the weight of cheese in kg produced from 100 kg of milk. This trait can also be expressed as the volume of milk in litres required to manufacture one tonne of cheese (Banks, 2007). Obviously, the determination of actual CY requires the measurement of the weight of all inputs (milk, starter and salt) and outputs (cheese and whey) of the cheese-making process.

Recovery of individual milk constituents in the curd, and their loss in the whey define, with CY, the efficiency of cheese-making (Banks, 2007). Factors affecting these indices can be grouped in two main headings related to milk quality: 1) animal concerns, such as the species (Othmane et al., 2002a; Zicarelli et al., 2007), breed

(Malacarne et al., 2006; De Marchi et al., 2008; Martin et al., 2009),stage of lactation (Wedholm et al., 2006), parity (Wedholm et al., 2006), feeding (Banks et al., 1986) and health (Politis and Ng-Kwai-Hang, 1988); and 2) conditions, such as the handling and storage of the milk prior to cheese-making, and the technologies adopted (Lucey and Kelly, 1994).

One of the most used approach to study CY is the determination of predictive formulas. Predictive formulas for estimating CY have been based on knowledge of the protein (or casein/para-caseinate) and fat contents of milk (Van Slyke and Price, 1952; Banks et al., 1981 and 1984; Emmons et al., 1990), or the sum of the fat and protein contents (Verdier-Metz et al., 2001). All of these formulas assume that the recovery of milk protein and fat in the curd is constant. However, this assumption is contradicted by the results obtained by Aleandri et al. (1989), who presented a curvilinear relationship between the protein content of milk and the CY.

In general, experimental cheese-making (when carried out in cheese-making plants) trials are expensive, time-consuming, and only allow for a small number of replicates. In the last 30 years many different laboratory cheese-making procedures have been proposed, ranging from very simple protocols to techniques that simulate the industrial processes. Laboratory procedures present these advantages: the use of small quantities of milk; reduced time and costs required for experiments; more possible treatments or replications per day; and the ability to estimate CY from individual animals. It is generally agreed that individual CY is important for studies intended to test the existence of a genetic basis for these traits (Othmane et al., 2002b). Moreaver, individual bovine CY could be an economy parameter of maximum importance for dairy farmers and industries considering that in 2009 cheese produced from milk delivered to dairies (i.e. industrial cheeses) represented more than 80% of the global

natural cheese production (International Dairy Federation, 2010). However, most of the studies that involve micro cheese-making procedures have used bulk milk, largely because it is very labour intensive to produce a high number of model cheeses from individual milk samples.

Few studies (Hurtaud et al., 1995; Wedholm et al., 2006) presented results about individual bovine CY obtained using a micro cheese-making procedure but with a low or medium number of observations. Only Othmane et al. (2002) reported results of individual cheese yield with an high number of observations, using ewes milk: in this study an efficient procedure (60 observations simultaneously) using 10 ml of milk per sample was reported. This small amount of milk for sample did not allow the determination of the components of curd, and, therefore, authors estimate only individual CY without the determination milk nutrients recovery in the curd.

To our knowledge, genetic parameter for bovine CY and for the other traits that define efficiency of cheese-making process, have not yet been estimated. Othmane et al., (2002b) estimated heritability of individual CY for ewes milk obtaining values near to 9% and positive genetic correlations with milk components ranging from 0.60 to 0.78. Another approach was presented by Rosati and Van Vleck (2002): they used a prediction formula (Altiero et al., 1989) to estimate the mozzarella yield in an entire lactation for the Italian population of river buffaloes. Heritability for mozzarella yield was 14% showing positive genetic correlations with milk components (from 54% to 87%) and milk yield (95%).

SENSORY PROPERTIES

Sensory analysis of cheese and, in general, of dairy products, represents the last step for the quality evaluation (Drake, 2007). Generally, cheese sensory quality is influenced by two main groups of sources of variation: first, the quality of the raw material used; second, the cheese production techniques (pre-treatment of milk, cheese-making and ripening). In particular, the consumers are especially interested by the quality of the milk preferring final products resulting from the use of raw materials of high quality. The quality of the milk is affected by endogenous and exogenous factors. Endogenous factors are related to the animal and can be summarized as follows: genetic factors that comprehend the species, the breed and the individual, and physiological factors that include the state of animal health, lactation and order of parity. While, exogenous factors include: environment, feeding system and herd management.

Milk quality traits take special importance in the case of labelled cheese products such as Protected Designation of Origin production (PDO) because of the relevance of regulations and restrictions on the modifications of raw milk during the cheese-making process.

Generally, all studies conducted on cheese sensory properties have generally focused on cheeses produced in the industry (milk from one or more herds) and have not yet been taken into account sources of variations related to the productions at individual level. Considering only the effect of endogenous factors of milk quality, many reports have focused on the cheese sensory properties and studied species (Ha et al., 1991; Kondyli and Katsiari, 2001), breed (Verdier et al., 1995; Verdier-Metz et al., 1998; Martin et al., 2009), the health of the animal (with the study of relationship between somatic cell count and cheese sensory properties; Auldist et al., 1996), and the stage of lactation (Coulon et al., 1998).

The reason why sensory evaluations at individual level have not yet been performed is certainly due to the large amount of work involved in the sampling of milk, in the individual cheese-making, and in the individual assess of cheese sensory properties.

The study at the individual level requires a high number of observations (animals) and thus implies a large number of cheeses to be sensory analysed. Studies at the individual level would relate more precisely the relationship between the sources of variations of milk quality and the sensory properties of the cheeses. Furthermore, the collection of individual data implies the possibility to carry out genetic studies. To our knowledge, genetic parameters of cheese sensory properties have not yet been estimates.

AIMS AND OUTLINES OF THE THESIS

The main objective of this thesis is to investigate the variability of some "new phenotypes" related to the technological properties of individual milk of Brown Swiss cows. The study will be divided in three main parts. In the first part, milk coagulation properties (90 minutes of analysis) obtained by using different instruments, namely the Formagraph (mechanical instrument) and the Optigraph (optical instrument) were assessed to:

1. Compare results from two different instruments under the same lab conditions (type and concentration of rennet, analysis temperature, technician).
2. Investigate several sources of variation for individual MCP samples.
3. Estimate heritabilities of MCPs of these two devices.
4. Estimate their genetic correlations relationship between instruments within trait and between traits within instrument.
5. Obtain correlations for sire rankings based on the instruments used.

In the second part of the thesis, individual cheese yield and nutrients recoveries in the curd will be assessed using a cheese-making model manufacturing process in order to:

1. Characterize CY (of curd, dry matter and water), milk nutrient and energy recoveries in the curd at the individual level.
2. Investigate several sources of variation for CY and nutrient/energy recoveries in the curd.
3. Estimate genetic parameters of CY, curd nutrient/energy recoveries
4. Estimate their genetic relationships with milk yield and composition.

In the last part of the thesis, quality traits of individual cheese will be evaluated. Particularly, the aims of the last contribute were:

1. Assess the variability of cheese chemical components, of physical traits and of sensory properties at the individual level.
2. Investigate several sources of variation for cheese chemical components, of physical traits and of sensory properties

I CONTRIBUTION

COMPARISON BETWEEN MECHANICAL AND NEAR-INFRARED METHODS FOR ASSESSING COAGULATION PROPERTIES OF BOVINE MILK

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ABSTRACT

The aim of the present study was to compare milk coagulation properties measured through a traditional mechanical device, the Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark), and a near-infrared optical device, the Optigraph (OPT; Ysebaert SA, Frépillon, France). Individual milk samples of 913 Brown Swiss cows from 63 herds located in Trento Province (Italy) were analyzed for rennet coagulation time (RCT, min), curd-firming time (k_{20}, min), and 2 measures of curd firmness (a_{30} and a_{45}, mm) using the 2 instruments and under identical conditions. The trial was performed in the same laboratory, by the same technician, and following the same procedures. Extending the analysis by either instrument to 90 min permitted RCT and k_{20} values to be obtained even for late-coagulating milk samples. Milk coagulation properties measured using the OPT differed considerably from those obtained using the FRM. The average k_{20} values varied greatly (8.16 vs. 5.36 min for the OPT and the FRM, respectively), as did the a_{45} figures (41.49 vs. 33.66 mm for the OPT and the FRM, respectively). The proportion of noncoagulating samples for which k_{20} could be estimated differed between instruments, being less for the OPT. The between-instrument correlation coefficients were either moderate (0.48 for a30) or low (0.24 and 0.17 for k_{20} and a_{45}, respectively) when the same traits were compared. The correlations between k_{20} and a_{45}, and milk yield varied among instruments, as did the correlations between k_{20}, a_{30}, and a_{45} and milk composition, and the correlations between a_{45} and pH. The relative influence of days in milk on k_{20} and a_{45} varied, as did the effect of parity on a_{45} and that of the measuring unit of coagulation meter on k_{20} and a_{30}. The RCT estimated by the OPT was the only milk coagulation property to show good agreement with the FRM-derived value, although this was not true for the data from late-coagulating samples.

Key words: milk coagulation property, Optigraph, Formagraph

INTRODUCTION

Milk coagulation properties (**MCP**) are important measures of the technological quality of milk (Annibaldi et al., 1977). Good reactivity to rennet, high curd-firming capacity, good syneresis ability, and whey drainage are crucial features of milk for cheese making. The suitability of milk for cheese making is evaluated by measuring rennet coagulation time (**RCT**); the time required for curd-firming ($\mathbf{k_{20}}$); and the firmness ($\mathbf{a_{30}}$), elasticity, permeability, contractility, and syneresis of curd, as reviewed in detail by Mariani et al. (1997).

The methods used to assess MCP explore physicochemical changes occurring in milk during rennet-induced coagulation. Rennet modifies casein micelles, resulting in changes of milk viscosity and elasticity (Auldist et al., 2001; O'Callaghan et al., 2002). Several techniques have been used to measure MCP and a wide range of mechanical, vibrational, ultrasonic, thermal, and optical instruments are available (Laporte et al., 1998; O'Callaghan et al., 2002; Klandar et al., 2007). The most common approach, at both the research and industry levels, is to monitor milk viscosity following addition of rennet. Traditionally, MCP are evaluated over the testing time of 30 min using a lactodynamograph. This is a mechanical device customized to evaluate several milk samples (usually 10) simultaneously. The milk temperature is held constant during the analysis. The lactodynamograph measures the tiny forces that act on submerged pendula when samples of coagulating milk are oscillated in a linear manner. The outputs are firmness/time graphs. The common MCP discussed in the literature (Annibaldi et al., 1977; McMahon and Brown, 1982) are RCT (min), k_{20} (min), and a_{30} (mm).

Only a few studies have examined the repeatability and reproducibility of MCP obtained using traditional mechanical instruments. Although MCP are often expressed in various ways in the literature, their repeatability appears to be low (Caroli et al., 1990; Dal Zotto et al., 2008; Bittante, 2011). For many years, optical instruments using infrared analysis have been used to monitor milk coagulation, curd firming, and syneresis (Payne et al., 1993; Fagan et al., 2007; Mateo et al., 2009). Infrared instruments have been used also to predict the MCP, usually measured with a mechanical lactodynamograph. These studies can be divided into 2 categories. First, mid-infrared spectra (**MIRS**) of raw untreated milk have been used to calculate MCP, after appropriate instrument calibration (Dal Zotto et al., 2008; De Marchi et al., 2009). Second, the pendula submerged in oscillating milk samples during lactodynamography have been replaced by detectors that record absorbance at a single near-infrared (**NIR**) wavelength in a still sample during coagulation induced, as usual, by heating and enzyme addition (Kübarsepp et al., 2005).

The correlations between traditionally estimated MCP measures and MIRS predictions of such values are medium to high (Dal Zotto et al., 2008; De Marchi et al., 2009). Therefore, MIRS analysis cannot replace lactodynamography, but MIRS can be used at population level for genetic purposes (Cecchinato et al., 2009). Milk coagulation properties are influenced by species (Bencini, 2002; Park et al., 2007; Cecchinato et al., 2012b) and breed (Macheboeuf et al., 1993; De Marchi et al., 2008; Martin et al., 2009). Moreover, several studies showed that exploitable additive genetic variation exists for MCP measured with mechanical (Tyrisevä et al., 2008; Cassandro et al., 2008; Cecchinato et al., 2012c) and optical devices (Vallas et al., 2010).

However, from a phenotypic point of view, few comparisons between such instruments have been performed, and all were based on a small number of samples or

were conducted under different analytical conditions, or both (Panari et al., 2002; Kübarsepp et al., 2005; Pretto et al., 2011). Therefore, the aim of the present study was to compare MCP measures obtained from mechanical and NIR instruments using a large number of samples under the same experimental conditions.

MATERIALS AND METHODS

FIELD DATA

Nine hundred thirteen Brown Swiss cows from 63 herds located in Trento Province (Italy) were sampled between April 2010 and February 2011. Two milk subsamples per cow were collected. With few exceptions, 15 cows from each herd were individually sampled once during evening milking. After collection, samples (without preservative) were immediately refrigerated (4°C). One random subsample was transported to the Milk Quality Laboratory of the Breeders Association of Trento Province (Trento, Italy) for composition analysis. The other subsample was transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy) for MCP analysis. All samples were processed within 20 h after collection. Information on cows and herds were provided by the Breeders Association of Trento Province (Italy).

ANALYSIS OF MILK QUALITY TRAITS

Individual milk subsamples were analyzed for fat, protein, and casein percentages using a MilkoScan FT6000 apparatus (Foss Electric A/S, Hillerød, Denmark). Somatic cell count values were obtained from the Fossomatic FC counter (Foss Electric A/S) and were then converted to SCS by means of logarithmic

transformation (Ali and Shook, 1980). The pH values of the subsamples used for MCP analysis were measured before the analysis, using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain).

ANALYSIS OF MILK COAGULATION PROPERTIES

Measures of MCP were obtained using 2 different instruments: a Formagraph (**FRM**; Foss Electric A/S) and an Optigraph (**OPT**; Ysebaert SA, Frépillon, France). Both instruments were housed in the same laboratory and operated by the same technician. Each subsample was analyzed simultaneously on both instruments. All experimental conditions (milk temperature, and the concentration and type of rennet) were identical. Two racks containing 10 cuvettes (1 rack per instrument) were prepared; milk samples (10 mL) were heated to 35°C and 200 μL of a rennet solution [Hansen Standard 160, with 80 ± 5% chymosin and 20 ± 5% pepsin; 160 international milk clotting units (**IMCU**)/mL; Pacovis Amrein AG, Bern, Switzerland] diluted to 1.6% (wt/vol) in distilled water was added at the beginning of analysis. Both instruments analyzed 10 samples simultaneously, 1 sample for each measuring unit of the coagulation meter (**MUCM**; pendula for the FRM and monochromators for the OPT). These devices record the width (mm) of the graph during testing; the OPT records a datum every 6 s and the FRM every 15 s. The observation period continued for 90 min after rennet addition. Variations in absorbance, as detected by the OPT, were transformed using an appropriate calibration equation to mimic the shape of the graph afforded by traditional mechanical instruments (Kübarsepp et al., 2005). This means that the usual MCP can be calculated using either device. Rennet coagulation time (min) is defined as the time from addition of enzyme to the beginning of coagulation, k_{20} (min) is the interval from RCT to the time at which the width of the graph attains 20

mm, and a_{30} (mm) is a measure of the extent of curd firmness 30 min after coagulant addition. Moreover, prolongation of the duration of recording allowed curd firmness 45 min after enzyme addition (**a_{45}**, mm) to be calculated. Samples that did not coagulate within 30 min were classified as noncoagulating (**NC**; Ikonen et al., 1999), although extension of analysis allowed RCT and k_{20} values to be detected for all samples.

STATISTICAL ANALYSIS

The cumulative frequency distributions of sample number against RCT and k_{20} values were calculated. Fisher's exact test was used to determine whether the proportions of samples in particular frequency bands differed after 15, 20, 25, 30, 35, 40, and 45 min.

Additionally, linear regression (SAS Institute Inc., Cary, NC) was used to explore the relationship between MCP traits from the FRM and OPT. The *F*-test was used to test the significance of any slope that deviated from unity and any intercept that was not zero ($P < 0.05$). Relationships among different MCP obtained using the same device and among MCP and milk yield (**MY**), milk quality, and acidity, were investigated.

Variance homogeneity between FRM and OPT data was explored using Levene's test (Milliken and Johnson, 1984). To estimate effects of different factors on lactodynamographic variables (RCT, k_{20}, a_{30}, and a_{45}) obtained using the FRM and OPT, an ANOVA was conducted (SAS Institute Inc., Cary, NC) using the following linear model:

$$y_{ijklm} = \mu + herd_i + dim_j + parity_k + MUCM_l + e_{ijklm},$$

where y_{ijklm} is the observed trait (RCT, k_{20}, a_{30}, or a_{45}) from the FRM or OPT; μ is the overall mean; $herd_i$ is the fixed effect of the ith herd (i = 1 to 63); dim_j is the fixed effect

of the jth class of DIM (j = 1 to 6; class 1: <60 d, class 2: from 60 to 120 d, class 3: from 121 to 180 d, class 4: from 181 to 240 d, class 5: from 241 to 300 d, and class 6: >300 d); $parity_k$ is the fixed effect of the kth parity (k = 1 to 4 or more); $MUCM_l$ is the fixed effect of the lth MUCM (l = 1 to 10); and eijklm is the residual random error term ~ N (0, σ^2_e).

PHENOTYPIC PATTERN OF MILK COAGULATION AND CURD FIRMING FROM MECHANICAL AND NIR INSTRUMENTS

Descriptive statistics for investigated traits are in Table 1. Milk yield; fat, protein, and casein content; and SCS averaged 24.36 kg/d; 4.23, 3.71, and 2.89%; and 3.03, respectively. In general, MY and milk quality traits were higher than those reported by Samoré et al. (2007), but the extent of variability was similar, being comparable to findings from an earlier Italian context on Brown Swiss cows (ANARB, 2010). Somatic cell score and MY showed the largest coefficients of variation, and protein and casein content the smallest.

The mean values and standard deviations of MCP from FRM and OPT are shown in Table 2, along with results obtained for Levene's test. The average values for RCT and a_{30} obtained using either instrument were similar (RCT: 19.95 vs. 18.91 min, and a_{30}: 30.09 vs. 27.23 mm, for the FRM and OPT, respectively). The standard deviations of both MCP were higher when the FRM data were examined and this was statistically confirmed by Levene's test. Therefore, because the variances were heteroskedastic, MCP were separately analyzed via ANOVA. The distributions of k_{20} revealed opposite characteristics and the equality-of-variances hypothesis was not rejected. Analysis of a_{45} and k_{20} obtained using the 2 instruments showed that the OPT

yielded systematically higher values. The heteroskedasticity was similar to that observed for RCT and a_{30}, although for a_{45}, the mechanical instrument yielded the lowest variance.

A comparison between the distributions of RCT for the 2 instruments (Figure 1) highlighted that most of the observed differences were attributable to the relative frequency of late-coagulating samples; this was higher when the FRM rather than the OPT was used. This peculiarity rendered the FRM distribution more asymmetric than the OPT distribution. This was confirmed by the higher proportion of NC samples when FRM was used (6.57% vs. 2.08%, for the FRM and OPT respectively; $P < 0.001$).

The cumulative frequency distribution of RCT values against time, obtained using either instrument (Figure 2a), confirmed that, although the FRM detected more coagulated samples at 15 min, the number of such samples was lower with FRM rather than OPT testing at 20, 25, 30, and 35 min. Figure 2a also shows that, within 45 min after rennet addition, all samples coagulated using either instrument.

The distribution of k_{20} (Figure 1) showed that the values were, on average, higher when the OPT was used, and the extent of asymmetry was lower (the skewness was 3.46 vs. 1.51 for the FRM and OPT, respectively). Although the extent of data variability showed by either instrument did not differ, the kurtosis of k_{20} as measured by the FRM was 22.33, indicating that the distribution was leptokurtic. A longer k_{20} means that, even if RCT values are similar, a curd firmness of 20 mm was attained later when samples were analyzed with the OPT than the FRM. This also indicates that, at any given time point after rennet addition, lower proportions of OPT samples had successfully yielded k_{20} values. The graph of cumulative frequency distribution against time of the RCT + k_{20} values (Figure 2b) makes it clear that 30 min after rennet addition (the usual endpoint of lactodynamographic testing), significant proportions of samples

had not yet yielded k_{20} values. Further, this proportion was much higher when the OPT rather than the FRM was used (27.5% vs. 20.9%, respectively; $P < 0.001$). Extension of analysis to 45 min reduced the proportions of samples that did not yield k_{20} values (3.6 vs. 2.9% for the FRM and OPT, respectively).

Curd firmness at 30 min showed a bimodal distribution (Figure 1) because of the existence of milk samples with a_{30} values equal to zero (i.e., NC samples); this was true when either instrument was used. When NC samples were not considered, both instruments yielded skewness and kurtosis that were close to zero, being −0.61 and 0.08 for the FRM and 0.24 and 0.34 for the OPT, respectively. This suggests that a_{30} values are normally distributed. Similarly, the a_{45} distributions were close to normality. However, differences in average values and variability were evident (Figure 1).

RELATIONSHIPS BETWEEN MECHANICAL AND NIR INSTRUMENTS

Figure 3 shows linear regressions between values of each MCP obtained using either instrument. Rennet coagulation time showed the highest correlation (r = 0.82) and the regression equation had an intercept that did not significantly differ from zero. The regression coefficient (1.09; $P < 0.001$) was higher than unity, thus explaining the slightly higher average RCT value obtained using the FRM compared with the OPT (Table 2). It may also be noted that the extent of the discrepancy between the 2 methods is attributable to differences in the number of late-coagulating samples.

The between-instrument correlation for k_{20} was low (r = 0.49), and both the intercept and regression coefficient of the equation were much lower than expected. This explains the lower average value of the FRM compared with the OPT. The most significant discrepancies were evident when samples exhibiting very high k_{20} values were analyzed. In the case of a_{30}, the between-instrument correlation coefficient was

intermediate compared with the other MCP (r = 0.69); the intercept was greater and the slope was lower than the expected values.

Finally, when a_{45} data were analyzed, the correlation between results obtained using the 2 instruments was low (r = 0.41). We also sought quadratic relationships among data obtained using the FRM and OPT, but any increment in the coefficient of determination was trivial.

Pearson product-moment correlations among different MCP obtained using the same instrument are summarized in Table 3. All correlations were significant ($P < 0.001$), suggesting that different MCP obtained using either instrument exhibit linear dependency. The correlations among MCP tended to be of similar magnitude, with the exception of the correlations between RCT and k_{20}; these appeared to be higher when data were obtained using the FRM than the OPT (0.65 vs. 0.32, respectively), and when they involved a_{45}. The latter tended to be greater when OPT data were analyzed.

FACTORS AFFECTING VARIATION OF MILK COAGULATION PROPERTIES

The correlation coefficients between MCP and other milk traits are in Table 4. Milk yield was moderately correlated with RCT and with k_{20} obtained from the OPT. A moderately negative association with a_{45} from the OPT was evident. Milk fat content was favorably associated with MCP with the exception of RCT; the correlation coefficients were higher when the MCP data were obtained using the OPT rather than the FRM. Milk protein and casein contents were unfavorably associated with RCT obtained using either instrument but favorably with the other MCP. The correlation coefficients between lactose content and MCP were positive and moderate, and were similar when data from either instrument were analyzed. Somatic cell score showed a moderately positive correlation with RCT obtained using either instrument; this was

also generally true for other MCP, although only a_{30} showed significantly low (and contradictory) coefficients, being negative when the FRM was used but positive when the OPT was used. Lastly, the correlation coefficients between pH and MCP were the highest, with the exception of a_{45}, confirming the fact that low pH facilitates milk coagulation and curd firming.

Table 5 shows the importance of the various effects included in the linear model in explaining the variation of MCP. In general, the coefficients of determination were moderate and ranged from 0.14 to 0.30, regardless of the instrument used. Days in milk was the most important source of variation ($P < 0.01$). A tendency toward worsening of MCP during the first phase of lactation was noted, with recovery becoming evident during the second phase (Figure 4). The effect of DIM on k_{20} and a_{45} was more pronounced when OPT rather than FRM data were analyzed. All MCP were significantly influenced by herd ($P < 0.05$); the maximum differences between the least squares means of 63 herds were 10.45 min (FRM) and 6.52 min (OPT) for RCT, 5.14 min (FRM) and 4.59 min (OPT) for k_{20}, 22.48 mm (FRM) and 17.56 mm (OPT) for a_{30}, and 18.80 mm (FRM) and 20.22 mm (OPT) for a_{45} (data not shown). Parity attained significance only when RCT and a_{45} obtained using the FRM were analyzed. For RCT, the least squares means increased from the first to the second parity and fell thereafter. For a_{45}, a negative tendency was evident with increasing parity (Figures 5a and 5d).
Finally, the only source of variation directly associated with instruments, being the MUCM, significantly affected MCP, except RCT; this was especially true for k_{20} values obtained using the FRM and a_{45} values measured with the OPT.

DISCUSSION

RENNET COAGULATION TIME AND THE PROPORTION OF NC SAMPLES

One of the most significant problems in the analysis, statistical treatment, and interpretation of MCP is the existence of milk samples that do not coagulate within 30 min after rennet addition (i.e., NC samples; Cecchinato et al., 2011). Noncoagulating milk is a problem in the dairy industry, and delivery of NC milk can sometimes invoke a penalty in terms of payment to producers (Calamari et al., 2005; Bittante et al., 2011a,b). In many countries, it has been found that selective cattle breeding has increased the number of cows producing NC milk (Malossini et al., 1996; Tyrisevä et al., 2003).

Although NC milk is of considerable practical relevance, NC samples are simply ignored in most reports on MCP. Alternatively, a different trait definition (i.e., occurrence of coagulation at a given time), involving categorization on a binary scale, has been used (Tyrisevä et al., 2004). In other instances, the frequency of NC samples is indeed reported, together with the average RCT of coagulated samples. Finally, in some cases, the experimental conditions are modified to limit the incidence of NC samples. This may be achieved by increasing the concentration of rennet added to milk at the beginning of lactodynamographic testing. To the best of our knowledge, only 2 comparisons between the FRM and OPT have been reported in the literature. In the first study, Kübarsepp et al. (2005) compared results yielded by the 2 instruments operating in 2 different laboratories. In the trial, a solution with very high coagulating activity (0.150 IMCU/mL) was used. The authors did not clearly state the incidence of NC samples, but the figures of the cited work indicated that only 1 of 81 samples from cows of various breeds did not coagulate using either instrument. The second study (Pretto et al., 2011) compared 3 instruments, 2 of which were mechanical (the FRM and the

computerized renneting meter) and 1 was optical (the OPT), running in 3 different laboratories. All samples analyzed using the 2 mechanical instruments and a subsample of those analyzed with the OPT received calf rennet at a concentration identical to that of the present work (0.051 IMCU/mL) and the detected incidence of NC samples was 19/165 (11.5%) using the FRM and 30/60 (50%) using the OPT. The same authors obtained only 1 NC sample from 165 Holstein-Friesian cows (0.6%) analyzing all samples with the OPT but increasing by 135% the enzymatic activity (0.120 IMCU/mL) and using a microbial coagulant, making these results not comparable with those obtained with the FRM. In this trial, using the (low) IMCU activity recommended by the supplier (0.051 IMCU/mL), we obtained 52 and 19 NC samples out of 913 samples from Brown Swiss cows analyzed with the FRM (6.57%) and the OPT (2.3%), respectively. Both of these NC proportions are similar to that (3.5%) found by Cecchinato et al. (2011) on a large experiment on Brown Swiss cows using a mechanical computerized renneting meter and a rennet concentration slightly higher (0.061 IMCU/mL) than that used in the present report. These NC frequencies confirmed the lower NC incidence of milk from Brown Swiss cows with respect to Holsteins (Malossini et al., 1996; Malacarne et al., 2005, 2006). As no reason exists to suggest that the same sample of milk maintained in the same laboratory, at the same temperature, upon addition of the same quantity and quality of rennet by the same technician, should coagulate at different times in either instrument, we speculate that the large difference between the 2 instruments/laboratories/dates found by Pretto et al. (2011) could be attributed to experimental conditions (age and conservation of samples, operational conditions, or instrument setting, or all of these), whereas operating in the same conditions, the small difference between the 2 instruments found in the present trial should be due to an anticipated coagulation time prediction by optical

lactodynamography, at least when late-coagulating samples were analyzed. It is clear that the OPT did not simply yield an estimate of coagulation time, but rather reduced the variability of such estimates, especially when late-coagulating samples were analyzed (Figure 1 and Figure 2a). This was confirmed by Levene's test of equality of variance (Table 2). We were able to obtain RCT values for all samples because we prolonged the observation time after rennet addition to 90 min. The presence of late-coagulating samples (now not dismissed as NC samples) explains most of the higher true average RCT values that resulted in the present work; as expected, our figures are greater than previous estimates obtained using the same breed but excluding the NC samples (Cecchinato et al., 2009, 2011).

The work of Kübarsepp et al. (2005) highlighted the different operative principles of the 2 instruments. In fact, the authors used an OPT that was not equipped with the software designed to yield outputs similar to those of the FRM; this software was developed only later. The initial coagulation time measured by the cited authors, using the OPT, was (on average) 30% lower (with an SD 51% lower) than was the RCT yielded by FRM analysis of the same samples. Gelification of milk, and the sudden modification of milk viscosity detected by the FRM (the singular point that defines the RCT) occurs toward the end of the first phase of coagulation (during which most κ-CN tails are cleaved by chymosin); this marks the beginning of phase 2 of coagulation, which features micelle aggregation and curd firming. Optigraph measurements are not rheological, rather using NIR optical signaling. During coagulation, the extent of light transmission through milk becomes gradually less because of changes in the micelle structure of casein. Kübarsepp et al. (2005) referred gelification to the point at which the derivative of the signal intensity curve was maximal. Scher and Hardy (1993), using an NIR reflectance probe at 860 nm, found that the maximal rate of increase in turbidity

was evident before visible clotting occurred. To make possible the maximal first derivative of signal intensity yielded by the OPT comparable to RCT measured using the FRM, Kübarsepp et al. (2005) developed a linear regression equation featuring a regression coefficient of 1.784 and an intercept of −2.303. Use of this equation showed, for their 81 milk samples, that the average RCT predicted using the OPT was very close to that of RCT measured by the FRM, but the OPT standard deviation value was slightly lower.

It thus appears to be evident that RCT measured by the FRM and OPT are essentially different traits, not only because of underlying differences in methodology, but also because different technological features are measured. Nevertheless, the values afforded by either instrument are correlated. Kübarsepp et al. (2005) obtained a correlation coefficient of 0.973 when RCT data obtained using the FRM were compared with either original or transformed RCT estimated by the OPT. Our results are consistent with those reported by Pretto et al. (2011) who calculated a correlation coefficient of 0.879. The regression coefficient calculated in the present work was 1.09 (thus, significantly different from 1.00) and was very similar to the slope estimated by Kübarsepp et al. (2005) after transformation of original data (1.115). The slope calculated by Pretto et al. (2011) was very high (2.141), but they compared RCT measures obtained after addition of a solution with a very different rennet type and a different coagulation activity. The intercepts varied accordingly, being −0.67 min in the present trial (thus, not significantly different from zero), but −1.12 and −3.66 min, respectively, for the transformed and original data of Kübarsepp et al. (2005) and +1.16 when the values of Pretto et al. (2011) were analyzed.

DETECTION AND EXTENT OF THE CURD-FIRMING RATE

The k_{20} value is probably the MCP of greatest practical importance in the dairy industry, indicating the optimal time at which curd-cutting should commence. Following results of previous studies (Bynum and Olson, 1982; Riddell-Lawrence and Hicks, 1989), Fagan et al. (2007) concluded that an optimum firmness exists at which the gel should be cut to achieve maximum retention of fat as well as an optimum curd moisture content that will maximize product yield and quality. In any case, k_{20} is seldom studied. This is because, especially when milk from slow-coagulating breeds such as Holstein-Friesians and some Scandinavian breeds is examined, a considerable proportion of samples does not attain a curd firmness of at least 20 mm over the usual 30-min test duration. This may explain why no previous report has compared k_{20} values obtained using mechanical and optical instruments, even if Payne et al. (1993) explored the possibility of predicting the cutting time of curd using the time from rennet addition and the inflection point of the diffuse reflectance curve. In the present work, prolongation of the observation period allowed estimation of k_{20} values for all samples.

At the usual time of test completion (thus, 30 min), the proportion of samples that did not attain a 20-mm curd firmness value was large, being 20.9 and 27.5% for FRM and OPT, respectively. The main reason for the between-instrument difference was that, on average, k_{20} values yielded by the OPT were 52% greater than were those measured using the FRM (Table 2). O'Callaghan et al. (2002), who reviewed the available systems used to monitor curd-setting during cheese making, found that the effects of photon scattering are influenced by the size of casein micelles and the extent of lattice formation; geometric and structural effects were, thus, explored at the microscopic level. Although these effects on coagulation are stronger before gel formation, some microstructural changes continue during curd firming. Optical

measurements afford only indirect measures of gel strength. A particular gel structure may be stronger than is another because of differences in chemical detail, although the structural geometry may be similar. For 2 decades, Payne et al. (1993) found that the inflection point of the reflectance curve alone does not allow a precise identification of optimal cutting time, as done by mechanical lactodynamographs, and that this information could be used in a multiple regression equation including also the protein content of milk, at least.

When correlations between MCP, on the one hand, and milk content data on the other were compared, the OPT k_{20} associations tended to be higher than were those obtained when FRM data were used (Table 4). This may be because both MCP obtained by OPT and milk composition data are derived using sample infrared responses. The k_{20} value yielded by the OPT thus differed greatly from the supposedly equivalent value afforded by use of the FRM; the practical and scientific meanings of the OPT value require further study.

CURD FIRMNESS MEASURED AT DIFFERENT TIMES AFTER RENNET ADDITION

Curd firmness is usually evaluated 30 min after enzyme addition. However, and especially if milk from a slow-coagulating breed is under study, the interval between gelification (i.e., the RCT time) and measurement of curd firmness is often brief. Under such circumstances, the a_{30} value is strongly dependent on the time interval between RCT and the 30th minute; this means that the correlation between RCT and a_{30} values is very high and the a_{30} value, thus, fails to add information beyond that yielded by the RCT (Bittante, 2011). In efforts to define more independent traits, some authors have extended the interval between enzyme addition and curd firmness measurement to 45

min (Mariani et al., 1997; Cecchi and Leotta, 2002) or 60 min (O'Brien et al., 2002; Auldist et al., 2004). Under such circumstances, the 2 traits become less interdependent, but the problem with delayed measurement of curd firmness is that this trait does not continuously increase toward an asymptotic value, but, because of syneresis, rather attains a maximum level and next tends to decrease. In the present trial, we report curd firmness data recorded 30 min (a_{30}) and 45 min (a_{45}) after rennet addition.

When data from the 2 instruments were compared, it was evident that the average values of FRM a_{30} measurements were slightly higher than estimates made by the OPT (30.09 vs. 27.23 mm, respectively; Table 2). Previously, Kübarsepp et al. (2005) estimated an a_{30} value that was about half that obtained using a mechanical instrument, but the data were expressed as a signal strength (in V) and not in millimeters of curd firmness. The cited authors constructed an equation facilitating interconversion of the 2 data sets. In contrast, Pretto et al. (2011), using an OPT device calibrated to mimic the FRM, found that the average a_{30} value was very similar to that obtained using an FRM, when similar rennet concentrations were used. Moreover, in the present work we found that a_{30} values estimated using the OPT were associated with standard deviations that were lower than those allied with measurements made via the FRM (Levene's test for equality of variance).

The extent of agreement between a_{30} values obtained using the 2 instruments was higher than that noted when k_{20} values were compared, but less than that apparent when RCT were analyzed (Figure 3c). Neither the slope nor the intercept of the linear regression equation reflected theoretical values. Kübarsepp et al. (2005) described a quadratic relationship between a_{30} data derived using the 2 instruments; the coefficient of determination was high. Pretto et al. (2011) found that the 2 types of a_{30} values exhibited a much lower correlation.

At 45 min after rennet addition, the extent of curd firmness measured by the FRM had increased by only 11% with respect to the a_{30} value whereas, using the OPT, the average increase was 49%. This variability is remarkably high, and application of the Levene's test of variance equality showed that the difference was significant (Table 1). The correlations between the 2 a_{45} series was very low (Figure 3d) and the parameters of the equation differed greatly from theoretical values.

CONCLUSIONS

Optical instruments that record NIR signals are promising tools, allowing milk coagulation and curd firming to be evaluated during the cheese-making process. Our results, obtained using a large data set, revealed that the OPT was less influenced by sensor characteristics than was the FRM. Milk coagulation properties obtained using the OPT are distinct from those yielded by the FRM; large differences in k_{20} and a_{45} average values were apparent when data from the 2 instruments were compared. The variances in RCT, a_{30}, and a_{45} differed; all MCP varied in terms of normality of distribution; the incidence of NC samples (as detected via RCT measurement) differed; the proportion of samples yielding estimable k_{20} values at a given time was not the same; moderate (a_{30}) or low (k_{20} and a_{45}) correlations were observed when identical measures by either instrument were compared; important differences were evident when correlations between RCT and k_{20}, measured by the same instrument, were compared; the phenotypic correlations of MCP with MY, milk composition, and pH varied and the relative importance of DIM (on k_{20} and a_{45}), parity (on a_{45}), and MUCM (on k_{20} and a_{30}), were not the same. Rennet coagulation time obtained using the OPT is the only MCP that was in good agreement with the value measured by the FRM, with the exception of late-coagulating samples and those that did not coagulate 30 min after

addition of rennet. The curd firmness characteristics estimated using the OPT differed from those calculated by the FRM. The practical significance of FRM data are well understood; data afforded by the OPT require further evaluation toward this end.

Finally, the OPT algorithm has apparently been constructed to mimic traditional lactodynamography, especially over the usual test duration of 30 min. Beyond this time (when RCT values for late-coagulating samples and a_{45} figures may be calculated), discrepancies between FRM and OPT data increased notably; the optical instrument needs to be carefully calibrated if it is to operate in this time region. Possibly, new optical instruments, rather than mimicking traditional mechanical lactodynamographs, could model the signals obtained to derive new parameters correlating with useful milk technological properties, especially to its modification during the first phase of coagulation, before visible clotting.

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TABLES AND FIGURES

Table 1. Descriptive statistics (n = 913) of milk yield and milk quality traits[1]

Trait[2]	Mean	SD	P1	P99
Milk Yield, kg/d	24.36	8.02	8.50	45.00
DIM, d	196	135	12	656
Milk fat, %	4.23	0.70	2.66	6.15
Milk protein, %	3.71	0.42	2.86	4.72
Casein, %	2.89	0.32	2.26	3.68
SCS,[2] units	3.03	1.88	-0.56	7.86

[1]P1 = first percentile; P99 = 99th percentile.
[2]SCS = log2(SCC/100,000) + 3.

Table 2. Descriptive statistics (n = 913) of milk coagulation properties obtained by using Formagraph (Foss Electric A/S, Hillerød, Denmark) and Optigraph (Ysebaert SA, Frépillon, France) instruments

	Formagraph		Optigraph		Equality of
Trait[1]	Mean	SD	Mean	SD	variance test[2]
RCT, min	19.95	5.81	18.91	4.40	***
k_{20}, min	5.36	3.12	8.16	2.97	NS
a_{30}, mm	30.21	12.90	27.92	11.35	***
a_{45}, mm	33.66	8.43	41.49	11.54	***

[1]RCT = rennet coagulation time of all samples (including those coagulating after 30 min after enzyme addition); k_{20} = curd-firming time (including those reaching 20 mm of curd firmness after 30 min after enzyme addition); a_{30} = curd firmness at 30 minutes (excluding 47 and 18 samples samples coagulating after 30 min after enzyme addition for Formagraph and Optigraph instruments, respectively); a_{45} = curd firmness at 45 minutes.
[2]Levene's test.
*** $P < 0.001$; NS = not significant.

Table 3. Pearson product-moment correlations among milk coagulation properties (MCP) obtained by using a Formagraph (Foss Electric A/S, Hillerød, Denmark) and among MCP obtained by using an Optigraph (Ysebaert SA, Frépillon, France)

Trait[1]		Formagraph	Optigraph
RCT with:			
	k_{20}	0.65^{***}	0.32^{***}
	a_{30}	-0.83^{***}	-0.75^{***}
	a_{45}	-0.18^{***}	-0.28^{***}
k_{20} with:			
	a_{30}	-0.74^{***}	-0.72^{***}
	a_{45}	-0.57^{***}	-0.82^{***}
a_{30} with:			
	a_{45}	0.51^{***}	0.78^{***}

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 minutes; a_{45} = curd firmness at 45 minutes.
$^{***}P < 0.001$.

Table 4. Pearosn product moment correlations between milk coagulation properties obtained using a Formagraph (FRM; Foss Eletric A/S, Hillerød, Denmark) or an Optigraph (OPT; Ysebaert SA, Frépillon, France) and milk production, composition, and acidity

	MILK COAGULATION PROPERTY[1]							
	RCT		k_{20}		a_{30}		a_{45}	
Trait	FRM	OPT	FRM	OPT	FRM	OPT	FRM	OPT
Milk Yield, kg/d	-0.14^{***}	-0.14^{***}	-0.02^{ns}	0.15^{***}	0.04^{ns}	-0.04^{ns}	-0.06^{ns}	-0.17^{***}
Milk fat, %	0.02^{ns}	0.04^{ns}	-0.08^{**}	-0.31^{***}	0.07^{*}	0.20^{***}	0.17^{***}	0.33^{***}
Milk protein, %	0.27^{***}	0.36^{***}	-0.18^{***}	-0.47^{***}	0.04^{ns}	0.13^{***}	0.40^{***}	0.50^{***}
Casein, %	0.25^{***}	0.34^{***}	-0.18^{***}	-0.49^{***}	0.06^{ns}	0.15^{***}	0.41^{***}	0.51^{***}
Lactose, %	-0.22^{***}	-0.23^{***}	-0.19^{***}	-0.09^{**}	0.15^{***}	0.14^{***}	0.09^{**}	0.05^{ns}
SCS	0.14^{***}	0.18^{***}	0.03^{ns}	-0.06^{ns}	-0.07^{*}	0.10^{**}	0.007^{ns}	0.01^{ns}
pH	0.41^{***}	0.39^{***}	0.27^{***}	0.33^{***}	-0.35^{***}	-0.42^{***}	-0.07^{*}	-0.29^{***}

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 minutes; a_{45} = curd firmness at 45 minutes.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{ns}not significant.

Table 5. Significance (Fisher exact test values and *P*-values) of model effect for ANOVA of milk coagulation properties obtained using a Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark) or an Optigraph (OPT; Ysebaert SA, Frépillon, France)

	MILK COAGULATION PROPERTY[1]							
	RCT		k_{20}		a_{30}		a_{45}	
Item[2]	FRM	OPT	FRM	OPT	FRM	OPT	FRM	OPT
Herd	3.29^{***}	2.87^{***}	1.37^{*}	1.67^{**}	2.06^{***}	2.19^{***}	3.87^{***}	2.13^{***}
DIM	22.25^{***}	25.19^{***}	3.35^{**}	13.84^{***}	6.75^{***}	4.27^{***}	4.96^{***}	17.78^{***}
Parity	2.71^{**}	2.22^{ns}	0.23^{ns}	2.52^{ns}	1.02^{ns}	0.52^{ns}	4.12^{**}	0.79^{ns}
MUCM	0.84^{ns}	0.53^{ns}	2.43^{**}	1.62^{ns}	2.59^{**}	0.85^{ns}	2.86^{***}	2.26^{**}
R^2, %	0.30	0.29	0.14	0.19	0.20	0.18	0.28	0.25
RMSE	5.11	3.88	3.03	2.80	12.09	10.81	7.50	10.51

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 minutes; a_{45} = curd firmness at 45 minutes.
[2]Measuring unit of the coagulation meter; RMSE = root mean square error.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{ns}not significant.

Figure 1. Distribution of rennet coagulation time (RCT, min), curd-firming time (k_{20}, min), curd firmness at 30 (a_{30}, mm) and 45 minutes (a_{45}, mm) obtained by using Formagraph (Foss Electric A/S, Hillerød, Denmark) and an Optigraph (Ysebaert SA, Frépillon, France). For a_{30}, the null values relative to noncoagulating milk after 30 min also are plotted

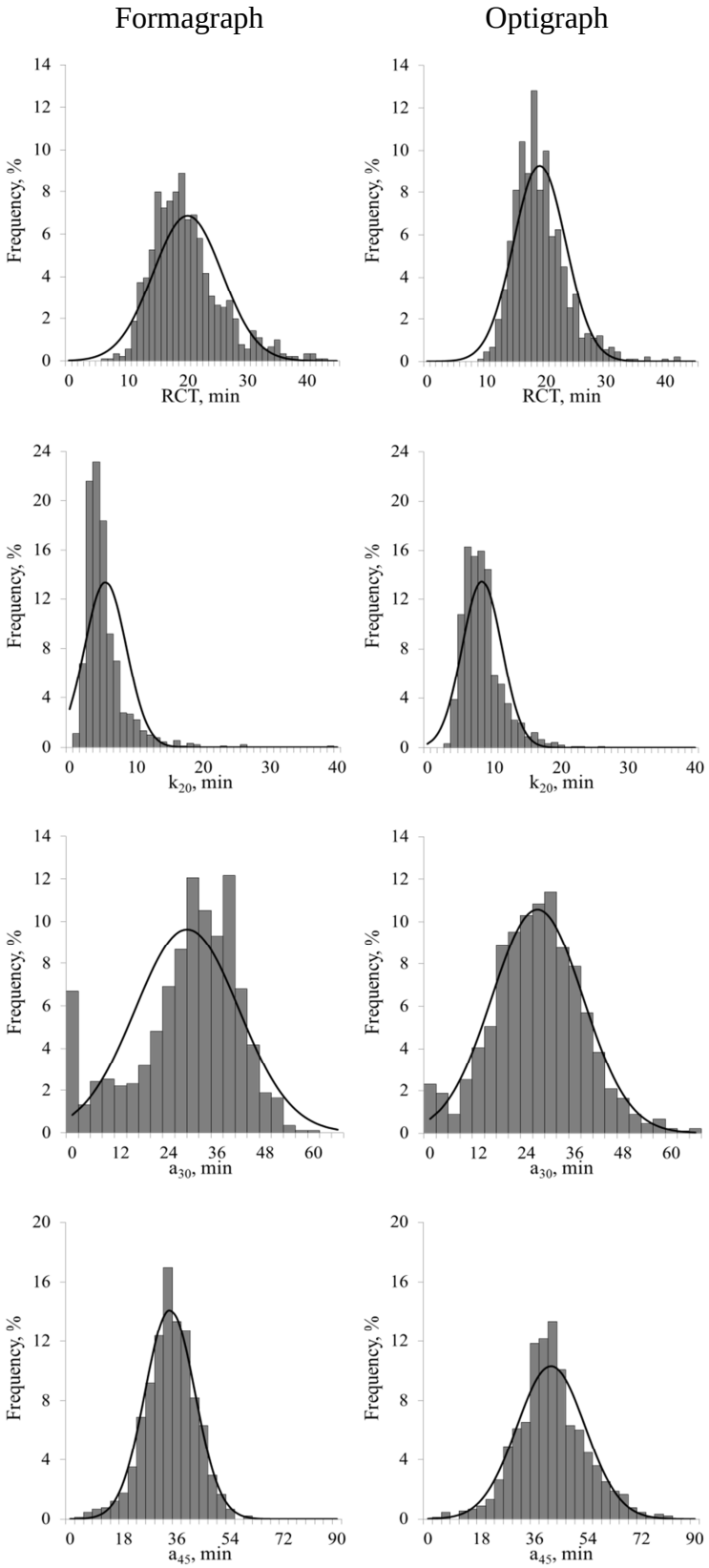
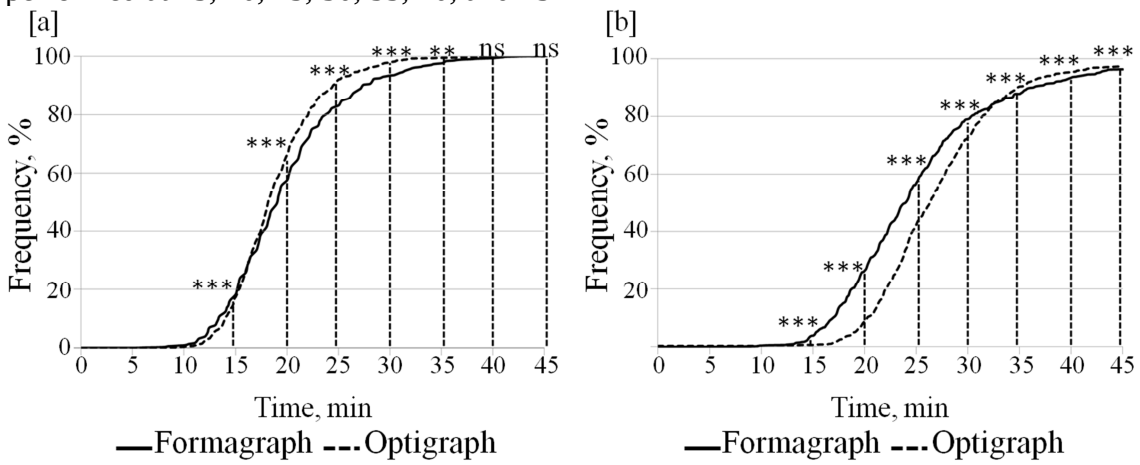


Figure 2. Cumulative frequency distribution against [a] time from enzyme addition of rennet coagulation time (RCT, min) and [b] RCT+curd-firming time (RCT+k_{20}, min) obtained by using a Formagraph (Foss Electric A/S, Hillerød, Denmark) and an Optigraph (Ysebaert SA, Frépillon, France). The Fisher's exact test ($P < 0.05$) was performed at 15, 20, 25, 30, 35, 40, and 45 min



***$P < 0.001$; **$P < 0.01$; ns = not significant.

Figure 3. Relationship between milk coagulation properties obtained by using Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark) and Optigraph (OPT; Ysebaert SA, Frépillon, France) instruments: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k_{20}, min), (c) curd firmness at 30 min (a_{30}, mm), and (d) curd firmness at 45 min (a_{45}, mm). Significance of the *F*-test was computed for a slope different from 1 and intercept different from 0 ($P < 0.05$). a = intercept; b = slope; ns = nonsignificant. ***$P < 0.001$

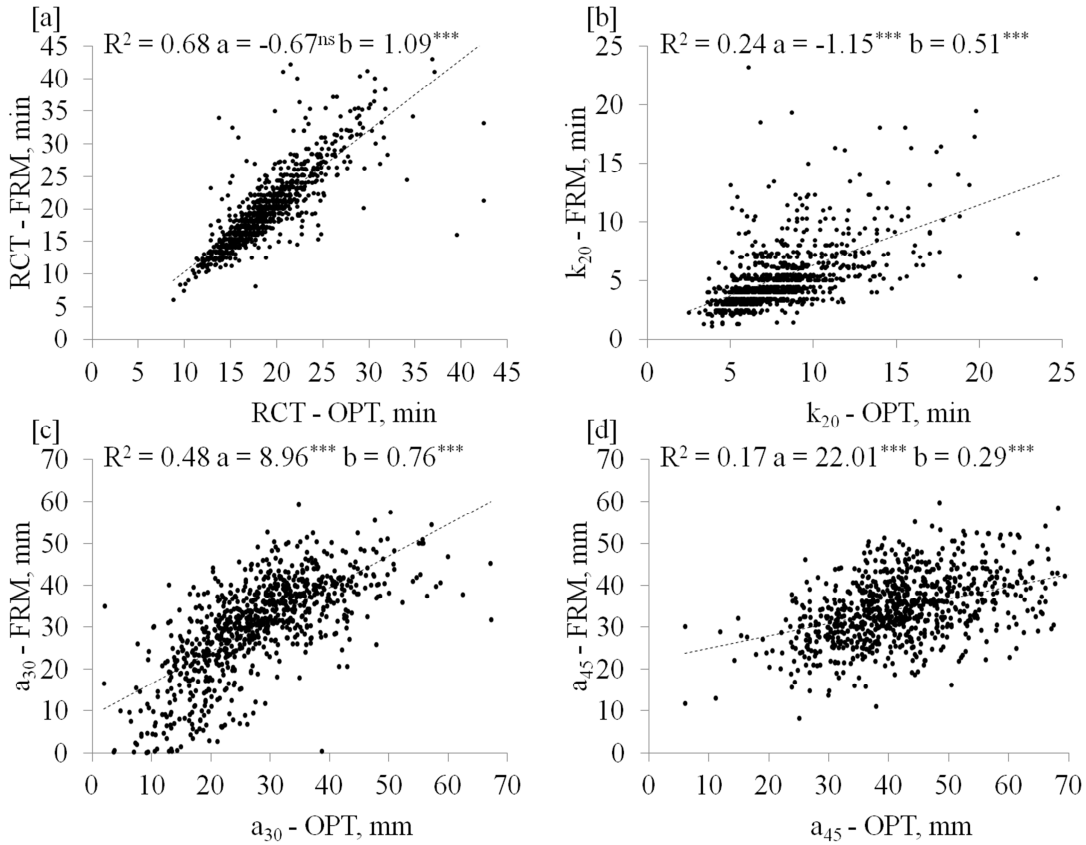


Figure 4. Least squares means of milk coagulation properties (MCP) obtained with 2 different instruments across DIM: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k20, min), (c) curd firmness at 30 min (a30, mm), and (d) curd firmness at 45 min (a45, mm). Solid lines represent data from a Formagraph (Foss Electric A/S, Hillerød, Denmark) and dashed lines represent data from an Optigraph (Ysebaert SA, Frépillon, France). Error bars represent the SEM

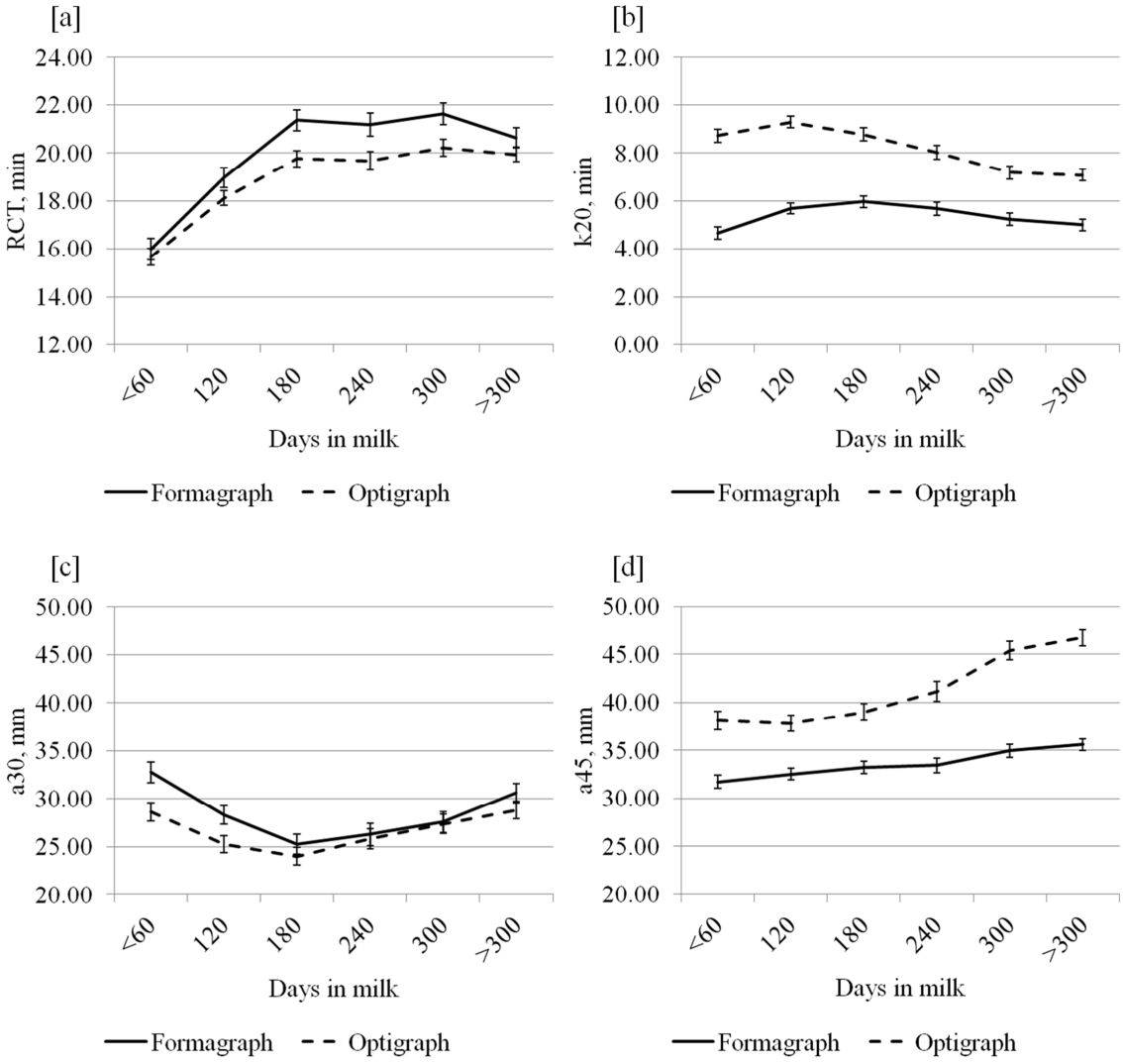
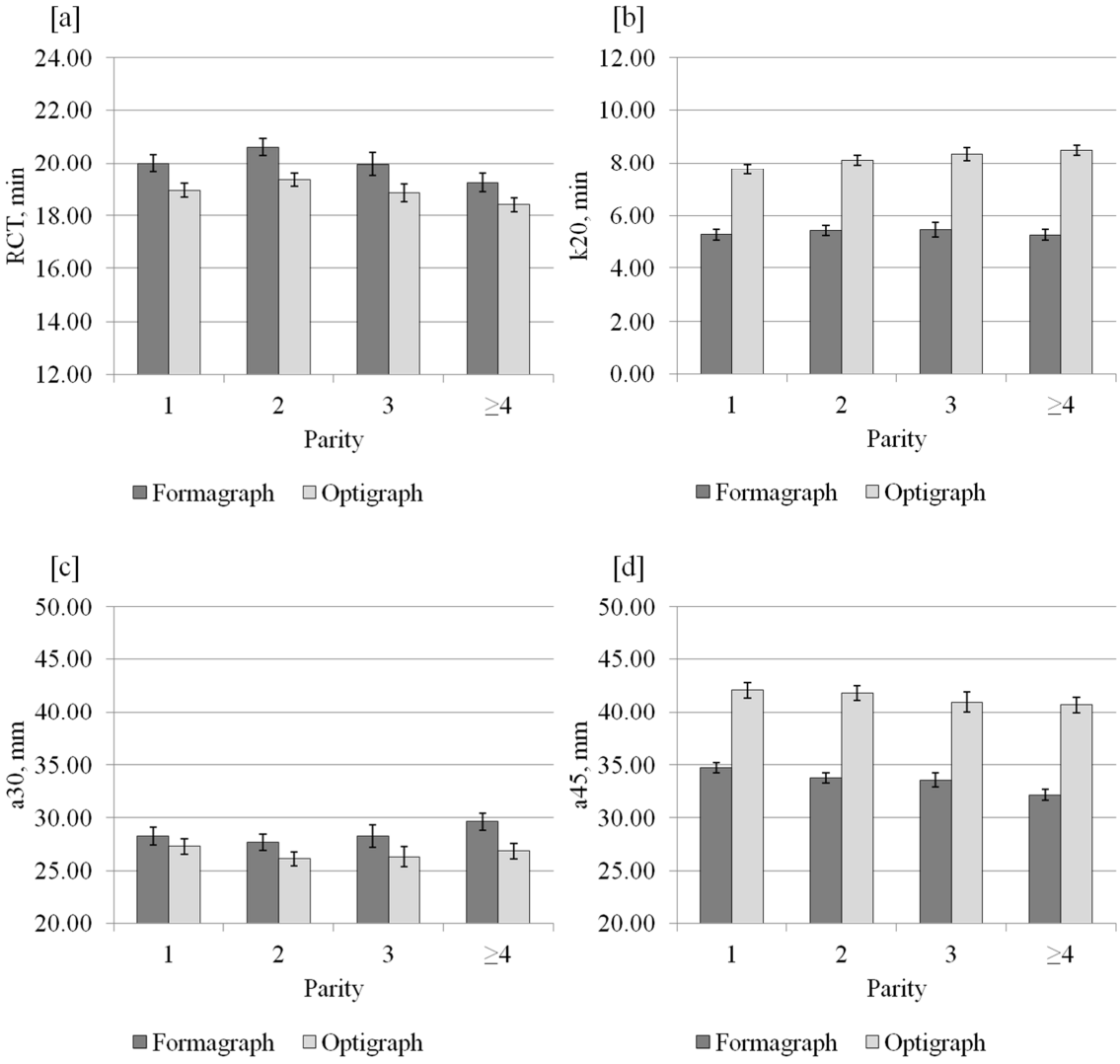


Figure 5. Least squares means of milk coagulation properties (MCP) obtained with 2 different instruments across parities: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k_{20}, min), (c) curd firmness at 30 min (a_{30}, mm), and (d) curd firmness at 45 min (a_{45}, mm). Dark gray bars represent data from a Formagraph (Foss Electric A/S, Hillerød, Denmark) and light gray bars represent data from an Optigraph (Ysebaert SA, Frépillon, France). Error bars represent the SEM



II CONTRIBUTION

GENETIC ANALYSIS OF RENNET COAGULATION TIME, CURD-FIRMING RATE, AND CURD FIRMNESS ASSESSED OVER AN EXTENDED TESTING PERIOD USING MECHANICAL AND NEAR-INFRARED INSTRUMENTS

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ABSTRACT

The aims of this study were (1) to analyze rennet coagulation time (RCT), curd-firming rate, and curd firmness obtained by extending the standard 30-min testing time to 45 min; (2) to estimate heritabilities of the aforementioned traits determined by mechanical (Formagraph; Foss Electric, Hillerød, Denmark) and near-infrared optical (Optigraph; Ysebaert, Frépillon, France) instruments, and to assess the statistical relevance of their genetic background by using the Bayes factor procedure, the deviance information criterion, and the mean squared error; (3) to estimate phenotypic and genetic relationships between instruments within trait and between traits within instrument; and (4) to obtain correlations for sire rankings based on the used instruments. Individual milk samples were collected from 913 Brown Swiss cows reared in 63 herds located in Trento Province (Italy). Milk coagulation properties (MCP) were measured using 2 different instruments: Formagraph and Optigraph. Both instruments were housed in the same laboratory and operated by the same technician. Each sample was analyzed simultaneously on each instrument. All experimental conditions (milk temperature and the concentration and type of rennet) were identical. For the analysis, univariate and bivariate animal models were implemented using Bayesian methods. Univariate analyses were conducted to test the hypothesis that the traits showed additive genetic determination. Deviance information criterion, Bayes factor, and mean squared error were used as model choice criteria. The main results were that (1) RCT could be measured on all samples by extending the observation time to 45 min, and its genetic parameters ($h^2 = 0.23$) and breeding values could be estimated while avoiding the bias of noncoagulating samples; (2) curd-firming rate could be measured on almost all milk samples, and its genetic parameters could be estimated for the first time on a field data set ($h^2 = 0.21$); (3) for the first time, genetic parameters of

curd firmness 45 min after rennet addition (h^2 = 0.12) were estimated, and they were compared with curd firmness 30 min after rennet addition (h^2 = 0.17); and (4) MCP estimated using the Optigraph appeared to be genetically different from those determined by Formagraph, with the partial exception of RCT (genetic correlation = 0.97). Breeding strategies for the improvement of MCP must be planned with caution. Currently, the high throughput, ease of use, and reduced costs of analysis make predictions obtained from mid-infrared spectroscopy (MIRS) on untreated milk samples a promising alternative to produce relevant data at the population level. The use of mechanical lactodynamographs to establish reference data for MIRS calibrations have been already studied, whereas the use of near-infrared optical lactodynamographs as a reference method for MIRS calibrations needs to be investigated.

Key words: milk coagulation property, mechanical and optical lactodynamograph, heritability, Bayes factor

INTRODUCTION

Measurement of milk coagulation properties (**MCP**) is of special relevance for cheese manufacturing. The most used instruments to assess MCP are lactodynamographs (renneting meters) by which rennet coagulation time (**RCT**, min), curd-firming time ($\mathbf{k_{20}}$, min), and curd firmness are measured after addition of the clotting enzyme to raw milk (Annibaldi et al., 1977; Zannoni and Annibaldi, 1981; McMahon and Brown, 1982). Lactodynamographs record physicochemical changes occurring in milk during the coagulation process when the enzyme hydrolyzes κ-casein aggregates and induces changes in milk viscosity and elasticity (Auldist et al., 2001; O'Callaghan et al., 2002).

Several systems have been adopted to determine MCP in cow's milk. Traditionally, laboratory mechanical instruments such as the Formagraph (**FRM**; Foss Electric, Hillerød, Denmark) and the Computerized Renneting Meter (Polo Trade, Monselice, Italy) have been used, whereas optical instruments based on infrared technologies have been often used to monitor MCP directly in the cheese-making vats (Payne et al., 1993; Laporte et al., 1998; O'Callaghan et al., 2002). Recently, 2 categories of infrared optical instruments have been adopted to predict MCP at laboratory level. The first includes medium infrared spectrometers (**MIRS**) to predict MCP from raw milk samples analyzed without induction of rennet coagulation (Dal Zotto et al., 2008; De Marchi et al., 2009). The second includes lactodynamographs such as the Optigraph (**OPT**; Ysebaert, Frépillon, France), which has been proposed to determine MCP through induction of rennet coagulation of milk (Panari et al., 2002; Kübarsepp et al., 2005). The 2 types of lactodynamographs record the same parameters on coagulating samples but using different principles. Mechanical measures are based on continuous recording of the movement, after the immersion of small loop pendulum in linearly oscillating samples of coagulating milk, induced by minute forces applied to the pendulum as a consequence of the milk coagulation (McMahon and Brown, 1982). The optical instrument continuously measures the optical signal in the near-infrared (NIR) region (820 nm) and estimates the MCP by means of specific calibration equations. Recently, a phenotypic study by Cipolat-Gotet et al. (2012a) demonstrated that FRM and OPT yield different results with the partial exception of RCT.

Several studies have been carried out to estimate genetic parameters of MCP measured by mechanical lactodynamographs (e.g., Lindström et al., 1984; Ikonen et al., 2004; Cecchinato et al., 2012c), and recently one study dealt with OPT (Vallas et al., 2010). No direct comparisons between genetic parameters of MCP obtained from

mechanical and optical instruments are currently available. Moreover, past research on genetic aspects of MCP dealt with renneting parameters determined for 30 min after the addition of the clotting enzyme, and faced the problem of milk samples that do not coagulate within the testing time of 30 min (the so-called noncoagulating samples, NC) and of the potential bias in the estimation of genetic parameters of MCP and breeding values of animals (Cecchinato and Carnier, 2011). A significant fraction of milk samples do not usually attain curd firmness of 20 mm within the 30-min testing time; hence, k_{20} is often excluded from genetic analyses. Only a few studies have estimated genetic parameters for k_{20}, and they were based on a small number of cows reared on experimental farms (Tervala et al., 1985; Ikonen et al., 1997).

Therefore, the objectives of this study were (1) to analyze RCT, k_{20}, and curd firmness obtained by extending the standard 30-min testing time (a_{30}) to 45 min (a_{45}); (2) to estimate heritabilities of the aforementioned traits measured by FRM and OPT instruments, and to assess the statistical relevance of their genetic background by using the Bayes factor (**BF**) procedure, the deviance information criterion (**DIC**), and the mean squared error (**MSE**); (3) to estimate phenotypic and genetic relationships between instruments within trait and between traits within instrument; and (4) to obtain correlations for sire rankings based on the instruments used.

MATERIALS AND METHODS

FIELD DATA

In total, 913 Brown Swiss cows from 63 herds located in Trento Province (Italy) were sampled once during the evening milking between April 2010 and February 2011. Within a given day, only one herd was sampled. Two milk subsamples per cow were

collected and immediately refrigerated at 4°C without preservative. One random subsample was transported to the Milk Quality Laboratory of the Breeders Federation of Trento Province (Trento, Italy) for composition analysis. The other subsample was transferred to the Cheese-Making laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy) for MCP analysis. All samples were processed within 20 h of collection. Information on cows and herds were provided by the Breeders Federation of Trento Province (Italy). Pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy) and included cows with phenotypic records for the investigated traits and all their known ancestors.

ANALYSIS OF MILK QUALITY

Individual milk subsamples were analyzed for fat, protein, and casein contents using MilkoScan FT6000 (Foss). Somatic cell count was obtained from the Fossomatic FC counter (Foss) and was then converted to SCS by means of logarithm transformation (Ali and Shook, 1980). The pH of the subsamples was measured before MCP analysis, using a Crison Basic 25 electrode (Crison, Barcelona, Spain).

ANALYSIS OF MILK COAGULATION PROPERTIES

Milk coagulation properties were determined using FRM and OPT. Both instruments were housed in the same laboratory and operated by the same technician. Each subsample was analyzed simultaneously on each instrument. All experimental conditions (milk temperature and the concentration and type of rennet) were identical. Two racks containing 10 cuvettes (one rack per instrument) were prepared; milk samples (10 mL) were heated to 35°C and 200 μL of rennet solution (Hansen Standard

160, with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.6% (wt/vol) in distilled water, to yield 0.051 international milk clotting units (IMCU)/ mL, was added to samples after milk heating. Both instruments yield the width (mm) of the oscillatory graph during testing: the OPT records a datum every 6 s and the FRM every 15 s. The observation period continued for 90 min after rennet addition but, for the purposes of the present work, only the first 45 min were considered. Variations in absorbance, as detected by OPT, were transformed by instrument software using an appropriate calibration equation to mimic the shape of the graph afforded by traditional mechanical instruments (Kübarsepp et al., 2005). This means that MCP can be calculated using either device. The MCP recorded were (1) the time from addition of enzyme to the beginning of visible coagulation (gelification) within a time interval of 45 min (RCT, min); (2) the interval from gelification (RCT) to the time at which the width of the graph attained 20 mm (k_{20}, min); (3) the firmness of the curd at 30 min from rennet addition (a_{30}, mm); and (4) the firmness of the curd at 45 min from rennet addition (a_{45}, mm). Samples that did not coagulate within 30 min were classified as NC (Ikonen et al., 1999), although extension of analysis allowed RCT and k_{20} to be recorded for all samples.

STATISTICAL ANALYSIS

Nongenetic Effects. Nongenetic effects to be included in mixed models to estimate genetic parameters for MCP determined by FRM and OPT were identified through preliminary analysis based on the GLM procedure (SAS Inst. Inc., Cary, NC). For all traits, the model accounted for the effects of herd (63 levels), DIM (class 1: <60 d, class 2: 60–120 d, class 3: 121–180 d, class 4: 181–240 d, class 5: 241–300 d, and class 6: >300 d), parity (1 to 4 or more), and renneting meter sensor (10 levels) of the

lactodynamograph, being the pendula of FRM and the monochromators of OPT. All these effects were important sources of variation ($P < 0.05$) except for the renneting meter sensor for RCT and k20.

Univariate Models for Testing the Hypothesis of Additive Genetic Determination. The genetic background of the MCP (y) was investigated by analyzing data under the following hierarchical model:

$$y = Xb + Z_1h + Z_2a + e, \qquad [1]$$

where y was the vector of phenotypic records with dimension *n*; X, Z_1, and Z_2 were appropriate incidence matrices for systematic effects (b), herd-date effects (h), and polygenic additive genetic effects (a), respectively; and e was the vector of residual effects. Specifically, b included nongenetic effects of DIM, parity, and renneting meter sensor (only for a_{30} and a_{45}).

All models were analyzed under a standard Bayesian approach. The joint distribution of the parameters in the model was proportional to

$$p(\mathbf{b}, \mathbf{p}, \mathbf{a}, \sigma_e^2, \sigma_p^2, \sigma_a^2|\mathbf{y}) \propto p(\mathbf{y}|\mathbf{b}, \mathbf{p}, \mathbf{a}, \sigma_e^2)p(\sigma_e^2)p(\mathbf{b})$$
$$\times p(\mathbf{p}|\sigma_p^2)p(\sigma_p^2)p(\mathbf{a}|\mathbf{A}, \sigma_a^2)p(\sigma_a^2),$$

where A was the numerator relationship matrix between individuals (Wright, 1922), and σ_e^2, σ_h^2, and σ_a^2 were the residual, herd, and additive genetic variance, respectively. The a priori distribution of **p** and **a** were assumed to be multivariate normal as follows:

$$p(\mathbf{p}|\sigma_p^2) \sim N(\mathbf{0}, \mathbf{I}\sigma_p^2)$$

$$p(\mathbf{a}|\sigma_a^2) \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2),$$

where **I** was an identity matrix with dimensions equal to the number of elements in **h**. Priors for **b** and variance components were assumed to be flat.

The univariate model was used to test for additive genetic determination of each trait. Different criteria were used for this purpose. The DIC (Spiegelhalter et al., 2002)

was computed both for the model including the additive genetic effect and for the reduced model without this effect; differences in DIC of more than 7 units were considered important (Spiegelhalter et al., 2002). The BF (Kass and Raftery, 1995; García-Cortés et al., 2001; Casellas et al., 2010) was computed as a pair-wise comparison by calculating the ratio between the posterior probabilities of 2 competing models, taking any positive value between >0 and +∞. In this case, a linear mixed model with additive polygenic effects (numerator model) was compared against a model without additive polygenic effects (denominator model), where >1 BF favored the numerator model and <1 BF favored the denominator model. In this report, the BF results were discussed within the context of the Jeffreys (1984) discrete scale of evidences. This scale classifies the BF according to 6 levels of evidence for the numerator model, objectively classifying the BF as follows: denominator model supported, not worth more than a bare mention, substantial evidence, strong evidence, very strong evidence, and decisive evidence. From now on, this terminology will be systematically used when referring to the BF. The MSE between real and predicted phenotypic records was also used to compare models (i.e., the one with additive polygenic effects and the same model without additive polygenic effects). For all MCP traits, the expectation of the predictive distribution of a given record was computed as in Varona et al. (1999):

$$\hat{y}_{MCPi=}\mathbf{x}_i\hat{\beta} + \mathbf{z}_{1i}\hat{\mathbf{h}} + \mathbf{z}_{2i}\hat{\mathbf{a}} - \hat{\mathbf{e}}_i$$

Where $\hat{y}_{MCPi}$ is the expectation fro the *i*th MCP record, $\mathbf{x}_i$, $\mathbf{z}_{1i}$, $\mathbf{z}_{2i}$ are the *i*th rows of the incidence matrices that link systematic, herd/date, and additive genetic effects and $\hat{\mathbf{e}}_i$ are the residuals for the *i*th MCP record. Note that $\hat{\beta}$, $\hat{\mathbf{h}}$, $\hat{\mathbf{a}}$ and $\hat{\mathbf{e}}_i$ are posterior median estimates. The MSE was defined as:

$$\text{MSE} = \frac{\sum_{i=n}^{n}(y_i - \hat{y}_i)^2}{n}$$

Bivariate Models for Estimating Correlations Between Traits. To estimate genetic correlations between MCP variables, a set of bivariate analyses were conducted, implementing model [1] in its multivariate version. In this case, the involved traits were assumed to jointly follow a multivariate normal distribution as well as the additive genetic, herd/date, and residual effects. For these effects, the corresponding prior distributions were:

$$\mathbf{a}|\mathbf{G}_0, \mathbf{A} \sim MVN(0, \mathbf{G}_0, \otimes\mathbf{A}),$$

$$\mathbf{h}|\mathbf{H}_0, \sim N(0, \mathbf{H}_0, \otimes\mathbf{I}_n), \text{ and}$$

$$\mathbf{e}|\mathbf{R}_0, \sim N(0, \mathbf{R}_0, \otimes\mathbf{I}_m),$$

where $\mathbf{G}_0, \mathbf{H}_0, \mathbf{R}_0$ were the corresponding variance-covariance matrices between the involved traits, and $\mathbf{a}$, $\mathbf{h}$, and $\mathbf{e}$ were vectors of dimension equal to the number of animals in the pedigree (n and m) times the number of traits considered.

Gibbs Sampler. Marginal posterior distributions of unknown parameters were estimated by performing numerical integration using the Gibbs sampler (Gelfand and Smith, 1990). This was employed to obtain auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and of the burn-in period were assessed by visual inspection of trace plots, as well as by the diagnostic tests of Geweke (1992) and Gelman and Rubin (1992). After a preliminary run, we decided to construct a single chain consisting of 850,000 iterations and to discard the first 50,000 iterations as a very conservative burn-in. Subsequently, one of every 200 successive samples was retained, to store draws that were more loosely correlated. Thus, 4,000 samples were used to determine posterior distributions of unknown parameters. The lower and upper bounds of the highest 95% probability density regions (**HPD95%**) for parameters of concern were obtained from the estimated marginal densities. The posterior median was

used as the point estimate for all parameters. Auto-correlations between samples and estimates of Monte Carlo standard error (Geyer, 1992) were calculated.

Heritability was computed as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_H^2 + \sigma_E^2}$$

where σ_A^2, σ_H^2, and σ_E^2 are additive genetic, herd/date, and residual variances, respectively.

Additive genetic correlations were estimated as:

$$r_A = \frac{\sigma_{A1,A2}}{\sigma_{A1} \cdot \sigma_{A2}}$$

where $\sigma_{A1,A2}$ is the additive genetic covariance between trait 1 and 2, and σ_{A1} and σ_{A2} are the additive genetic standard deviations for traits 1 and 2, respectively.

RESULTS

MILK COAGULATION PROPERTIES AND PHENOTYPIC VARIATION

Descriptive statistics for MCP from FRM and OPT are summarized in Table 1. A comprehensive discussion on the phenotypic pattern of milk coagulation and curd firming from mechanical and NIR instruments has been reported by Cipolat-Gotet et al. (2012a). In general, the average RCT from OPT was slightly shorter (1 min) than the value from FRM. Despite this, a_{30} was approximately 3 mm smaller when assessed by OPT than by FRM. Curd-firming time was notably longer when evaluated by OPT (8.16 min) than by FRM (5.36 min). Finally, a_{45} was about 8 mm greater when assessed by OPT (41.49 mm) than by FRM (33.65 mm). Rennet coagulation time and a_{30} from OPT were less variable than the corresponding MCP from FRM, whereas the opposite was found for a_{45}. Variances of MCP from FRM and OPT were statistically different

according to Levene's test ($P < 0.05$; data not shown) with the exception of k_{20}. In fact, despite mean values being notably different between instruments, k_{20} values exhibited similar standard deviations. Consequently, the proportion of NC samples at 30 min (samples without a detectable a_{30}) was 6.57% for FRM and 2.08% for OPT ($P < 0.05$; data not shown). The extension of MCP analysis up to 45 min allowed the RCT recording for all samples and k_{20} recording for most late-coagulating milks. The late-coagulating milks also affected the distribution of a_{30} and k_{20}, whereas a_{45} showed close to Gaussian distribution.

HERITABILITY OF MILK COAGULATION PROPERTIES

Point estimates (median of the marginal posterior density of the parameter) for the additive genetic, herd/date, and residual variances, and heritabilities of MCP measured by FRM and OPT are shown in Table 2, and the estimated posterior densities of the heritabilities are depicted in Figure 1. All Monte Carlo standard errors were very small, and a lack of convergence was not detected by the Geweke test (data not shown; Geweke, 1992). Marginal posterior distributions were approximately normal; thus, mode, mean, and median were similar, and only the posterior median is reported.

Rennet coagulation time was moderately heritable, and estimates were very similar between instruments (0.230 and 0.241 for FRM and OPT, respectively) with HPD95% between 0.10 and 0.40 both for FRM and OPT. The posterior densities were symmetric either for FRM and OPT and their shape was similar and almost overlapping. Even though heritability estimates were similar, the additive genetic, herd-date, and residual variances were 41.4, 51.1, and 43.5% lower when RCT was determined by OPT than by FRM.

Posterior density distribution and point estimates of heritability for k_{20} yielded by FRM were very close to those obtained for RCT, whereas the corresponding features from OPT data were much different. Heritability of k_{20} from OPT was almost twice the value found for the corresponding MCP of FRM and of RCT of both instruments. Moreover, the posterior densities (Figure 1) of the heritability for k_{20} obtained using OPT were more dispersed (HPD95% between 0.15 and 0.61) than those obtained by FRM, indicating more uncertainty in the estimation of this parameter. The high value of heritability was the consequence of the notably higher additive genetic and lower residual variance of OPT compared with FRM (+48.8% and −33.4%, respectively; Table 2). Estimates of variance due to herd-date effects on this MCP were also higher for OPT than for FRM (+26.9%).

Point estimates of heritability for a_{30} were slightly lower than those estimated for RCT and were comparable between instruments (0.171 for FRM and 0.205 for OPT; Table 2 and Figure 1). In addition, the HPD95% were similar between instruments, varying from 0.04 to 0.40. Estimates of additive genetic and residual variance were not very different between instruments (+13.2% and −14.3%, respectively), whereas herd-date variance was much higher (+64.1%) for OPT than for FRM.

Results for a_{45} were extremely variable and inconsistent between instruments. Point estimate of additive genetic variance from OPT was almost 5 times that from FRM. This explains the large differences in heritability for a_{45} assessed by FRM (0.120) and OPT (0.309), also in terms of variation of the point estimate (HPD95% from 0.02 to 0.27 for FRM, and 0.13 to 0.51 for OPT) as clearly depicted in Figure 1. Even in this case, estimates of heritability for a_{45} obtained using OPT were characterized by more uncertainty.

The inclusion of the additive polygenic effect improved the goodness of fit of the model for all MCP, regardless of the instrument used to assess MCP (Table 3). In particular, DIC and MSE decreased when the additive polygenic effect was accounted for in the analysis, suggesting a better fitting. The decrease in DIC ranged between 60 and 144 units. The BF confirmed the relevance of including the polygenic effect in the model, particularly in the analysis of RCT, and of a_{30} and a_{45} assessed using OPT. The BF between models with and without a genetic component, in fact, gave values >1 for all traits, providing evidence that the model was preferable when additive polygenic effects were included. The BF >100 indicated "decisive evidence" of genetic determinism for RCT yielded by both lactodynamographs, and for a_{30} and a_{45} obtained with OPT.

RELATIONSHIPS AMONG MILK COAGULATION PROPERTIES

Point estimates (posterior medians) and HPD95% for genetic (rg) and phenotypic (rp) correlations between the same MCP trait assessed using FRM and OPT are reported in Table 4. The estimated phenotypic correlations were moderate to high and ranged from 0.426 (a_{45}) to 0.806 (RCT). The estimated genetic relationships were always high and were between 0.764 (k_{20}) and 0.974 (RCT).

Within instrument, the phenotypic correlations between the MCP were moderate to high, with some differences between the 2 instruments (Table 5). Genetic correlations between traditional MCP (RCT, k_{20}, and a_{30}) were very high, with the only exception being the relationship between RCT and k_{20} yielded by OPT (0.415). Curd firmness measured at 45 min from rennet addition yielded very low (and opposite in sign) genetic correlations with RCT with both instruments. The correlations of a_{45} with k_{20} and a_{30} were both low in the case of FRM and very high in the case of OPT.

RELATIONSHIPS BETWEEN SIRE RANKINGS

The relationships between sire rankings based on EBV for each MCP measured using FRM and OPT are depicted in Figure 2. The sire ranking for RCT was only marginally affected by the instrument used to assess the trait, as the correlation between EBV based on measures of RCT determined by the 2 instruments was 0.99. For a_{30} (r = 0.95), a_{45} (r = 0.94), and k_{20} (r = 0.87), reranking was more pronounced.

DISCUSSION

HERITABILITY OF RCT MEASURED OVER AN EXTENDED TESTING PERIOD

To our knowledge, this is the first study dealing with estimation of genetic parameters of MCP obtained on a testing period extended to 45 min to avoid NC and to allow calculation of k_{20} on almost all milk samples. Most estimates of genetic parameters for RCT found in the literature have been obtained after discarding NC samples. As the risk of NC milk is higher for cows and progeny characterized by a slow coagulation process, it is clear that both genetic parameters and EBV for RCT can be biased. This risk is particularly high for breeds characterized by slowly coagulating milk, such as Holstein-Friesian and some Scandinavian breeds (Ikonen et al., 1997; Tyrisevä et al., 2004; De Marchi et al., 2007).

To account for the NC samples, Cecchinato and Carnier (2011) compared different statistical models (linear, right-censored linear, survival, and threshold) and concluded that the best approach is to treat NC samples as censored records. They used individual RCT data from 1,025 Holstein-Friesian cows determined through a 30-min testing-period analysis and found that both additive genetic and error variances

estimated using a right-censored linear model were approximately twice the values found when ignoring NC samples and using a linear model. Consequently, the heritability estimates were very similar.

Cecchinato et al. (2011) applied a right-censored linear model to RCT data from Brown Swiss cows and compared the results with findings from a previous study that was based on the same data but that ignored NC samples and used a linear instead of a censored linear model to analyze the records (Cecchinato et al., 2009). The use of the right-censored linear model led to an increase in the additive genetic variance (4.96 to 5.39 min^2) and to a even more pronounced increase of the residual variance, so that heritability estimate decreased from 0.34 (Cecchinato et al., 2009) to 0.24 (Cecchinato et al., 2011).

Milk from Holstein-Friesian is usually characterized by a slower coagulation process than milk from Brown Swiss cows, even if the incidence of NC samples is seldom reported in literature (Malacarne et al., 2005, 2006). It is worth mentioning here that milk protein genetic variants play an important role in explaining the additive genetic variance of MCP (Penasa et al., 2010) and that this effect, and consequently the differences among breeds, depends on the relative frequencies of the genetic variants, especially those relative to κ-casein alleles (Ikonen et al., 1999; Auldist et al., 2002; Bittante et al., 2012). A summary of literature on the effects of genetic variants of milk protein fractions on MCP has recently been reviewed by Bittante et al. (2012), whereas an extensive discussion on the role of κ-casein gene allelic variants on MCP has been reported by Bonfatti et al. (2010).

In the present study, extending the observation period to 45 min allowed us to obtain RCT values for all samples, even if 6.67% of milks coagulated after 30 min from rennet addition. As expected, the estimate of genetic variance of RCT measured by

FRM for 45 min (7.09 min^2) was higher than those (4.40–5.48 min^2) obtained from a linear model on different subsamples of the same breed, but determined for 30 min (Cecchinato et al., 2009). Moreover, the estimate of the additive genetic variance was higher than that previously reported by Cecchinato et al. (2011) on the same breed using the right-censored linear model. A possible explanation is that the distribution of RCT of the entire population measured extending the observation period is not perfectly Gaussian, showing a skewness due to a larger than-expected right tail. As a result, the assumption of normality of the right-censored linear model can lead to underestimation of the contribution of both additive genetic and residual variances induced by slowly coagulating samples. The heritability estimate of RCT determined for 45 min was very similar to the estimate found by Cecchinato et al. (2011) from RCT measured for 30 min and with NC samples treated as censored.

GENETIC PARAMETERS OF CURD-FIRMING TIME

Curd-firming time is valuable at the industry level because it defines the optimal moment for curd cutting, limiting the fines losses with early cutting and the excess moisture of the curd with late cutting (Janhøj and Qvist, 2010). Only 2 studies estimated heritability for k_{20}: Tervala et al. (1985) reported very low heritability (0.021) using 319 milk samples from Finnish Ayrshire, Finnish Friesian, and Finncattle cows reared in an experimental farm and sampled once, whereas Ikonen et al. (1997) found very high estimates using 174 samples from 59 Finnish Ayrshire (0.540) and 155 samples from 55 Finnish Friesian (0.660) cows, again from an experimental farm.

No estimates are available on field data primarily because not only NC samples but also many slowly coagulating samples do not reach curd firmness of 20 mm within

the usual testing time of 30 min, so that large biases can be expected from both genetic parameters and EBV.

Heritability of k_{20} from FRM (0.212; Table 2) was close to the average heritability of the other MCP and was intermediate between findings from Tervala et al. (1985) and Ikonen et al. (1997). Tervala et al. (1985) defined k_{20} differently from our research and from Ikonen et al. (1997). Moreover, the data used by Ikonen et al. (1997) were measured on samples reaching the target value (20 mm) within 30 min from gelification, whereas the time limit was 45 min in the present study. Differences in type and activity of coagulant and in statistical analysis were also found in the studies, making difficult the comparison among them.

No estimates of genetic correlations between k_{20} and other MCP or milk production or composition traits are available in the literature. In the present study, k_{20} showed high positive phenotypic and genetic correlations with RCT and very high negative correlations with a_{30}, confirming that late-coagulating samples are characterized by slow firming rate and low a_{30}. With a genetic correlation of −0.979, k_{20} seems to add no valuable information, from a genetic point of view, beyond that yielded by a_{30}.

GENETIC PARAMETERS OF CURD FIRMNESS EVALUATED 30 MINUTES AFTER RENNET ADDITION

In addition to RCT, a_{30} is also affected by the problem of NC samples. Samples that do not coagulate within 30 min from coagulant addition do not have a curd firmness value over the baseline, which is assumed to be zero. Most published studies report estimates of genetic parameters for a_{30} that were obtained without the inclusion of NC samples. On the contrary, Ikonen et al. (1999) and Tyrisevä et al. (2004) reported

estimates of genetic parameters that were obtained with the inclusion of NC samples. Again, Ikonen et al. (2004) faced this problem comparing 2 approaches. The first was based on the inclusion of NC samples attributing to a_{30} a zero value; results showed a higher heritability estimate compared with that found using only coagulated samples (from 0.22 to 0.39, respectively). The second approach treated a_{30} as a binary trait (occurrence of coagulation); heritability (0.26) was higher than that obtained excluding NC samples but much lower than that found including them as zero values.

The inclusion of samples coagulating after 30 min (with a zero value) led to an estimate of additive genetic variance slightly larger than that found in the same breed by Cecchinato et al. (2009, 2011) excluding NC samples, but the estimate of the residual variance was even larger so that heritability estimate was relatively low (0.171), similar to the value found by Cassandro et al. (2008) in Holstein-Friesians but lower than the estimates of previous studies in the same breed (Oloffs et al., 1992; Ikonen et al., 1997) and other northern breeds (Tervala et al., 1985; Oloffs et al., 1992; Tyrisevä et al., 2004).

Moreover, despite the different definition of RCT and the use of the zero a_{30} for NC samples, the present work confirmed the very high genetic and phenotypic correlations between the 2 MCP traits found in most previous studies. It is clear that the longer the RCT, the shorter the time available for curd firming and the smaller the curd firmness measured at a fixed time, thus confirming the poor informative value of a_{30} beyond RCT information and the need for new modelling of the curd-firming process and traits that are less interdependent (Bittante, 2011).

GENETIC PARAMETERS OF CURD FIRMNESS EVALUATED 45 MINUTES AFTER RENNET ADDITION

To overcome the problem of NC samples and define more independent traits, some researchers have extended the interval between enzyme addition and curd firmness measurement to 45 min (Mariani et al., 1997; Cecchi and Leotta, 2002) or 60 min (O'Brien et al., 2002; Auldist et al., 2004), but no estimates of genetic parameters for a_{45} or a_{60} are available in the literature.

The a_{45} measured by the mechanical instrument is more independent than a_{30} from the other MCP (RCT and k_{20}), both at the phenotypic and genetic levels. Moreover, the correlations between curd firmness measured 30 and 45 min after rennet addition are relatively low, at both the genetic (+0.269) and phenotypic (+0.476) levels. This low dependence from other traditional MCP is probably because curd firmness tends to increase after gelification to a maximum value and later tends to decrease due to syneresis. The time at which maximum curd firmness is reached can differ greatly in different samples (Bittante et al., 2012), so that a_{45} can be measured in the ascending or descending phase of the curd-firmness curve. Consequently, the same a_{45} value can be observed in very late coagulating samples as in very early coagulating samples (characterized by rapid syneresis). Because of these considerations, a_{45} does not seem very useful at the industry level, but more research on this topic is needed.

HERITABILITY OF MCP MEASURED BY NIR LACTODYNAMOGRAPH

In previous research (Cipolat-Gotet et al., 2012a), MCP determined by mechanical and NIR lactodynamographs on the same samples, in the same laboratory, and by the same technician, have been shown to be different traits, with the partial exception of RCT. Differences were observed in mean values (especially for k_{20}),

variability (with the exception of k_{20}), distribution of the data, and correlations with other MCP and with milk yield and composition traits.

The present study highlighted that the optical device yields MCP that are different from the mechanical device, from the genetic point of view (Table 2). It is worth noting that the NIR lactodynamograph does not measure curd firmness; rather, it predicts curd firmness based on an optical signal that is modified by chemical changes that happen mainly during the first phase of coagulation process (before gelification), not during the second phase, when the physical properties (firmness) change dramatically (O'Callaghan et al., 2002). Thus, the ability of OPT to mimic mechanical instruments is expected to decrease after gelation.

Vallas et al. (2010) found heritability of 0.28 for log-transformed RCT and 0.41 for a_{30} in Estonian Holstein-Friesian cows. Comparison with the present study is not very useful because the Estonian study used a microbial enzyme (instead of calf rennet) at a very high activity level. As expected, within 30 min of coagulant addition, Vallas et al. (2010) obtained very short RCT but a_{30} similar to that found in the present research using a much lower enzyme activity. The use of a high concentration of enzyme is not advisable at the industry level because extra enzyme would reduce cheese yield, change the balance between coagulation time and acidification, and increase the production of bitter peptides beyond the capacity of the enzymes of the cheese microflora to degrade them (Law, 2010). This is particularly true for the production of Protected Designation of Origin cheeses, where production processes cannot be altered to address milk defects and, thus, such cheese-makers require (and pay for) milk of high technological quality (Bertoni et al., 2005; Bittante et al., 2011a,b).

It is worth noting that optical measurements have been used without inducing and monitoring rennet coagulation of milk samples, by predicting MCP on the basis of

the MIRS spectra of fresh milk samples through a proper calibration with MCP measured with mechanical lactodynamographs (Dal Zotto et al., 2008; De Marchi et al., 2009). Cecchinato et al. (2009), comparing the genetic parameters of MCP measured by a mechanical lactodynamograph with those of MCP predicted by MIRS spectra, found results similar to those obtained from the present study, with a slight increase of heritability for RCT and a more pronounced increase of heritability for a_{30}.

Relationships Between MCP Measured by Mechanical and Optical Lactodynamographs

The genetic correlations between FRM and OPT for the same MCP were much higher than the corresponding phenotypic relationships (Table 4). A similar result was obtained by Cecchinato et al. (2009) for both RCT and a_{30} predicted by MIRS or measured by mechanical lactodynamograph.

The genetic correlation between RCT yielded by FRM and OPT was very high (0.974), as was the rank correlation of EBV of sires, and the genetic correlation is only slightly higher than that found in the same breed by Cecchinato et al. (2009), who compared measured and MIRS-predicted RCT (0.93, average of 4 subsets). We can assume that the use of MIRS prediction on fresh noncoagulated milk samples is almost as efficient as, but much less expensive and time consuming than, NIR lactodynamograph estimates in a breeding program aimed at improving RCT as an alternative to the use of traditional mechanical lactodynamographs.

The genetic correlation between a_{30} from FRM and OPT (0.917) was lower than the corresponding value exhibited by RCT on the same instrument (0.974), but much higher than the genetic correlation between measured and MIRS predicted a_{30} obtained by Cecchinato et al. (2009). The genetic correlations between k20 and a_{45} from FRM

and OPT are lower than for RCT and a_{30} (Table 4), but no studies are currently available in literature for comparison. The genetic correlation between RCT and a_{30} yielded by OPT was slightly lower than that obtained by FRM (−0.769 vs. −0.856, respectively), but indeed very high and much different from the genetic correlation (−0.160) estimated by Vallas et al. (2010) comparing log-transformed RCT and a_{30} obtained by OPT. No literature comparisons are possible for the other MCP.

CONCLUSIONS

Extending the standard 30-min testing time to 45 min allowed us to measure RCT of all milk samples and k_{20} for most late-coagulating milks, avoiding NC records. The use of all RCT data (included those larger than 30 min) led to higher additive genetic and residual variances compared with those found in literature, but the heritability remained almost unchanged. For FRM, heritability of k_{20} was similar to that of RCT, but the genetic correlations with both RCT and a_{30} were very high, so that the value of k_{20} for breeding purposes, beyond RCT, is questionable. The relevance of a_{30} is also questionable because of the high genetic correlation with RCT. Genetic parameters for a_{45} have been estimated for the first time; this trait exhibited a lower correlation coefficient with RCT than a_{30}, but compared with a_{30} it was characterized by lower heritability (only for FRM). The MCP estimated by OPT appeared to be different traits from those measured by FRM with the exception of RCT. Breeding strategies for the enhancement of MCP must be planned with caution. Presently, the high throughput, ease of use, and reduced costs of analysis make predictions obtained from MIRS on untreated milk samples a promising alternative for the generation of relevant data at the population level. The use of mechanical lactodynamographs to establish the reference

data for MIRS calibrations have been already studied (De Marchi et al., 2009), whereas the use of NIR optical lactodynamographs as reference method for MIRS calibrations needs to be investigated.

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TABLES AND FIGURES

Table 1. Descriptive statistics (n = 913) of milk coagulation properties assessed using Formagraph (Foss Electric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments[1]

	Formagraph				Optigraph			
	Mean	SD	P1	P99	Mean	SD	P1	P99
RCT, min	19.95	5.81	10.31	38.00	18.91	4.40	11.40	31.70
k20, min	5.36	3.12	1.45	17.30	8.16	2.97	3.70	17.80
a30, mm	30.09	11.34	0.40	51.04	27.23	10.80	2.35	55.79
a45, mm	33.66	8.43	9.03	52.20	41.49	11.54	11.87	70.62

[1]P1 = 1st percentile; P99 = 99th percentile.
[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

Table 2. Features of marginal posterior densities of heritability (h^2), additive genetic (σ_a^2), herd/date (σ_h^2), and residual variances (σ_e^2) of milk coagulation properties assessed using Formagraph (Foss Electric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments

	Formagraph		Optigraph		
Trait[1]/parameter	Median[2]	HPD95%[3]	Median	HPD95%	Δ%[4]
RCT, min					
σ_a^2	7.092	3.12; 13.02	4.155	2.01; 7.32	-41.4
σ_h^2	4.343	2.52; 7.41	2.123	1.17; 3.63	-51.1
σ_e^2	19.435	14.46; 23.65	10.979	8.37; 13.29	-43.5
h^2	0.230	0.10; 0.41	0.241	0.12; 0.41	+4.8
k_{20}, min					
σ_a^2	2.102	0.74; 4.33	3.128	1.29; 5.52	+48.8
σ_h^2	0.286	2.52; 7.40	0.363	0.07; 0.82	+26.9
σ_e^2	7.535	5.73; 9.06	5.017	3.12; 6.71	-33.4
h^2	0.212	0.07; 0.41	0.368	0.15; 0.61	+73.6
a_{30}, mm					
σ_a^2	21.034	5.01; 46.23	23.815	5.94; 49.52	+13.2
σ_h^2	6.423	1.85; 13.57	10.541	5.08; 19.44	+64.1
σ_e^2	95.327	74.01; 114.2	81.673	61.01; 100.3	-14.3
h^2	0.171	0.04; 0.36	0.205	0.05; 0.40	+19.9
a_{45}, mm					
σ_a^2	8.368	1.74; 19.84	38.111	16.36; 67.22	+355.4
σ_h^2	12.535	7.78; 20.02	9.795	4.57; 18.32	-21.9
σ_e^2	48.96	39.38; 57.34	75.337	52.33; 96.87	+53.9
h^2	0.120	0.02; 0.27	0.309	0.13; 0.51	+157.5

[1]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.
[2]Median = median of the marginal posterior density of the parameter.
[3]HPD95% = lower and upper bounds of the 95% highest posterior density.
[4]Median of the marginal posterior density of the difference between variance components and heritabilities for milk coagulation properties assessed using Formagraph and Optigraph.

Table 3. Deviance information criterion (DIC), mean squared error (MSE), and Bayes factor[1] (BF) for analysis of milk coagulation properties under the model with (M+) and without (M-) additive polygenic effects[2]

	Formagraph		Optigraph	
Item[3]	M+	M-	M+	M-
RCT, min				
DIC	5,403.7	5,479.5	4,901.5	4,986.3
MSE	13.7	24.4	7.7	14.1
BF	239.8		12,193.2	
k_{20}, min				
DIC	4,512.5	4,565.3	4,258.1	4,402.4
MSE	5.6	9.1	3.1	7.4
BF	11.9		4.8	
a_{30}, mm				
DIC	6,796.2	6,841.7	6,670.9	6,730.9
MSE	70.3	102.1	58.7	94.1
BF	15.8		214.6	
a_{45}, mm				
DIC	6,161.2	6,189.6	6,655.8	6,767.4
MSE	38.64	52.2	49.1	102.7
BF	3.75		494.4	

[1]Bayes factor of the model with additive polygenic effects against the same model without additive polygenic effects following García-Cortés et al. (2001).

[2]Formagraph from Foss Electric (Hillerød, Denmark); Optigraph from Ysebaert (Frépillon, France).

[3]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

Table 4. Additive genetic (r_g) and phenotypic (r_p) correlations within milk coagulation properties assessed using Formagraph (Foss, Eletric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments[1]

	r_g		r_p	
Trait[2]	Median	HPD95%	Median	HPD95%
RCT, min	0.974	0.896; 0.997	0.806	0.779; 0.831
k_{20}, min	0.764	0.315; 0.992	0.518	0.462; 0.571
a_{30}, mm	0.917	0.610; 0.992	0.731	0.693; 0.764
a_{45}, mm	0.847	0.453; 0.991	0.426	0.359; 0.491

[1]Median = median of the marginal posterior density of the parameter; HPD95% = lower and upper bounds of the 95% highest posterior density.

[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

Table 5. Additive genetic (r_g) and phenotypic (r_p) correlations between milk coagulation properties within instrument[1] [Formagraph (Foss Eletric, Hillerød, Denmark) or Optigraph (Ysebaert, Frépillon, France)]

	Formagraph				Optigraph			
	r_g		r_p		r_g		r_p	
Trait[2]	Median	HPD95%	Median	HPD95%	Median	HPD95%	Median	HPD95%
RCT with								
k_{20}	0.792	0.43; 0.95	0.675	0.63; 0.71	0.415	-0.06; 0.74	0.416	0.34; 0.47
a_{30}	-0.856	-0.98; -0.64	-0.854	-0.87; -0.83	-0.769	-0.91; -0.50	-0.821	-0.84; -0.79
a_{45}	0.162	-0.41; 0.75	-0.213	-0.29; -0.12	-0.131	-0.53; 0.32	-0.397	-0.46; -0.32
k_{20} with								
a_{30}	-0.979	-0.99; -0.85	-0.847	-0.86; -0.82	-0.953	-0.99; -0.75	-0.757	-0.78; -0.72
a_{45}	-0.284	-0.68; 0.39	-0.583	-0.63; -0.52	-0.966	-0.99; -0.83	-0.825	-0.84; -0.80
a_{30} with								
a_{45}	0.269	-0.62; 0.79	0.476	0.40; 0.53	0.774	0.49; 0.92	0.798	0.76; 0.82

[1]Median = median of the marginal posterior density of the parameter; HPD95% = lower and upper bounds of the 95% highest posterior density.
[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

Figure 1. Marginal posterior distributions of the heritability for measures of rennet coagulation time of samples coagulating within 45 min from rennet addition [RCT, min], curd-firming time within 45 min from rennet addition [k_{20}, min], curd firmness at 30 min after rennet addition [a_{30}, mm], and curd firmness at 45 min after rennet addition [a_{45}, mm] assessed using Formagraph (FRM; Foss Eletric, Hillerød, Denmark) and Optigraph (OPT; Ysebaert, Frépillon, France) instruments

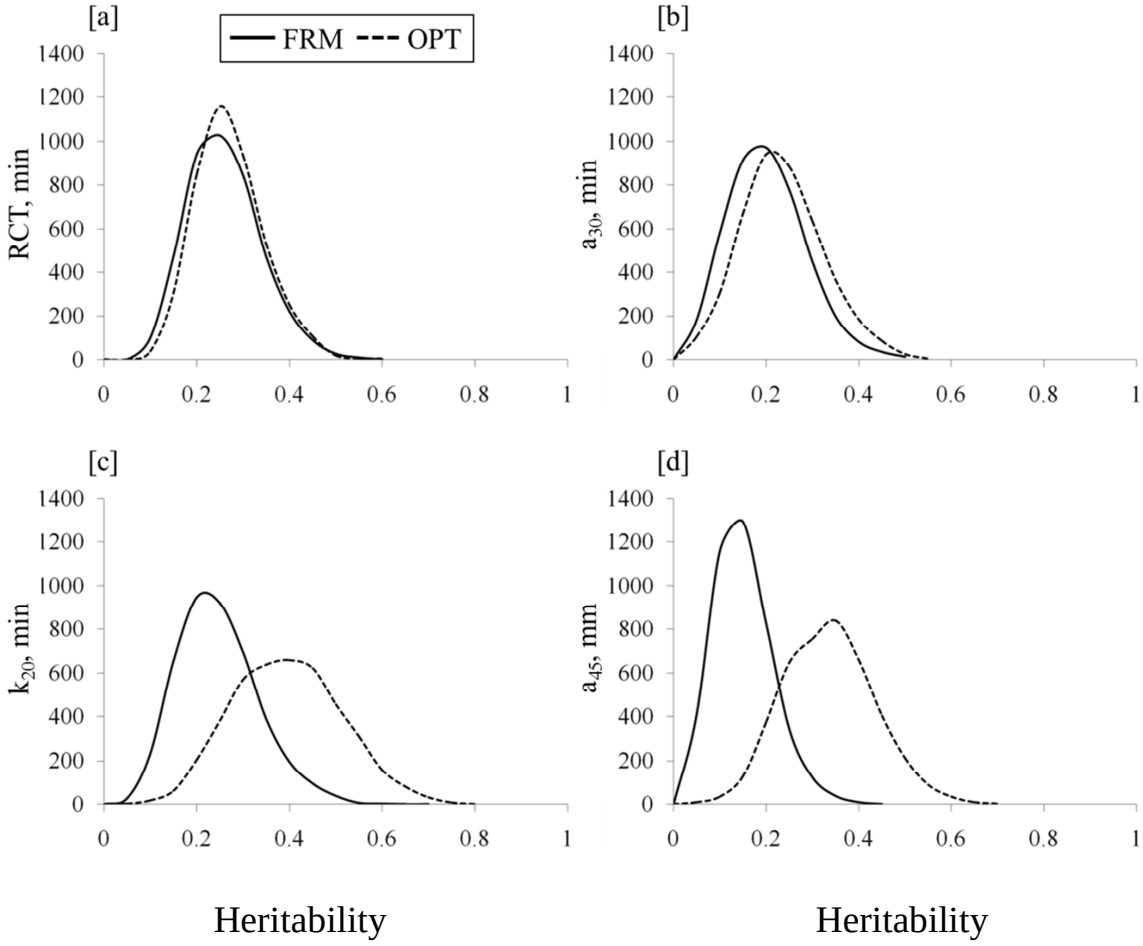
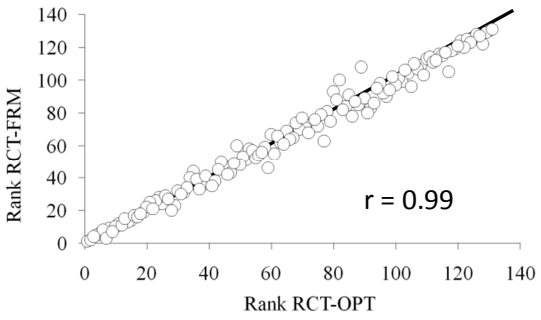
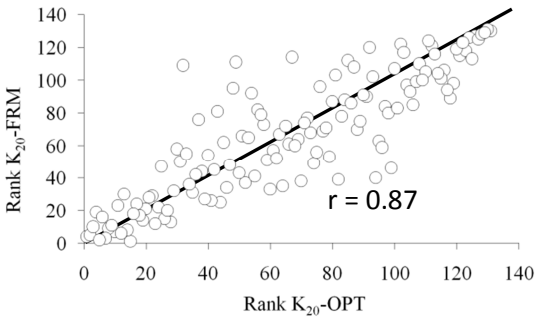


Figure 2. Relationships between sire rankings based on EBV for measures of rennet coagulation time of samples coagulating within 45 min from rennet addition (RCT, min), curd-firming time measured within 45 min from rennet addition (k_{20}, min), curd firmness at 30 min after rennet addition (a_{30}, mm), and curd firmness at 45 min after rennet addition (a_{45}, mm) assessed using Formagraph (FRM; Foss Eletric, Hillerød, Denmark) and Optigraph (OPT; Ysebaert, Frépillon, France) instruments

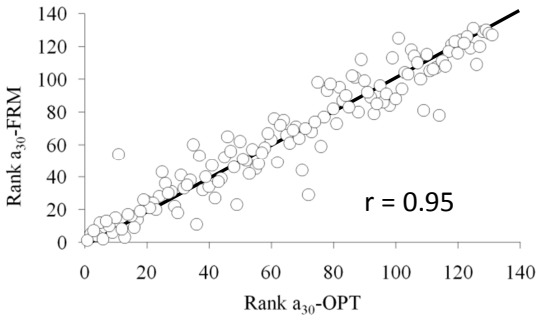
[RCT]



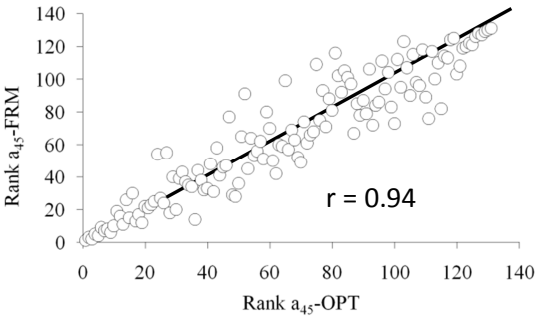
[k_{20}]



[a_{30}]



[a_{45}]



III CONTRIBUTION

FACTORS AFFECTING DIFFERENT MEASURES OF CHEESE YIELD AND NUTRIENT RECOVERY FROM AN INDIVIDUAL MODEL CHEESE-MANUFACTURING PROCESS

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ABSTRACT

Cheese yield is the most important technological trait of milk, as cheese-making uses a very high proportion of the milk produced worldwide. Few studies have been carried out at the level of individual milk-producing animals, due to a scarcity of appropriate procedures for model-cheese production, the complexity of cheese-making, and the frequent use of the fat and protein (or casein) contents of milk as a proxy of cheese yield. Here, we report a high-throughput cheese manufacturing process that mimics all phases of cheese-making, uses 1.5-L samples of milk from individual animals, and allows the simultaneous processing of 15 samples per run. Milk samples were heated (35°C for 40 min), inoculated with starter culture (90 min), mixed with rennet (51.2 IMCU×L-1 of milk), and recorded for gelation time. Curds were cut twice (10 and 15 min after gelation), separated from the whey, drained (for 30 min), pressed (three times, 20 min each, with the wheel turned each time), salted in brine (for 60 min), weighed and sampled. Whey was collected, weighed and sampled. Milk, curd, and whey samples were analyzed for pH, total solids, fat content, and protein content. The energy contents of the milk, curd and whey were estimated from their chemical compositions. Three measures of cheese yield (CY) were calculated, CY_{CURD}, CY_{SOLIDS}, and CY_{WATER}, which represented the ratios between the weight of fresh curd, the total solids of the curd, and the water content of the curd, respectively, and the weight of the milk processed. Three measures of nutrient recovery were computed, REC_{FAT}, $REC_{PROTEIN}$, and REC_{SOLIDS}, which represented the ratio between the weights of the fat, protein, and total solids in the curd, respectively, and the corresponding nutrient in the milk. The energy recovery, REC_{ENERGY}, represented the energy content of the cheese versus that in the milk. This procedure was used to process individual milk samples obtained from 1,167 Brown Swiss cows reared in 85 herds of the Alpine province of

Trento (Italy). The assessed traits exhibited almost normal distributions, with the exception of REC_{FAT}. The average values ± SD were: CY_{CURD} = 14.97±1.86, CY_{SOLIDS} = 7.18±0.92, CY_{WATER} = 7.77±1.27, REC_{FAT} = 89.79±3.55, $REC_{PROTEIN}$ = 78.08±2.43, REC_{SOLIDS} = 51.88±3.52, and REC_{ENERGY} = 67.19±3.29. All traits were highly influenced by the herd/test date and days in milk of the cow, moderately influenced by parity and daily milk yield, and weakly influenced by the utilized vat. Comparisons among the analyzed traits indicated the following: CY_{CURD} depends not only on the on fat and protein (casein) contents of the milk, but also on their proportions retained in the curd; the water trapped in curd has a variability higher than that of CY_{SOLIDS} (with which it is moderately correlated) and significantly contributes to explaining the individual variability of CY_{CURD}; REC_{FAT} and $REC_{PROTEIN}$ are variable, independent of one another, and affect all cheese yield measures; REC_{SOLIDS} and REC_{ENERGY} are variable, highly correlated with each other, and strongly affect all cheese yield measures. The described model cheese-producing procedure and the obtained results provide new insight into the variation and relationships among different cheese yield and recovery traits at the individual level. Clearly, additional research on this topic is warranted, especially in terms of assessing the genetic background of these traits and methods for their indirect prediction.

Keywords: individual cheese yield; cheese-making; whey losses; fat recovery; protein recovery

INTRODUCTION

Cheese yield (CY) is a key factor in the economics and profitability of dairy industries. Cheese yield, the recovery of individual milk constituents in the curd, and their loss in the whey all define the efficiency of the cheese-making process (Banks,

2007). These indices are influenced by two main aspects of milk quality: 1) animal concerns, such as the species (Othmane et al., 2002a; Zicarelli et al., 2007), breed (Malacarne et al., 2006; De Marchi et al., 2008; Martin et al., 2009), stage of lactation (Wedholm et al., 2006), parity (Wedholm et al., 2006), feeding (Banks et al., 1986) and health (Politis and Ng-Kwai-Hang, 1988); and 2) conditions, such as the handling and storage of the milk prior to cheese-making, and the technologies adopted (Lucey and Kelly, 1994).

In general, experimental cheese-making trials are expensive, time-consuming, and only allow for a small number of replicates. In the past 30 years, many studies (Hicks et al., 1981; Hurtaud et al., 1995; Cologna et al., 2009) have described the production of model cheeses through laboratory micro cheese-making processes that allow CY to be assessed from samples ranging from 1 mL to more than 10 L per sample. Compared to trials carried out in cheese-making plants, these laboratory procedures offer the following benefits: the use of small quantities of milk; reduced time and costs required for experiments; more possible treatments or replications per day; and the ability to estimate CY from individual animals.

It is generally agreed that individual CY is an important parameter for studies intended to test the existence of a genetic basis for these traits (Othmane et al., 2002a). However, most of the studies that involve micro cheese-making procedures have used bulk milk, largely because it is very labor-intensive to produce a high number of model cheeses from individual milk samples. Numerous steps are involved, as follows: individual milk sampling; milk analysis; milk weighing and heating; starter culture preparation and inoculation; pH measurement; rennet preparation and addition; gelation-time recording and curd cutting; whey drainage, sampling, and weighing; curd

sampling and analysis; wheel formation, compression, salting, and weighing; and model-cheese ripening, weighing and analysis.

Two previous studies (Hurtaud et al., 1995; Wedholm et al., 2006) estimated CY from individual cows using a micro cheese-making procedure; however, these studies examined only 6 and 45 animals, respectively. Therefore, the aims of the present study were: 1) to develop a model cheese-manufacturing process that supports the measurement of CY at the individual level; 2), to characterize milk nutrient and energy recoveries in the curd at the individual level; and 3) to investigate several sources of variation for CY and nutrient/energy recoveries in the curd, using milk from individual cows.

MATERIALS AND METHODS

ANIMALS AND MILK SAMPLING

Individual milk samples were obtained from a total of 1,167 Brown Swiss cows during the evening milking. Cows (two subsamples per cow) were sampled from in 85 herds (15 cows per herd, with few exceptions) located in Trento province (Italy). The present study is part of the Cowability–Cowplus projects. The sampling procedure was described in detail by Cipolat-Gotet et al. (2012a) and Cecchinato et al. (2012a), and the production environment was as described in Sturaro et al. (2012). After collection, milk samples (without any preservative) were immediately refrigerated at 4°C. One subsample (50 ml; designated for milk composition analysis) was transported to the Milk Quality Laboratory of the Trento Breeders Association. The second subsample (about 2,000 ml; designated for cheese making) was transferred to the Milk Laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment

(DAFNAE) of the University of Padova (Legnaro, Padova, Italy). All samples were analyzed and processed the following morning, within 20 h from collection. Information about cows and herds was obtained from the Superbrown Consortium of Trento (Trento, Italy).

Individual milk samples were analyzed for fat, protein, casein and somatic cell score (SCC) using a MilkoScan FT6000 (Foss, Hillerød, Denmark). SCC values were log-transformed to SCS (Ali and Shook, 1980). The milk pH values were obtained after the heating step of the cheese-making process, using a Crison Basic 25 electrode (Crison, Barcelona, Spain).

MICRO CHEESE-MAKING PROCEDURE

The laboratory micro-manufacturing procedure for assessing CY proposed by Cologna et al. (2009), which showed a good repeatability and reproducibility, was modified for a larger quantity of milk per sample (1,500 mL). The cheese-making facility consisted of three water baths (WBs) provided with supplementary temperature controllers and pumps for water mixing. Each WB contained five stainless steel micro-vats (1,500-mL capacity). Thus, a single cheese-making session allowed analysis of up to 15 (3 WB x 5 vats) individual milk samples. Each milk sample (1,500 mL) was subjected to the procedure summarized in Figure 1.

Briefly, raw milk was heated to 35°C, the pH was recorded, and the sample was inoculated with starter culture, which consisted of a freeze-dried formulation of thermophilic lactic bacteria (DELVO-TEC TS-10A DSL; DSM) that was solubilized with skim milk prior to use. The starter culture was used at a concentration 8-fold higher than the recommended by the, to reduce the acidification time to 90 minutes and minimize the role of the microflora present in the milk samples. Commercial rennet

[Hansen standard 160 with 80 ± 5% chymosin and 20 ± 5% pepsin; 160 international milk clotting units (IMCU) ×mL-1; Pacovis Amrein AG, Bern, Switzerland] was diluted 20:1 with distilled water, and 9.6 mL of rennet solution was added to each vat (final concentration, 51.2 IMCU ×L-1 of milk). The milk rennet coagulation time (RCT) was detected by visual observation. A double orthogonal vertical cut was made 10 minutes after RCT. Five minutes after the first cut, the curd was reduced into cubes of about 1 cm3. After 5 minutes, the curd was separated from the whey and suspended on a cheese mold for 30 minutes; the mold was suspended over the whey-containing vat and the curd was turned every 2 minutes to facilitate draining. After draining, the whey was weighed and sampled for analysis of pH and composition using a MilkoScan FT2 (Foss, Hillerød, Denmark). The curd was pressed for 60 minutes at 2.5 bar with turning every 20 minutes. Finally, the curd was salted for 60 minutes in brine (saturated solution; 20% NaCl). After brining, the cheese wheel was weighed, and the pH was measured and the composition was determined using a FoodScan (Foss, Hillerød, Denmark).. The acidity (pH) values of the curd and whey were measured using a Crison Basic 20 electrode (Crison, Barcelona, Spain).

DEFINITION OF TRAITS

All assessed traits were based on the weights and chemical compositions of the milk, and whey. The classical formula for cheese yield (CY_{CURD}, %) can be written as follows:

$$CY_{CURD}(\%) = \frac{\text{weight of curd (g)}}{\text{weight of milk (g)}} * 100$$

The weight of the curd was taken after brining.

Cheese yield was also calculated for the total solids (CY_{SOLIDS}, %) and water (CY_{WATER}, %) of the curd, as:

$$CY_{SOLIDS}\,(\%) = \frac{\text{milk total solids (g)} - \text{whey total solids (g)}}{\text{weight of milk (g)}} * 100$$

$$CY_{WATER}\,(\%) = \frac{\text{milk water (g)} - \text{whey water (g)}}{\text{weight of milk (g)}} * 100$$

Considering the weight (g) of the individual components of the milk and curd, the recoveries (%) of milk protein, fat and total solids in the curd were calculated as:

$$REC_{PROTEIN}\,(\%) = \frac{\text{milk protein (g)} - \text{whey protein (g)}}{\text{milk protein (g)}} *100$$

$$REC_{FAT}\,(\%) = \frac{\text{milk fat (g)} - \text{whey fat (g)}}{\text{milk fat (g)}} * 100$$

$$REC_{SOLIDS}\,(\%) = \frac{\text{milk total solids (g)} - \text{whey total solids (g)}}{\text{milk total solids (g)}} * 100$$

The recovery of energy in the curd was also calculated using:

$$REC_{ENERGY} = \frac{\text{milk energy (kJ)} - \text{whey energy (kJ)}}{\text{milk energy (kJ)}} * 100$$

The energy of milk and whey was calculated using the values proposed by the National Research Council (2001) and converted to kJ×g-1 (fat = 38.89 kJ×g-1; protein = 23.90 kJ×g-1; lactose = 16.53 kJ×g-1). The energy of the curd (kJ×g-1) was estimated as the difference between the energy of the milk and whey.

STATISTICAL ANALYSIS

Sources of variation of CYs and RECs were investigated using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The basic model (Model 1) was:

$$y_{ijklm} = \mu + HTD_i + DIM_j + parity_k + vat_l + e_{ijklm},$$

where y_{ijklm} is the observed trait (CY_{CURD}, CY_{SOLIDS}, CY_{WATER}, $REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS} or REC_{ENERGY}); μ is the overall intercept of the model; HTD_i (herd/test date) is the fixed effect of the ith herd-sampling date (i = 1 to 85); DIM_j is the fixed effect of the jth class of days in milk (j = 1 to 11; class 1: < 30 days, class 2: 30 to 60 days, class 3: 61 to 90 days; class 4: 91 to 120 days; class 5: 121 to 150 days; class 6: 151 to 180 days; class 7: 181 to 210 days; class 8: 211 to 240 days; class 9: 241 to 270 days; class 10: 271 to 300 days; class 11: > 300 days); $parity_k$ is the fixed effect of the kth parity of the cow (k = 1 to 5 or more); vat_l is the fixed effect of the lth number of the vat (l = 1 to 15); and eijklm is the residual random error term ~ N (0, σ2e). Then, another model (Model 2) was fitted by taking the basic model and introducing the effect of milk production (Milk Yield, MY; kg×d-1; 7 classes: class 1 ≤14.48; class 2: 14.49 to 18.43; class 3: 18.44 to 22.37; class 4: 22.38 to 26.31; class 5: 26.32 to 30.26; class 6: 30.27 to 34.20; class 7 ≥ 34.21) to highlight any potential multicollinearity with the explanatory variables of Model 1.

RESULTS

CHARACTERISTICS OF INDIVIDUAL SAMPLES

Table 1 shows descriptive statistics for the various production traits. The sampled cows represented different stages of lactation (25-388 days in milk; DIM) and parities (1-5). The mean of milk yield (MY) and SCS were 24.34 kg×d-1 and 2.98, respectively, and showed large coefficients of variation (32.4 and 62.4%, respectively).

The mean values and SD of the milk, whey and curd compositions are given in Table 2. The milk fat, protein, casein (data not shown) and lactose contents of Brown

Swiss milk averaged 4.38, 3.75, 2.88, and 4.77%, respectively. The contents of fat and protein in the whey were, respectively, slightly higher than 0.5% and slightly lower than 1.0%; together with lactose and minerals, they contributed to a total solid average content of 7.79%.

The average cheese fat, protein and lactose (lactose curd content was determined by the difference between the milk lactose content and the whey lactose content) contents obtained using our individual model-cheese production procedure were 26.17, 19.51 and 2.59%, respectively, contributing to a total solid content of almost 50% (Table 2).

The coefficients of variation for milk, whey, and curd were greater than that of fat, followed by protein, total solids, lactose and pH; the only exception was the lactose content of curd, which showed a high coefficient of variability but a low average value.

Table 3 shows descriptive statistics for the CY and REC values, while Figures 2 and 3 give the corresponding distributions of the individual observations. The means value for CY_{CURD} after brining was 14.97%, while those of CY_{SOLIDS} and CY_{WATER} were 7.18 and 7.77%, respectively, each representing about half of CY_{CURD}; this was confirmed by the total solids content of the curd, which was 48.38% (Table 2). The coefficient of variation for CY_{WATER} was higher (16.34%) than the corresponding values for CY_{CURD} and CY_{SOLIDS} (12.42 and 12.81, respectively). All of these traits were almost normally distributed (Figure 2), with kurtosis and skewness values close to zero. The recoveries of protein, fat, total solids and energy averaged 78.08, 89.79. 51.88 and 67.19%, respectively. All of the recoveries exhibited SDs higher than those of the CYs, but had lower coefficients of variation (3.1% for protein recovery and 6.8% for total solids). As shown in Figure 3, only REC_{FAT} yielded a clearly non-normal distribution,

with kurtosis and skewness of 1.119 and -0.999, respectively; this may be explained by the proximity of the mean value to 100%.

The Pearson product-moment correlations and residual correlations for the CYs and RECs are summarized in Table 4. The correlations between the different CYs were all significant ($P < 0.001$); they showed higher values when CY_{CURD} was related to its components, CY_{SOLIDS} and CY_{WATER} (77-87%), whereas the two components of the curd showed less correlation with each other (42-44%).

For the recoveries, the Pearson and residual correlations between $REC_{PROTEIN}$ and REC_{FAT} were not significant (-3 and -6%), indicating that the recovery of these two components in the curd is independent. The recoveries of both curd components were moderately correlated to REC_{SOLIDS} and REC_{ENERGY}, and were more highly correlated with fat than protein; this was expected, given the greater variability and energy content of fat. The correlations between REC_{SOLIDS} and REC_{ENERGY} were strong (91-93%); this was expected, given that both recoveries depend on the fat and protein contents of the curd.

The dependences of CYs on the RECs of protein and fat were positive and moderate, while the dependences of CY_{CURD} and CY_{SOLIDS} on total solids and energy were higher than those of CY_{WATER}.

FACTORS THAT AFFECT THE VARIATION OF CYs AND RECs

The results from our analysis of the effects included in the linear models (M1 and M2) are given in Table 5. For Model 1, the coefficients of determination for the CYs ranged from 0.39 for CY_{SOLIDS} to 0.53 for CY_{WATER}. Days in milk (DIM) was the most important source of variation, showing the highest F-values ($P < 0.001$). The inclusion of daily milk yield (MY) in the model approximately halved the F values of

DIM, but the latter still remained the most important factor affecting variation of all CYs. For all three CYs, lactation number showed a trend opposite that of milk yield, with CYs decreasing from the first to second month of lactation and gradually increasing thereafter; this was evident both when MY was included in the model (Figure 4a) and when it was not (data not shown). When MY was included, the difference between the second and the last class of DIM was lower than when MY was not included. In the former case, the maximum differences between the least square means of the peak and the end of lactation were 2.01, 0.87 and 1.14%, for CY_{CURD}, CY_{SOLIDS} and CY_{WATER}, respectively. The corresponding values when MY was excluded from the model were 2.41, 1.11, and 1.29%, respectively.

In Model 2, the effect of MY was significant for CY_{CURD} ($P < 0.01$) and CY_{SOLIDS} ($P < 0.001$), but not for CY_{WATER}, although the ΔR^2 between the two linear models (including or excluding MY) for both dependent variables was close to zero. The least square means for MY (Figure 5a) showed that the CYs decreased with increasing of milk production.

All CYs were influenced by the herd/test date (HTD; $P < 0.001$); the maximum differences of the least squares means (Model 1) from the 85 herds were 4.84% for CY_{CURD}, 2.10% for CY_{SOLIDS} and 4.31% for CY_{WATER} (data not shown).

The effect of parity was not significant for CY_{SOLIDS}, while CY_{CURD} and CY_{WATER} decreased from first- to third-parity cows (Figure 6a).

Considering the recoveries of milk constituents in the curd, all the coefficients of determination (Model 1) exhibited R^2 values of 0.31 for REC_{ENERGY} and values equal or close to 0.40 for the other traits. Lactation stage (DIM) was the most important effect for all of the RECs, except for $REC_{PROTEIN}$, which was highly influenced by parity ($P < 0.001$). Whereas REC_{FAT} showed important differences during lactation, decreasing

3.23% until mid-lactation, $REC_{PROTEIN}$ had a much lower variability with respect to lactation (Figure 4b). Both REC_{SOLIDS} and REC_{ENERGY} exhibited trend similar to those of the CYs, with the highest values seen at the end of lactation (Figure 4c).

Regarding the effect of MY (Model 2), the ANOVA results were similar to those obtained for the CYs, with the ΔR^2 very small and the F-value halved for DIM. Milk yield was significant only for REC_{SOLIDS} ($P < 0.01$), with total solid recovery decreasing as milk production increased. Although the effect of MY was not significant for $REC_{PROTEIN}$, this trait tended to increase as MY increased.

For the RECs, the effect of herd/test date showed a significant variability; the maximum differences between the least squares means (Model 1) of 85 herds were 7.18% for $REC_{PROTEIN}$, 12.86% for REC_{FAT}, 7.25% for REC_{SOLIDS} and 7.33% for REC_{ENERGY} (data not shown).

We observed an important effect of parity for $REC_{PROTEIN}$ ($P < 0.001$), with this trait decreasing in the older cows (Figure 6b). For the other RECs, this source of variation was not significant.

The effect of vat was significant only for CY_{SOLIDS} ($P < 0.05$), REC_{SOLIDS} ($P < 0.01$ Model 1; $P < 0.05$ Model 2) and REC_{ENERGY} ($P < 0.05$; Model 1), emphasizing that the utilized micro cheese-making procedure exhibited an acceptable reproducibility between vats.

DISCUSSION

MODEL CHEESE-MAKING PROCEDURE

Numerous attempts have been made to mimic the complex processes of cheese-making on a small scale in the laboratory setting. Many different procedures have been

proposed, ranging from very simple protocols to techniques that resemble the industrial processes. All of these methods have been based on the steps of milk heating, enzyme addition, whey separation and curd weighing.

In general, the simpler protocols use very small amounts of milk, stop the coagulation and syneresis processes at a fixed time from enzyme addition rather than from coagulation time (which is not recorded), and use centrifugation for whey separation. These techniques allow the simultaneous processing of many samples but do not allow researchers to analyze different characteristics of individual model cheeses. For example, Hurtaud et al. (1995) described a procedure in which 100-mL milk samples were incubated in an oven for 1 h after enzyme addition. Othmane et al. (2002a) simultaneously evaluated cheese yield from 60 individual samples of ewe's milk (10 mL), using Pyrex glass tubes as individual vats. The most extreme technique was used by Bachmann et al. (2009), who screened starter cultures using as little as 1.7 mL milk (average curd weight, 0.17 g). Instead of individual vats, the authors used the wells of a 2-mL deep-well microplate, which allowed them to process up to 600 samples per run.

The more protocols more close to industrial processes are typically carried out on larger amounts of milk; in them, the coagulation and syneresis processes are stopped at a fixed time from enzyme addition or from coagulation time (which may be recorded), and the curd is drained and pressed for whey separation. These techniques allow the simultaneous processing of relatively few samples, but do enable researchers to analyze individual model cheeses. The quantity of milk utilized in such processes has varied. Pereira et al. (2010) processed 200-mL samples in glass flasks using an oven; Cologna et al. (2009) processed 500-mL samples in stainless steel mini-vats using a water bath; Marziali and Ng-Kwai-Hang (1996) processed 2,000-mL samples in plastic

containers in a water bath; Milesi et al. (2007) processed 2,000-mL samples in large glass flasks incubated in a water bath; Wedholm et al. (2006) processed 4,000-mL samples in laboratory-scale cheese vats; and Fagan et al. (2007) and Jacob et al. (2010) processed 7,000-mL samples in laboratory-scale cheese vats. Some authors have applied even larger scales for the production of model cheeses, such as the use of more than 30 L milk in pilot-scale cheese plants to study the effects of breed (Verdier et al., 1995; Mistry et al., 2002; Martin et al., 2009), feeding regime (Kefford et al., 1995; Verdier et al., 1995; Hurtaud et al., 2009), and cheese-making technique (Johnson et al., 2001; Jacob et al., 2010).

CHEESE YIELD

The average fresh cheese yield obtained in the present study (15.0%) is higher than that often found in the literature. This is primarily because we used milk from Brown Swiss cows.

The Italian Brown Swiss cattle breed ranks second among all dairy breeds in Italy in terms of both the number of cows reared and the average milk yield (ANARB, 2011). This breed is generally found in areas where environmental constraints limit potential milk production, such as in the mountains and southern portions of the country (ANARB, 2011). These constraints were reflected in the average milk yield of the cows used for the present study (Table 1). The milk produced by Brown Swiss cows is used mainly for the production of traditional cheeses that can obtain the Protected Designation of Origin (PDO) certification from the European Union (Bittante et al., 2011a and b). In this case, the quality of the milk (in terms of contents and technological properties) is fundamental, and the cheese yield and quality are the most important traits in determining milk price (Sturaro et al., 2012).

Italian Brown Swiss cows are currently selected mainly according to the protein yield and percentage of milk (ANARB, 2011). This selection strategy explains the high protein and casein contents of the milk used in the present research (Table 2) and confirms results obtained by other authors in the same breed (Samorè et al., 2007, 2012). The B allele of the CSN3 gene (encoding κ-casein) is also included in the selection index. This is intended to favor the further improvement of the strong coagulation properties (Comin et al., 2008; Penasa et al., 2010) that characterize milk from Brown Swiss cows (De Marchi et al., 2007; Cecchinato et al., 2011; Bittante et al., 2012b). Moreover, the breed has favorable genetic characteristics with regard to milkability, fertility, and longevity (Povinelli et al., 2003; Dal Zotto et al., 2007; Tiezzi et al., 2011 and 2012).

The effect of herd/test date was important, as the CY_{CURD} of the best herd/test date was almost 5% higher than the worst, even after we corrected for parity, days in milk and milk yield. Herd/test date is generally accepted as summarizing the effects on CY_{CURD} of the farm type, feeding regime (Kefford et al., 1995; Verdier-Metz et al., 1998) and season (Bynum and Olson, 1982; Summer et al., 2003), as well as the reproducibility, which was previously established to be quite good for a cheese-making procedure very similar to that used in the present study (Cologna et al., 2009).

For the causes of variation related to the individual animal, an increase in MY is known to reduce the protein and fat content of milk, so its negative effect on CY_{CURD} (Figure 6a) is not unexpected. Parity and DIM affect MY and composition (Kefford et al., 1995), so the lower CY_{CURD} of older cows and those in the first half of lactation is also not unexpected (data not shown). The previously reported effects of parity and lactation stage on cheese yield were generally not corrected for MY, so these effects remained somewhat obscure. In the present study, the effect on CY_{CURD} of both parity

and DIM remained significant even after we included MY in the model, even though the maximum differences were reduced. In fact, the corrected CY_{CURD} was significantly higher in first and second calving cows versus older cows (Figure 5a), and increased almost linearly from the second month to the end of lactation (Figure 4a). These results seem to indicate that milk yield and composition are not the only factors that affect cheese yield.

TOTAL SOLIDS AND WATER RETENTION IN THE CHEESE

Water retention in cheese varies and is influenced mainly by processing conditions, such as the type and concentration of rennet, the cutting time and intensity, the draining and pressing of wheels, the salting technique, and the length and climatic conditions of ripening (Remeuf et al., 1991; Janhøj and Qvist, 2010; Everard et al., 2011). To exclude the effect of variations in the water content of the cheese, some authors have calculated the total solid cheese yield (or dry matter cheese yield), which is expressed as the ratio between the dry matter content of the cheese and the weight of the processed milk (Fagan et al., 2007).

In the case of model cheeses, it may not be feasible to analyze the chemical composition of the curd if a very small quantity of milk is used. In addition, the use of centrifugation to separate the whey from the curd means that the water retention is not representative of that achieved through a standard cheese-making process. CY_{SOLIDS} is more often obtained in larger model cheese-making processes, such as those performed in pilot plants during the study of processing techniques (Fagan et al., 2007; Jacob et al., 2010).

In the case of model cheeses produced using very small amounts of milk, only the procedure proposed by Melilli et al. (2002) is based on the direct estimation of

CY_{SOLIDS}. However the authors used this procedure to predict the results obtained with the formula of Van Slike and Publow (1910), thus avoiding the need to analyze milk fat and protein. The average CY_{SOLIDS} obtained by Melilli et al. (2002) was 6.59%, whereas that found in the present study was 7.18%; this difference may be largely explained by differences in milk composition (3.83 vs. 4.38% for fat and 2.98 vs. 3.75% for protein, respectively).

The effect of herd, parity, DIM and MY on the amount of water retained in the curd as a fraction of the weight of processed milk (CY_{WATER}) had not previously been studied at the individual level. Total solids represented 48% of the fresh curd after brining, which was similar to the proportions found by other authors using similar conditions (Verdier-Metz et al., 2001; Martin et al., 2009). Thus, water contributed slightly more than total solids to cheese yield (7.8 vs. 7.2%, respectively; Table 1). CY_{WATER} was also characterized by a higher phenotypic coefficient of variation with respect to CY_{SOLIDS} (16.3 vs. 12.8%, respectively).

PROTEIN RECOVERY IN THE CHEESE AND LOSSES IN WHEY

Proteins play a fundamental role in the coagulation and syneresis processes that characterize cheese-making (Emmons et al., 2003), and the loss of proteins in whey reduce the cheese yield (Hallen et al., 2009).

Since 1895 (Emmons and Modler, 2010), almost all the predictive formulas for estimating cheese yield have been based on knowledge of the protein (or casein/para-caseinate) and fat contents of milk (Van Slyke and Price, 1952; Banks et al., 1981 and 1984; Emmons et al., 1990), or the sum of the fat and protein contents (Verdier-Metz et al., 2001). All of these formulas assume that the recovery of milk protein and fat in the curd is constant. However, this assumption is contradicted by the results obtained by

Aleandri et al. (1989), who found a curvilinear relationship between the protein content of milk and the Parmesan cheese yield. The same authors and others (Bynum and Olson, 1982; Ng-Kwai-Hung et al., 1989; Ikonen et al., 1999; Johnson et al., 2001) found that cheese yield is also affected by the coagulation properties of milk, although this has been disputed by some (Riddell-Lawrence and Hicks, 1989). Bittante et al. (2012b) reviewed the complex relationships between technological traits and other milk traits, particularly acidity, the casein proportion, and genetic variants.

The effects of farming conditions, cow feed (Summer et al., 2003), and cheese-making technologies (Bynum and Olson, 1982) on $REC_{PROTEIN}$ have been widely studied, but few studies have examined the effects of individual sources of variation on this trait. Kefford et al. (1995) failed to find differences in $REC_{PROTEIN}$ when examining cheese made from milk of mid- or late- lactation cows. This is consistent with the results of the present study, as we found that $REC_{PROTEIN}$ increased only during the initial stage of lactation.

The descriptive statistics in Table 3 show that the average recovery of milk protein in curd after brining was almost identical to the casein index (78.08 vs. 78.05%, respectively). However, the former trait had an appreciable variability coefficient (3.1%), and previous studies showed that the recovery of casein in cheese can be substantially lower than 100%. For example, Bynum and Olson (1982) studied typical Cheddar cheese production and obtained a crude protein recovery of 88.6±2.34% with respect to the casein content of the processed milk. Ikonen et al. (1999) studied Emmental cheese production and obtained $REC_{PROTEIN}$ values of 72.9 and 71.7% for milk with good coagulation and a casein index of 79.7 versus milk with poor coagulation and a casein index of 79.1, respectively. Summer et al. (2003), in a study on seasonal variations, and Malacarne et al. (2006), in a study comparing milk from Italian

Friesian and Italian Brown Swiss cows, examined in Parmigiano-Reggiano cheese production and found protein losses in whey of 26.8% and 26.8±1.0%, respectively, casein indices of 77.4±0.9, and 77.3±0.7, respectively, and no significant effect of season or breed. Moreover, Bittante et al. (2012a) analyzed the same dataset used in the present study and obtained high estimates for the across-herd and intra-herd heritability of $REC_{PROTEIN}$ (35.3 and 49.0%, respectively).

FAT RECOVERY IN CHEESE AND LOSSES IN WHEY

The average fat recovery in the curd was close to 90%. The reported variability in this measure is mainly related to the utilized cheese-making technology, season, farming conditions and feed (Bynum and Olson, 1982; Summer et al., 2003). Only a few results are available regarding individual phenotypic causes of variation. Among them, Kefford et al. (1995) found that the REC_{FAT} does not differ between milk from mid- and late- lactation cows.

The contribution of fat to coagulation and syneresis is less important than that of proteins, but fat recovery in the curd and losses in the whey are important for the final cheese yield, and are influenced by both coagulation and syneresis (Fagan et al., 2007).

The genetic effects of breed and milk protein variants have been discussed by Bittante et al. (2012a). The same authors also reported, from an estimation based on the same dataset used in the present study, that REC_{FAT} is heritable, though to a lesser degree than $REC_{PROTEIN}$ (0.14 and 0.21 for across-herd and intra-herd heritability, respectively).

TOTAL SOLIDS AND ENERGY RECOVERY IN CHEESE AND LOSSES IN WHEY

Only slightly more than half of the total solids present in milk (on average) are captured in the curd coagulum (Verdier et al., 1995; Kefford et al., 1995; Verdier-Metz et al., 1998), but they represent about two thirds of the total energy content of milk (Table 3). Both REC_{SOLIDS} and REC_{ENERGY} are characterized by coefficients of variation (6.8 and 4.9%, respectively) greater than those of the major individual cheese components.

A study carried out by Verdier et al. (1995) did not show any effect of breed or diet on REC_{SOLIDS}. However, a few years later Verdier-Metz et al. (1998) found that both factors had significant effects on this trait.

A large effect of the stage of lactation on REC_{SOLIDS} and the recovery of non-fat solids was found by Kefford et al. (1995), although their study involved cheese-making carried out on bulk milk and the model did not include MY.

After correction for the fat and protein contents, nutrient recoveries from milk and water retention in the curd can explain a large part of the residual variability in cheese yield, both between and within breeds.

The importance of these findings was highlighted by research conducted in the same area as the present study (Trento Province, northeast Italy) on the production of three traditional PDO cheeses. De Marchi et al. (2008) found that the cheese yields obtained using milk from Brown Swiss cows were higher than those obtained using milk from Holstein Friesian cows reared in the same herds for the production of all the three cheese types. The same authors found that only part of the differences in cheese yield between the two breeds could be explained by differences in the fat and protein contents of the milk. Despite similar fat:protein ratios in the milk, comparison of Casolet cheese made from the milk of Brown Swiss cows versus Holstein Friesian cows

showed that the former had a higher superiority in cheese yield (+12%) than in the protein content of milk (+8.2%). The same was found for Vezzena cheese production (+17 vs. +10%, respectively) and Grana del Trentino (or Trentingrana) cheese production (+12 vs. +7.7%, respectively). Similar results were obtained by Martin et al. (2009) when comparing the cheese yields of Holstein and Montbeliarde cows in Cantal cheese production; only about half of the superiority of cheese yield from Montbeliarde milk was explained by its superiority in protein and fat content. In studies using milk standardized to a 1.15 fat:protein ratio from Holstein, Montbeliarde and Tarentaise cows for the production of Saint-Nectaire cheese, however, Verdier et al. (1995) and Verdier-Metz et al. (1998) failed to find significant differences among the breeds in terms of CY_{CURD}, though the latter authors found a difference in REC_{SOLIDS}. Comparing Holstein and Jersey cows, which have similar milk coagulation properties, Auldist et al. (2004) found a higher CY_{CURD} for Jersey cows, which was expected given their differences in milk composition, but a similar "moisture adjusted cheese yield/100 kg of milk solids" (similar to REC_{SOLIDS}). Furthermore, the difference in CY_{CURD} disappeared when the Holstein milk was modified to reach the fat/protein and total solid contents seen in Jersey milk.

These between-breed differences may be at least partially due to differences in the population frequencies of genetic variations in milk proteins. For example, Walsh et al. (1998) found that milk with the B variant of κ-casein is associated with a significantly greater cheese yield than milk with the A variant, even after the data were corrected for milk composition.

CONCLUSIONS

In conclusion, we herein describe a model cheese-producing process that mimics all phases of cheese-making, and show that it can be very useful for studying the variation among individual cows in terms of cheese yield and composition.

The ability to analyze milk, curd and whey samples allowed us to compute the complete nutrient balance and estimate the cheese yields (expressed as the ratios between the weight of fresh curd, dry matter of curd, or water content of curd and the weight of the milk), nutrient recoveries (expressed as the ratio between the content of protein, fat or total solids in curd and the content of the corresponding nutrient in the milk) and energy recovery (expressed as the ratio between the energy content of the curd and that of the milk).

All of the analyzed yield and recovery traits varied substantially among individual cows and showed an almost normal distribution, with the exception of REC_{FAT}. Herd/test date and days in milk affected nearly all of the analyzed traits. Parity and milk yield were much less important, and the effect of the utilized cheese-making vat was often not significant, confirming the good reproducibility of our technique.

Comparisons among the analyzed traits indicate that:

- the cheese yield does not depend solely on the fat and protein (casein) contents of milk;
- the water trapped in curd has a higher variability than the total solids (although the two show a moderate correlation), and the former contributes significantly to explaining the individual variability of cheese yield;
- milk fat and protein recoveries in curd, and their losses in whey, are variable, independent of each other, affected by herd/test date and

individual causes of variation (parity, DIM and MY), and strongly affect all of the tested cheese yield measures; and

- total solid and energy recoveries in curd, and their losses in whey, are variable, correlated with each other, affected by herd/date and individual causes of variation, and strongly affect all of the analyzed cheese yield measures.

The model cheese procedure described herein and the results obtained using it on 1,167 samples from individual cows should facilitate our understanding of the variability and relationships among different cheese yield and recovery traits at the individual level. Furthermore, our findings underscore the need for further research on this topic, especially into the genetic backgrounds of these traits and methods for their indirect prediction.

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TABLES AND FIGURES

Table 1. Descriptive statistics of production traits[1]

Trait[2]	N	Mean	SD	P5	P95
Milk Yield, kg×d^{-1}	1243	24.34	7.89	12.30	37.90
DIM, d	1201	179.47	110.38	25.00	388.00
Parity	1264	2.54	1.39	1.00	5.00[2]
SCS[3], units	1260	2.98	1.86	0.19	6.20

[1]P5 = 5th percentile; P95 = 95th percentile.
[2]class of parity = 5 includes also parities > 5.
[3]SCS = log2 (SCC×10^{-5}) + 3.

Table 2. Descriptive statistics of components of milk, whey and curd (n = 1264)

Trait	Milk		Whey		Curd	
	Mean	SD	Mean	SD	Mean	SD
TS, %	13.89	1.05	7.79	0.33	48.38	4.79
Fat, %	4.38	0.90	0.53	0.22	26.17	5.05
Protein, %	3.75	0.43	0.97	0.16	19.51	1.66
Lactose, %	4.77	0.24	5.15	0.21	2.59	1.30
pH	6.64	0.08	6.42	0.14	6.22	0.23

Table 3. Descriptive statistics of individual cheese yields (curd, solids and water) and milk component recoveries (protein, fat, solids, energy).[1]

Trait	N	Mean	SD	P5	P95
CY_{CURD}, %	1,162	14.97	1.86	12.03	18.12
CY_{SOLIDS}, %	1,153	7.18	0.92	5.75	8.73
CY_{WATER}, %	1,156	7.77	1.27	5.84	9.90
$REC_{PROTEIN}$, %	1,158	78.08	2.43	73.90	81.96
REC_{FAT}, %	1,143	89.79	3.55	82.67	94.41
REC_{SOLIDS}, %	1,157	51.88	3.52	46.01	57.64
REC_{ENERGY}, %	1,144	67.19	3.29	61.78	72.42

[1]P5 = 5th percentile; P95 = 95th percentile.

Table 4. Pearson product-moment correlations between individual cheese yields (curd, solids and water) and milk components recoveries (protein, fat, solids, energy) above diagonal and correlations between residuals below diagonal

	CY_{CURD}	CY_{SOLIDS}	CY_{WATER}	$REC_{PROTEIN}$	REC_{FAT}	REC_{SOLIDS}	REC_{ENERGY}
CY_{CURD}	-	0.77^{***}	0.87^{***}	0.39^{***}	0.24^{***}	0.78^{***}	0.66^{***}
CY_{SOLIDS}	0.78^{***}	-	0.44^{***}	0.21^{***}	0.31^{***}	0.95^{***}	0.85^{***}
CY_{WATER}	0.85^{***}	0.42^{***}	-	0.41^{***}	0.11^{***}	0.47^{***}	0.37^{***}
$REC_{PROTEIN}$	0.45^{***}	0.33^{***}	0.42^{***}	-	-0.03^{ns}	0.20^{***}	0.24^{***}
REC_{FAT}	0.34^{***}	0.38^{***}	0.21^{***}	-0.06^{ns}	-	0.46^{***}	0.64^{***}
REC_{SOLIDS}	0.78^{***}	0.93^{***}	0.44^{***}	0.31^{***}	0.54^{***}	-	0.91^{***}
REC_{ENERGY}	0.69^{***}	0.88^{***}	0.36^{***}	0.36^{***}	0.65^{***}	0.93^{***}	-

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ^{ns} not significant.

Table 5. Results of ANOVA of individual cheese yields (curd, solids and water) and milk components recoveries (protein, fat, solids, energy)

Model	Trait	EFFECT							
		R^2	ΔR^2	RMSE[1]	HTD	DIM	Parity	Vat	MY
M1	CY_{CURD}	0.48		1.40	6.76^{***}	28.59^{***}	6.07^{***}	1.32^{ns}	
M2		0.49	0.01	1.39	7.01^{***}	13.04^{***}	4.02^{***}	1.10^{ns}	3.33^{**}
M1	CY_{SOLIDS}	0.39		0.75	4.47^{***}	23.69^{***}	1.35^{ns}	1.95^{*}	
M2		0.41	0.02	0.74	4.83^{***}	9.85^{***}	1.34^{ns}	1.77^{*}	4.63^{***}
M1	CY_{WATER}	0.53		0.91	10.29^{***}	18.28^{***}	8.11^{***}	1.38^{ns}	
M2		0.53	0.00	0.91	10.30^{***}	8.98^{***}	5.85^{***}	1.21^{ns}	1.33^{ns}
M1	$REC_{PROTEIN}$	0.40		1.97	6.10^{***}	2.58^{**}	18.25^{***}	0.89^{ns}	
M2		0.41	0.01	1.97	6.08^{***}	3.16^{***}	20.36^{***}	0.79^{ns}	2.25^{*}
M1	REC_{FAT}	0.40		2.89	7.19^{***}	8.47^{***}	0.44^{ns}	1.31^{ns}	
M2		0.41	0.01	2.89	6.74^{***}	7.09^{***}	0.47^{ns}	1.27^{ns}	1.19^{ns}
M1	REC_{SOLIDS}	0.38		2.86	4.58^{***}	21.20^{***}	0.51^{ns}	2.10^{**}	
M2		0.39	0.01	2.85	4.75^{***}	10.17^{***}	1.45^{ns}	1.87^{*}	3.22^{**}
M1	REC_{ENERGY}	0.31		2.85	4.48^{***}	5.97^{***}	0.20^{ns}	1.80^{*}	
M2		0.32	0.01	2.84	4.40^{***}	4.11^{***}	0.27^{ns}	1.67^{ns}	1.49^{ns}

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ^{ns} not significant.
[1]RMSE = Root means square error.

Figure 1. Flow chart for the micro model cheese-making

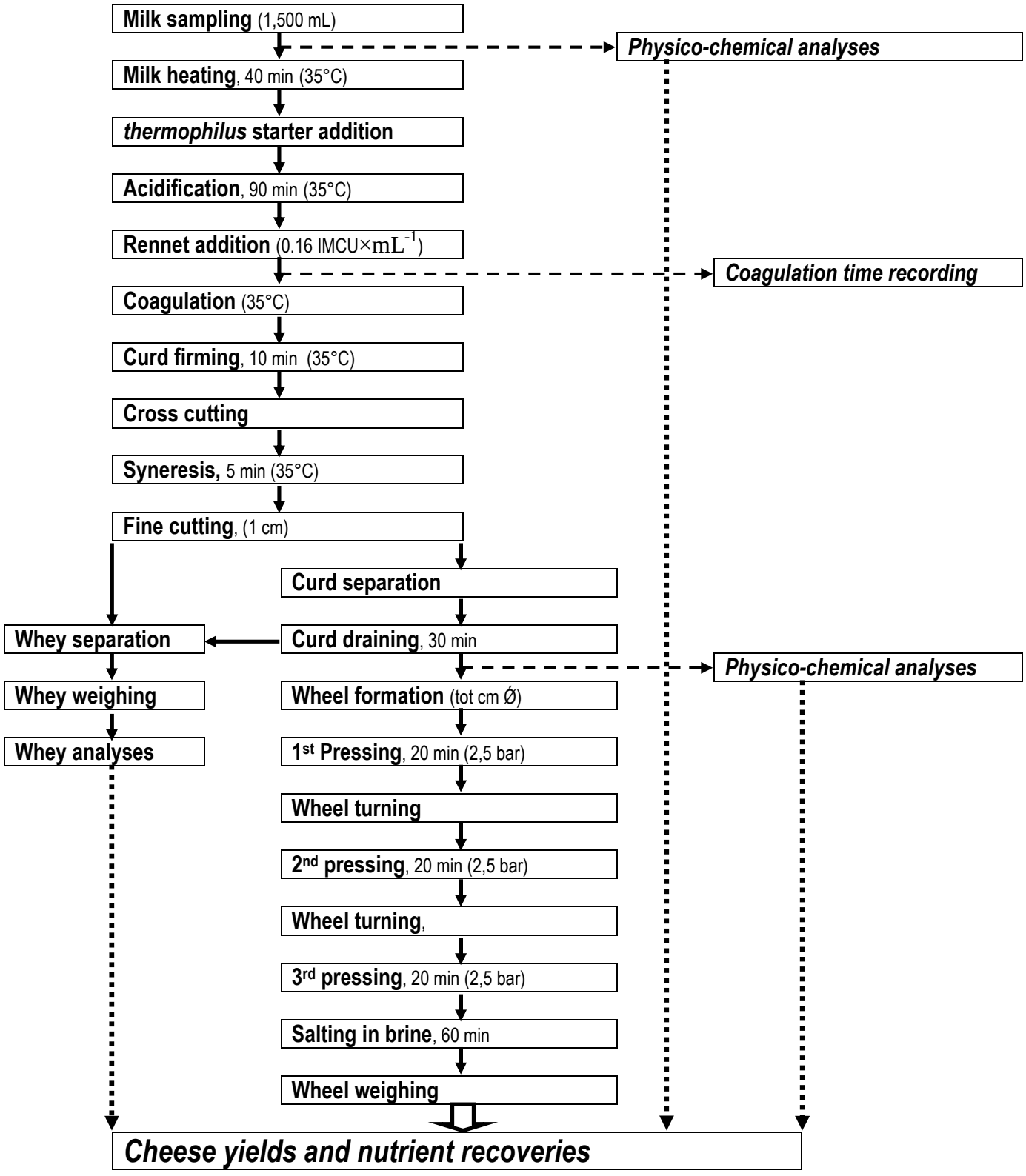


Figure 2. Distribution of individual CY_{CURD} [a] CY_{SOLIDS} [b] and CY_{WATER} [c]

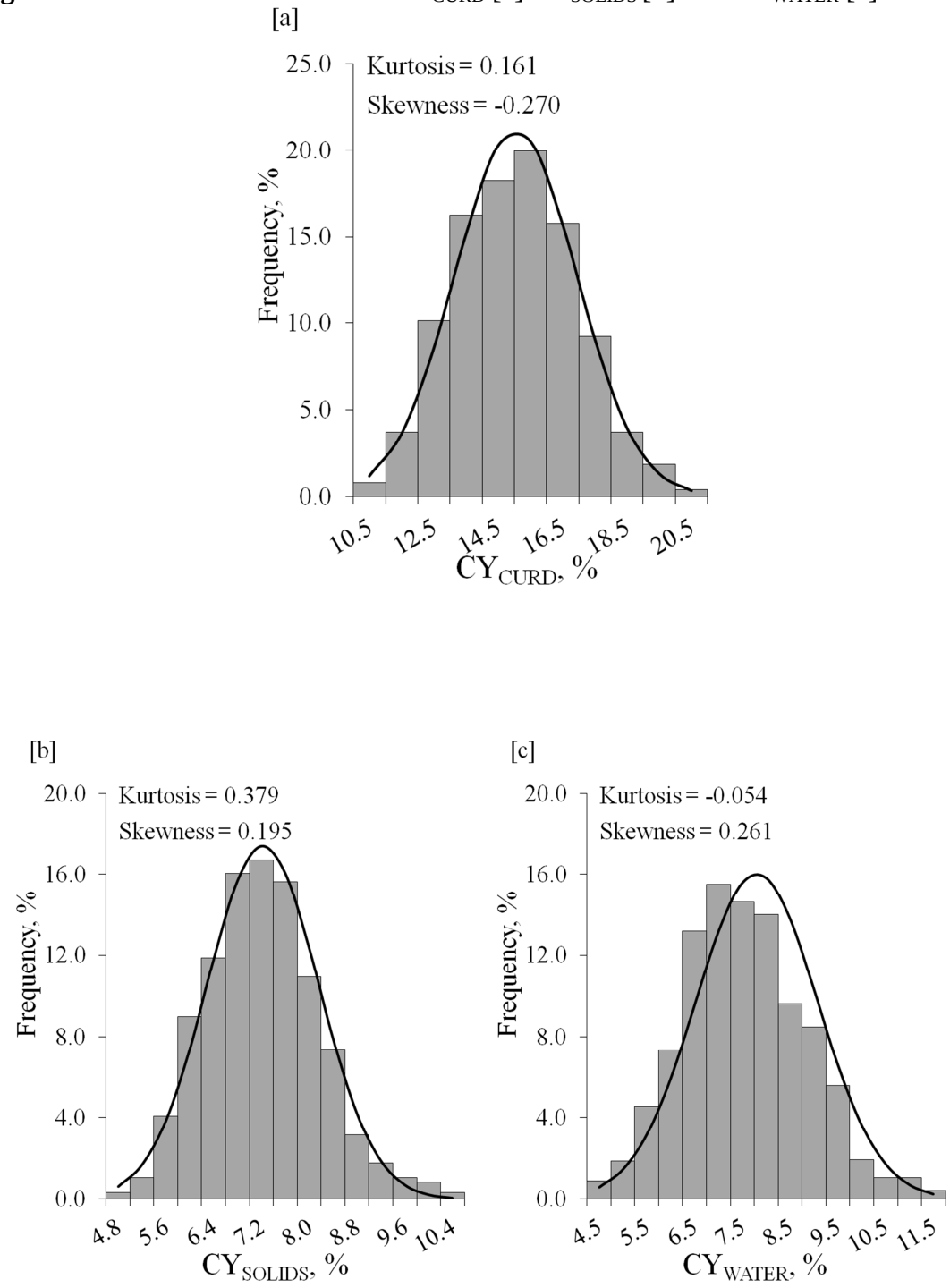


Figure 3. Distribution of $REC_{PROTEIN}$ [a], REC_{FAT} [b], REC_{SOLIDS} [c] and REC_{ENERGY} [d]

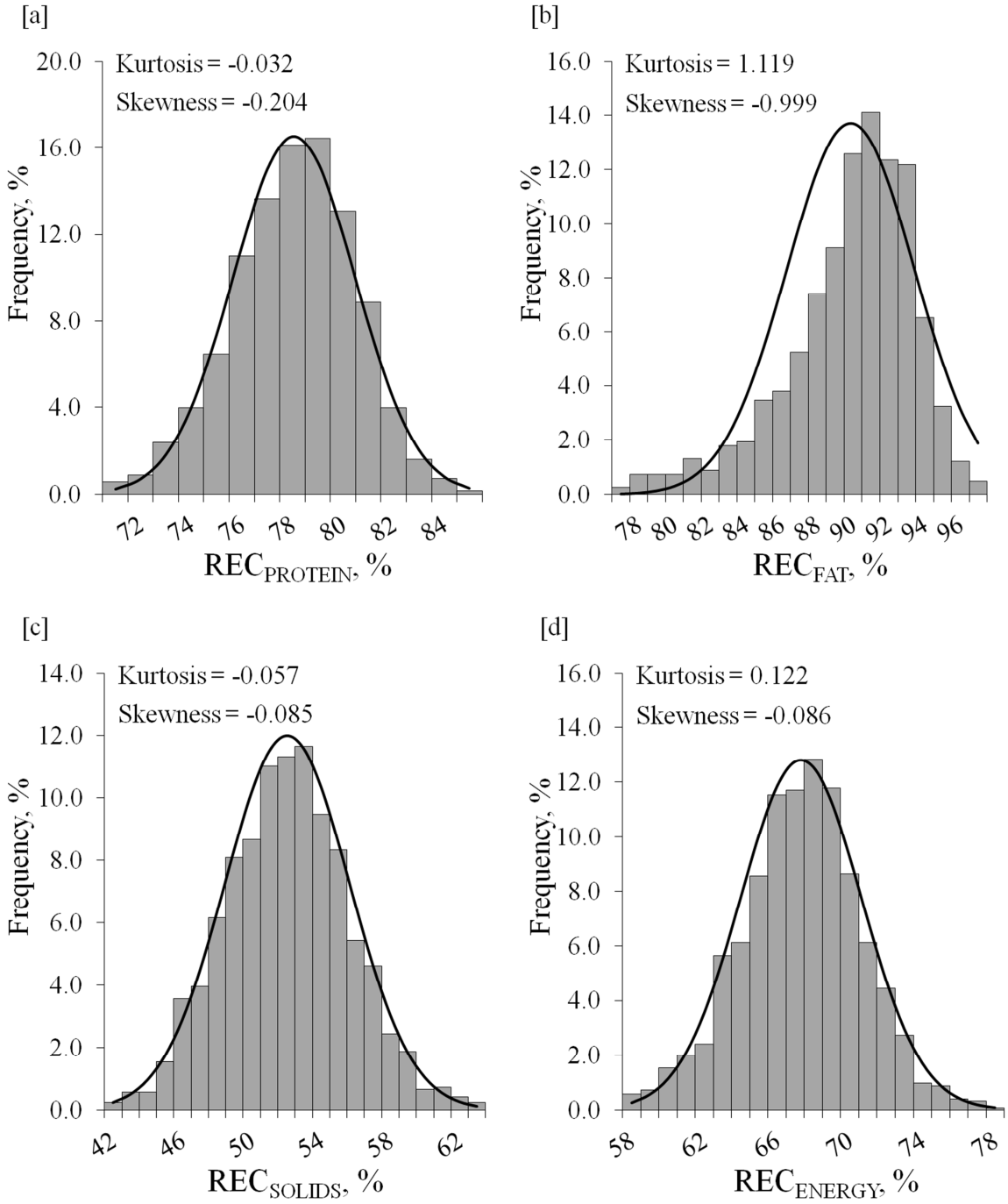


Figure 4. Least square means of CY_{CURD}, CY_{SOLIDS}, CY_{WATER} [a], $REC_{PROTEIN}$, REC_{FAT} [b], REC_{SOLIDS} and REC_{ENERGY} [c] over days in milk obtained with Model 2 (milk yield is included in the model)

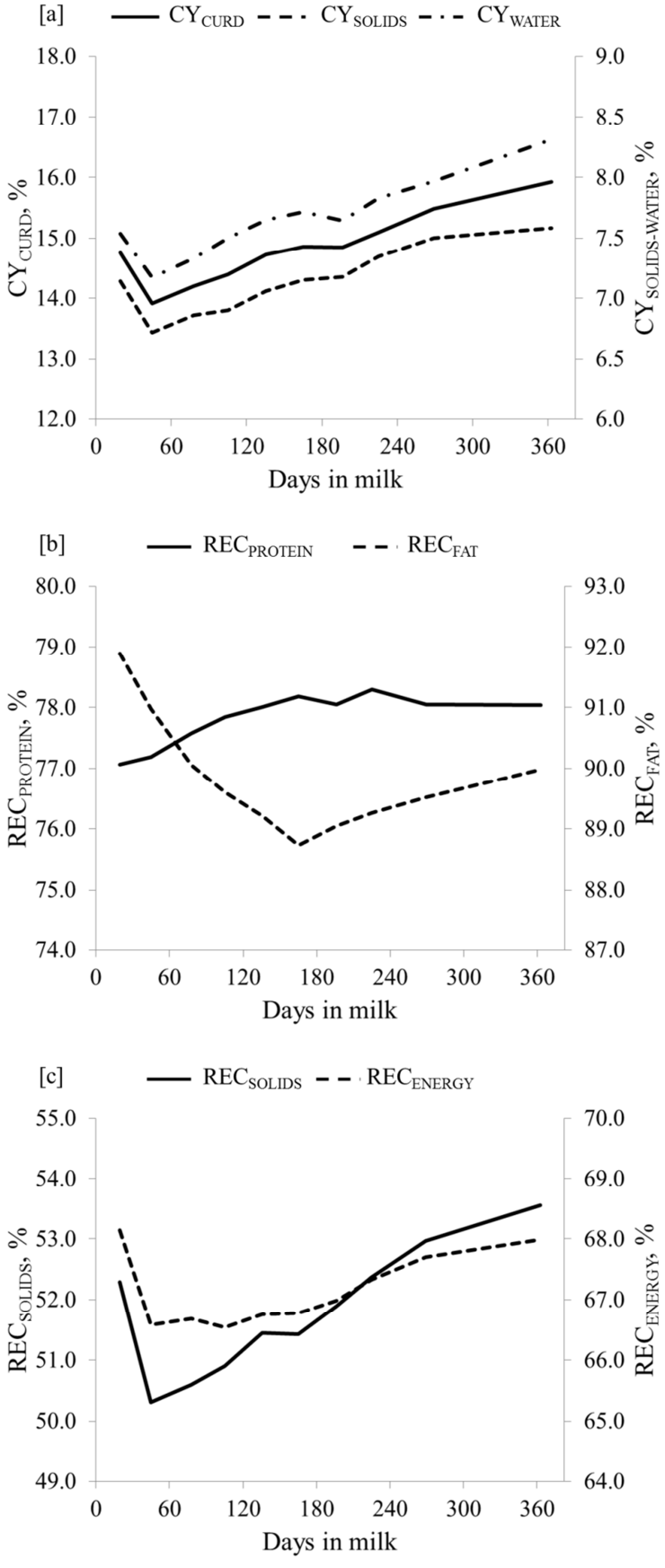


Figure 5. Least square means of CY_{CURD}, CY_{SOLIDS}, CY_{WATER} [a], $REC_{PROTEIN}$, REC_{FAT} [b], REC_{SOLIDS} and REC_{ENERGY} [c] across parities obtained with Model 2 (milk yield is included in the model)

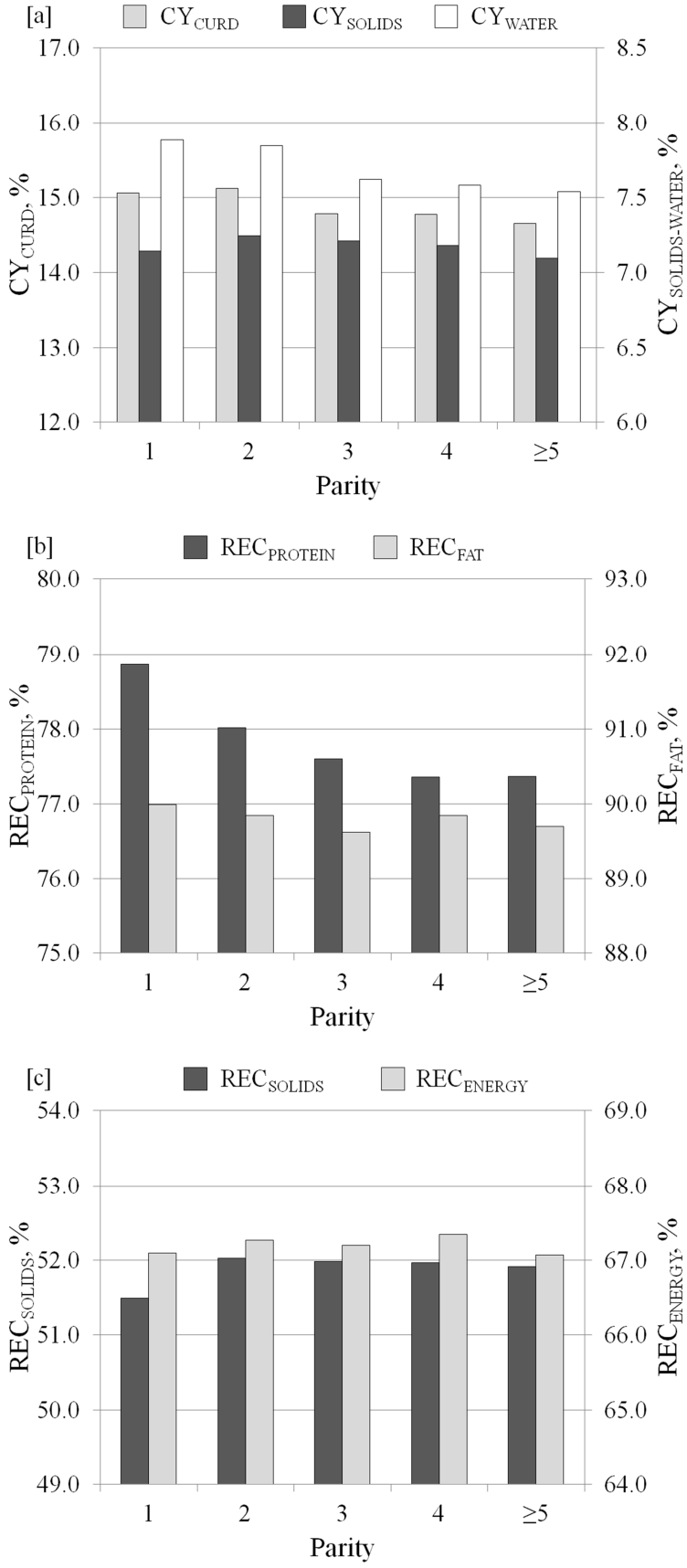
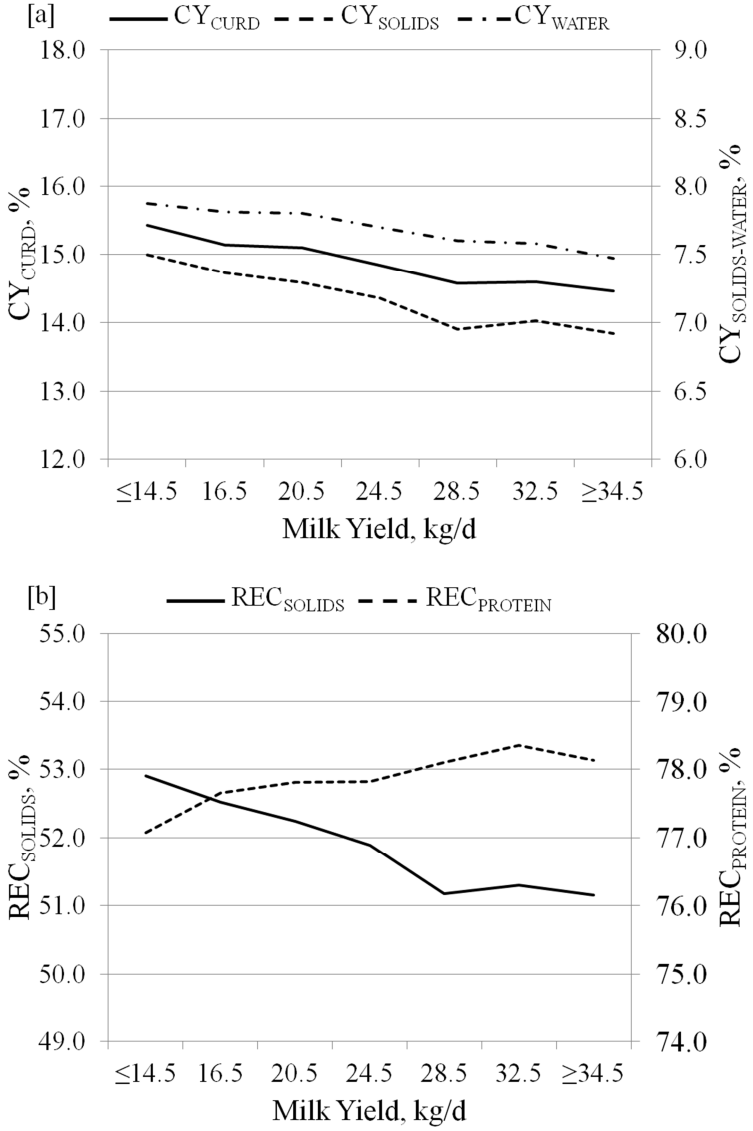


Figure 6. Least square means of CY_{CURD}, CY_{SOLIDS}, CY_{WATER} [a], REC_{SOLIDS}, $REC_{PROTEIN}$ [b], for MY ($kg \times d^{-1}$) obtained with Model 2



IV CONTRIBUTION

GENETIC ANALYSIS OF DIFFERENT MEASURES OF CHEESE YIELD AND NUTRIENT RECOVERY FROM INDIVIDUAL BOVINE MILK SAMPLES, AND THEIR GENETIC RELATIONSHIPS WITH MILK YIELD AND COMPOSITION

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ABSTRACT

Cheese yield (CY) is the most important technological trait in the dairy industry and the objective of this study was to estimate the genetic parameters of cheese yield for the first time, as measured in a dairy cattle population using an individual model-cheese production procedure. A total of 1,167 Brown Swiss cows belonging to 85 herds were sampled once (a maximum of 15 cows were sampled per herd on a single day, 1 or 2 herds per week). From each cow, 1,500 mL of milk was processed according to the following steps: milk sampling and heating, culture addition, rennet addition, gelation-time recording, curd cutting, whey draining and sampling, wheel formation, pressing, salting in brine, weighing, and cheese sampling. The compositions of individual milk, whey and curd samples were determined. The analyzed traits included: three different measures of cheese yield taken as the weight of the fresh salted cheese (CY_{CURD}), cheese total solids (CY_{SOLIDS}), and cheese water (CY_{WATER}) as a percentage of the weight of milk processed; and four nutrient recoveries taken as the weight of the fat (REC_{FAT}), protein ($REC_{PROTEIN}$), and total solids (REC_{SOLIDS}) in the cheese as a percentage of the same nutrient in milk; and the energy (REC_{ENERGY}) in the cheese as a percentage of that in milk. For statistical analysis, Bayesian univariate and bivariate animal models were implemented via Gibbs sampling. The effects of parity (1 to 4 and more), days in milk (6 classes), and laboratory vat (15 vats) were assigned flat priors; those of herd/test-date, animal, and residual were given Gaussian prior distributions. The results revealed the following: 1) The heritability estimates of CY_{CURD}, CY_{SOLIDS} and CY_{WATER} ranged from 0.224 to 0.267; these were larger than the estimates obtained for milk yield (0.182) and milk fat content (0.122), and similar to that for protein content (0.275). 2) CY_{WATER} showed a highly positive genetic correlation with CY_{SOLIDS} (0.87), whereas their phenotypic correlation was moderate (0.37). 3) The fat and protein

contents of milk showed high genetic correlations with the CYs, but the values were significantly less than unity. 4) The heritability estimates of $REC_{PROTEIN}$ and REC_{FAT} were larger (0.490 and 0.208, respectively) than those obtained for the protein and fat contents of milk, and the genetic relationships between $REC_{PROTEIN}$ and REC_{FAT} with milk protein and fat content were low or moderate. 5) $REC_{PROTEIN}$ and REC_{FAT} were moderately correlated with the CYs and highly correlated with REC_{SOLIDS} and REC_{ENERGY}. 6) REC_{SOLIDS} and REC_{ENERGY} were heritable (0.274 and 0.232), and showed high correlations to each other (0.96) and with the CYs (0.83 to 0.97). Together, these findings demonstrate the existence of economically important, genetically determined variability in cheese yield that do not depend solely upon the fat and protein content of milk, but also rely on the ability of the coagulum to retain the highest possible proportions of the available protein, fat, and water. The possible exploitation of this interesting genetic variation does not seem to be feasible through a direct measurement of the phenotype in cows at the population level. Instead, further research is warranted to examine possible means for indirect prediction, such as through assessing the mid-infrared spectra of milk samples.

Key words: individual cheese yield, fat recovery, protein recovery, whey losses, genetic parameters

INTRODUCTION

Cheese production is the most important use of milk produced in many countries (International Dairy Federation, 2011), and the technological parameter of cheese yield is of the highest economic importance for dairy industry (Emmons, 1993). Nevertheless, no previous study has estimated the genetic parameters of cheese yield at following cheese-making individual level. This lack of knowledge can be attributed to two main

issues: difficulties in individually measuring this trait at the population level, and the availability of phenotypically correlated traits for the indirect selection of cheese yield.

Cheese is traditionally obtained from the bulk milk of one or more herds. To obtain cheese yield measures at the level of individual animals, a model cheese-making procedure must be set up. This becomes labor-intensive due to the many manual steps required, which include: individual milk sampling; milk analysis; milk weighing and heating; starter culture preparation and addition; pH measurement; rennet preparation and addition; gelation-time recording and curd cutting; whey drainage, sampling and weighing; curd sampling and analyses; wheel formation, compression, salting, and weighing; and whey collection, weighing and analyses (Cipolat-Gotet et al., 2012b). Moreover, the smaller the volume of the model cheese, the less it represents conditions in the industry.

The most important indirect traits used to improve cheese yield are the milk contents of fat and protein or casein. Almost all of the selection indices for dairy breeds around the world include milk fat and protein content (kg and/or %) (VanRaden, 2004; Miglior et al., 2005). The relative weights of the fat and protein contents within these selection indices are based on the relative economic and/or technical importance of these two nutrients in the cheese-making process (Weigel et al., 1997; Rosati and Van Vleck, 2002). The inclusions of fat and protein in the selection indices are based on the following implicit assumptions: 1) the different proteins and fats have the same value; and 2) the recoveries of milk fat and protein in curd are both constant.

Caseins are the proteins that cause milk to coagulate; they form the basis of cheese production, while the other milk proteins remain primarily within the whey. Despite this, caseins are seldom included in selection indices because: 1) the casein ratio (the ratio between caseins and total protein) is not very variable (Schopen et al.,

2009); and 2) the casein and protein contents present genetic correlations close to unity (Ikonen et al., 2004; Cassandro et al., 2008; Samorè et al., 2012). However, the recovery of milk protein in curd has a higher variability than the casein index (Cipolat-Gotet et al., 2012b), indicating that some whey proteins can be entrapped in the curd and some caseins can escape coagulation. Furthermore, the recovery of milk fat in curd shows some variability that can significantly affect cheese yield (Fagan et al., 2007). The recovery of total solids is influenced by the fat and protein recoveries, the fat-to-protein ratio, and the lactose and mineral contents of the curd. Moreover, the recovery of different nutrients also influences the recovery of milk energy in curd. Finally, although the compositions and recoveries of the different nutrients determine the total solid cheese yield, the cheese yield is also influenced by the ability of the curd to retain water and its solutes.

Cheese yields and nutrient recoveries are influenced by many factors, such as the milk composition, the technological properties of the milk, the utilized cheese-making process, the time and size of curd cutting, etc. (Janhøj and Qvist, 2010). However, we have no information on the heritability of these parameters or their genetic correlations with other traits.

The aims of this study were to use model cheese from individual milk samples in a population of Brown Swiss cows to estimate the genetic parameters for different measures of cheese yields and curd nutrient/energy recoveries, and to estimate their genetic relationships with milk yield and composition.

MATERIALS AND METHODS

ANIMALS AND MILK SAMPLING

Milk samples were obtained from a total of 1,167 Brown Swiss cows from 85 herds (a maximum of 15 cows per herd) located in the Alpine province of Trento (Italy); milk samples were obtained once per cow during evening milking. Within a given day, only one herd was sampled.

The present study is part of the Cowability–Cowplus projects. Detailed descriptions of the sampling procedure may be found in Cipolat-Gotet et al. (2012a) and Cecchinato et al. (2012a), and the production environment was as described in Sturaro et al. (2012). Briefly, the collected samples (without preservative) were immediately refrigerated at 4°C and transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy).

Data on the cows and herds were provided by the Superbrown Consortium of Bolzano and Trento (Italy), and pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy). We included cows with phenotypic records available for the investigated traits and all known ancestors.

MODEL CHEESE-MAKING PROCEDURE

All samples were processed within 20 h after collection. Individual milk samples were analyzed for fat, protein, and casein percentages using a MilkoScan FT6000 (Foss, Hillerød, Denmark).

The procedure used for individual model-cheese production was based on that described by Cologna et al. (2009), which showed good repeatability. A detailed description of the modified cheese-making procedure was previously reported (Cipolat-

Gotet et al., 2012b). Briefly, 1,500 mL of milk was heated to 35°C in a stainless steel micro-vat, supplemented with thermophilic starter culture, mixed with rennet, and controlled for coagulation time. The resulting curd of each vat was cut, drained, shaped in wheels, pressed, salted, weighed, sampled, and analyzed. The whey collected from each vat was also weighed, sampled, and analyzed.

TRAIT DEFINITIONS

All of the measured traits were based on the weights (W, g) and chemical compositions of milk, whey and curd, as detailed by Cipolat-Gotet et al. (2012b). The energy of the curd ($kJ \times g^{-1}$) was estimated as the difference between the energy of the milk and whey. The measured traits were as follows:

- cheese yield (CY_{CURD}, %) as W of curd × 100 / W of milk;
- total solid (TS) cheese yield (CY_{SOLIDS}, %) as (W of milk TS – W of whey TS) ×100 / W of milk;
- water cheese yield (CY_{WATER}, %) as (W of milk water – W of whey water) × 100 / W of milk;
- fat (F) recovery (REC_{FAT}, %) as (W of milk F – W of whey F) × 100 / W of milk F;
- protein (P) recovery ($REC_{PROTEIN}$, %) as (W of milk P – W of whey P) × 100 / W of milk P;
- TS recovery (REC_{SOLIDS}, %) as (W of milk TS – W of whey TS) × 100 / W of milk TS;
- energy recovery (REC_{ENERGY}, %) as (milk energy – whey energy) × 100 / milk energy.

NON-GENETIC EFFECTS

Non-genetic effects were included in the mixed models designed to estimate heritability and genetic correlations for the measures of cheese yield and nutrient recovery. These non-genetic effects were identified in preliminary analyses based on the GLM procedure (SAS Inc., Cary, NC, USA). For all traits, the model accounted for the effects of the herd/sampling-processing date (85 levels), days in milk of the cow (DIM;

class 1: < 60 d, class 2: from 60 to 120 d, class 3: from 121 to 180 d, class 4: from 181 to 240 d, class 5: from 241 to 300 d, and class 6: >300), the parity of the cows (1 to 4 or more), and vats (15 levels).

GENETIC ANALYSIS

Statistical inferences were based on a set of bivariate analyses that considered pairs of traits. These traits were individual cheese yields (i.e., CY_{CURD}, CY_{SOLIDS}, CY_{WATER}), nutrient recoveries (i.e., $RECP_{ROTEIN}$, REC_{FAT}, REC_{SOLIDS}, REC_{ENERGY}) and daily milk production (MY) and composition (i.e., fat, protein and casein). Each bivariate analysis was based on the following linear mixed model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1 h} + \mathbf{Z_2 a} + \mathbf{e}$$

where **y** is a vector of records for traits 1 and 2; $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are appropriate incidence matrices for systematic effects in **b**, herd effects in **h**, and animal additive genetic effects in **a**, respectively; and **e** is a vector of random residuals.

Bayesian Inference. (Co)variance components and related parameters were estimated using a Bayesian approach and Markov-chain Monte Carlo methods (Sorensen and Gianola, 2002). All traits were taken as continuous variables, and their values were assumed to be sampled from the following multivariate normal distribution:

$$p(\mathbf{y}|\mathbf{b}, \mathbf{h}, \mathbf{a}, \mathbf{R}) \sim \text{MVN}(\mathbf{Xb} + \mathbf{Z_1 h} + \mathbf{Z_2 a}, \mathbf{I} \otimes \mathbf{R}),$$

where $\mathbf{y}, \mathbf{b}, \mathbf{h}, \mathbf{a}, \mathbf{X}, \mathbf{Z_1}$ and $\mathbf{Z_2}$ are as defined above, **R** is a 2 × 2 matrix of residual (co)variances, and **I** is a 2 × 2 identity matrix. The data were properly ordered within the vectors, and vectors **a** and **h** contained the effects for both traits individual by individual.

In a Bayesian setting, we assumed that:

$$p(\mathbf{a}|\mathbf{G}) \sim \text{MVN}(\mathbf{0}, \mathbf{A} \otimes \mathbf{G}) \text{ and}$$

$$p(\mathbf{h}|\mathbf{H}) \sim \text{MVN}(\mathbf{0}, \mathbf{I} \otimes \mathbf{H}),$$

where **G** is a 2 × 2 matrix of additive-genetic (co)variances, **A** is the numerator of the Wright's relationship matrix between individuals, **H** is a 2 × 2 (co)variance matrix for herd effects, and **I** is a 2 × 2 identity matrix. Flat priors were assumed for the effects in **b**, as well as for **G**, **H**, and **R**.

Gibbs Sampler. Marginal posterior distributions of unknown parameters were estimated by performing numerical integration using the Gibbs sampler (Gelfand and Smith, 1990) to obtain auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and of the burn-in period were assessed by visual inspection of trace plots, as well as by the diagnostic tests described by Geweke (1992) and Gelman and Rubin (1992). After a preliminary run, we decided to construct a single chain consisting of 850,000 iterations and discard the first 50,000 iterations as a very conservative burn-in. Subsequently, one in every 200 successive samples was retained, in order to store draws that were more loosely correlated. Thus, 4,000 samples were used to determine the posterior distributions of the unknown parameters. The lower and upper bounds of the highest 95% probability density regions for the parameters of interest were obtained from the estimated marginal densities. The posterior median was used as the point for all parameters. Auto-correlations between samples and estimates of Monte Carlo Standard Error (Geyer, 1992) were calculated.

Across-herd heritability was computed as:

$$h^2_{AH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_H + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are additive genetic, herd/test-date, and residual variances, respectively.

Intra-herd heritability was computed as:

$$h^2_{IH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are additive genetic, herd/test-date, and residual variances, respectively.

Additive genetic correlations were estimated as:

$$r_A = \frac{\sigma_{A1,A2}}{\sigma_{A1} \cdot \sigma_{A2}}$$

where $\sigma_{A1,A2}$ is the additive genetic covariance between traits 1 and 2, and σ_{A1} and σ_{A2} are the additive genetic standard deviations for traits 1 and 2, respectively.

RESULTS

DESCRIPTIVE STATISTICS

Table 1 shows descriptive statistics for the analyzed traits. The average cheese yield (CY_{CURD}) obtained using the individual model-cheese production procedure was 15.0% and the coefficient of variation was 12.4%. CY_{SOLIDS} represented slightly less than half the CY_{CURD}, while CY_{WATER} was slightly more than half. The coefficient of variation of CY_{WATER} was higher than that of CY_{SOLIDS} (16.3% and 12.8%, respectively). The nutrient recoveries averaged 51.9% (REC_{SOLIDS}) to 89.8% (REC_{FAT}), while their coefficients of variation were lower than those of the cheese yields at 3.1%, 4.0%, 6.8%, and 4.9% for $REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS}, and REC_{ENERGY}, respectively.

The milk production and composition (fat, protein and casein content) traits (Table 1) were representative of the Italian Brown Swiss population (Samorè et al., 2007; Samorè et al., 2012; Cecchinato et al., 2011).

VARIANCE COMPONENTS AND HERITABILITY

The point estimates and features of the marginal posterior densities for the additive genetic, herd/test-date and residual variances, as well as the across-herd and intra-herd heritabilities for the investigated traits, are reported in Table 2. The herd/test-date variance for CY_{CURD} was slightly larger than the variances attributed to the polygenic effect. The variability represented by the individual environmental causes of variation (within herd/test-date, parity, days in milk and laboratory vat) was slightly higher (1.22 residual standard deviation). From this, the across-herd heritability (h^2_{AH}) of CY_{CURD} was equal to 18.5% while the within-herd heritability (h^2_{IH}) was much higher at 26.7%. Analyzing the components of CY_{CURD} (Table 2), CY_{SOLIDS} showed, as expected, lower variances than CY_{CURD}, but the three random sources of variation accounted for similar proportions of the variance, and thus the two traits had similar heritability estimates (20.6% and 26.3%, respectively). All variances of CY_{WATER} were higher than those of CY_{SOLIDS}, especially that of herd/test-date. As a consequence, CY_{WATER} yielded an h^2_{AH} estimate (13.0 %) much lower than the h^2_{IH} (22.4%), and both were lower than the corresponding estimates for CY_{SOLIDS}.

In the context of nutrient recoveries, $REC_{PROTEIN}$ had very high (and similar) genetic and individual residual components, both of which were higher than the herd/test-date source of variation (Table 2). As a consequence, the h^2_{AH} was high (35.3%) and the h^2_{IH} was very high (49.0%).

REC_{FAT} showed an additive genetic variance similar to that of $REC_{PROTEIN}$ but had higher herd/test-date and residual variances, and thus yielded much lower h^2_{AH} (14.1%) and h^2_{IH} (20.8%) estimates.

The overall recoveries (REC_{SOLIDS} and REC_{ENERGY}) showed genetic variances similar to those of the individual nutrient recoveries, herd/test-date variances intermediate with respect to the previous examined traits, and residual variances similar to those of REC_{FAT}. The resulted heritability estimates of REC_{SOLIDS} and REC_{ENERGY} were intermediate between those of the two individual nutrients: 21.6% and 18.4% for h^2_{AH} and 27.4% and 23.2% for h^2_{IH}, respectively.

Figures 1 and 2 clearly show that the marginal posterior densities of the h^2_{AH} estimates for the different cheese yields and nutrient recoveries overlap, and also that the probability to be higher than 10% s almost 100% for all traits except for CY_{WATER} and REC_{FAT}, which were 97% and 95%, respectively. As expected, the probability of being higher than 10 % was much greater for the h^2_{IH} estimates of all traits (data not shown).

Comparison with the heritability estimates for the milk production and composition traits (Table 2) revealed that the heritability estimates of the cheese yields and nutrient recoveries were higher than that of milk yield and comparable with those of the milk contents. The recoveries of individual nutrients (protein and fat) were more heritable than the corresponding milk contents, and the recoveries of overall nutrients (total solids and energy) yielded heritability estimates similar to those of the protein and casein contents in milk.

PHENOTYPIC, GENETIC AND RESIDUAL CORRELATIONS

As expected, CY_{CURD} showed high phenotypic, genetic and residual correlations with its two components, CY_{SOLIDS} and CY_{WATER} (Table 3). With respect to the phenotypic and residual correlations between the two major cheese yield components, the retention of water in curd was moderately correlated with the retention of total

solids (+37% and +31%, respectively). In contrast, they were much more highly correlated from the genetic point of view (+87%).

The recovery of protein (Table 4) was totally independent from that of fat from the phenotypic and residual points of view (-2% and -7%, respectively), and it showed only moderate genetic relationship (+32%). Both individual nutrient recoveries were moderately correlated with both overall recoveries (REC_{SOLIDS} and REC_{ENERGY}), with REC_{FAT} showing higher correlations than $REC_{PROTEIN}$ in both cases. As would be expected, the two overall recoveries were highly correlated with each other (Table 4).

Considering the relationships between the cheese yields and nutrient recoveries (Table 5), the phenotypic, genetic, and residual correlations of CY_{CURD} and CY_{SOLIDS} with the individual nutrient recoveries were moderately positive (22% to 58%), while those with the overall recoveries were high (64% to 97%).

The retention of water by the curd presented low to moderate phenotypic and residual correlations (+13% to +40%) with all of the nutrient recoveries, while the genetic correlations were moderate with $REC_{PROTEIN}$ and REC_{FAT} (+38% and +50%, respectively) and high with REC_{ENERGY} and REC_{SOLIDS} (+83% and +88%, respectively).

The genetic correlations of milk yield and composition with cheese yields and nutrient recoveries are shown in Table 6. Milk yield tended to have a low and unfavorable additive genetic correlation with all of the individual cheese yield and nutrient recovery measures (-20% to -47%), with the exception of a low and favorable genetic correlation with $REC_{PROTEIN}$ (+27%). Milk fat, protein and casein content showed very high and positive additive genetic correlations with all of the measures of cheese yield, especially CY_{SOLIDS} (92% to 97%) and the recovery of total solids and energy in the curd. Fat content exerted low genetic correlations with REC_{FAT} (-19%) and $REC_{PROTEIN}$ (+21%). The protein and casein contents of milk tended to show

positive moderate genetic correlations with REC_{FAT} (+40% and +39%, respectively) and almost no genetic correlation with $REC_{PROTEIN}$ (3% and 6%, respectively).

DISCUSSION

GENETIC PARAMETERS OF CHEESE YIELD

As summarized by Cipolat-Gotet et al. (2012b), the cheese yield from the milk of Brown Swiss cows, as calculated on the basis of the weight of the curd after brining, was higher (15.0% on average, Table 1) than the estimates obtained in other breeds (Verdier-Metz et al., 1998 and 2001; Martin et al., 2009; Glantz et al., 2011). The main reasons for this are the high fat, protein and casein contents and the good coagulation properties (Cecchinato et al, 2011; Bittante et al., 2012) that characterize the milk from Brown Swiss cows and make it particularly suited for the production of traditional cheeses that come under the Protected Designation of Origin, as defined by the European Union (De Marchi et al., 2008; Bittante et al., 2011a and 2011b).

The heritability estimates obtained in the present study for milk yield and composition are close to those reported in previous studies of Brown Swiss population (Samorè et al., 2007 and 2012;., 2011). It is interesting to note that the heritability estimates obtained in the present study for cheese yields were much higher than the corresponding estimates of daily milk yield and milk fat content, and close to the estimates obtained for milk protein content.

To our knowledge, the present study offers the first heritability estimates of cheese yield obtained from individual cheese making in the bovine species. The only other heritability estimate of cheese yield found in the literature comes from a laboratory test carried out on very small quantities of ewe's milk (10 mL) that were mixed with a very high concentration of chymosin (2.4 IMCU/mL) and measured after

a fixed time from rennet addition (1 hour), trough centrifugation (15 min) and draining (45 min), as described in Othmane et al. (2002a). A large data set was obtained with this procedure (7,492 samples from 1,119 Spanish Churra ewes), and Othmane et al. (2002b) obtained a test-day heritability estimate of individual cheese yield that was much lower (8%) than that found in the present work. In contrast, their heritability estimates for the daily milk yield (15%), fat content (6%) and protein content (23%) of ewe's milk were similar to those obtained in the present study on cow's milk. Based on repeated sampling of animals, the authors also estimated the heritability of lactation data (Othmane et al., 2002c), and obtained a heritability (9%) that was very similar to test day heritability obtained in the present work on cow's milk. It seems likely that the very small amount of milk used in cheese-yield estimation and/or their operating conditions could have caused repeatability issues, accounting for the low heritability estimate obtained from ewe's milk.

In the present work, the cheese yield showed very high positive genetic correlations with the fat, protein and casein contents of milk (+88%, +87%, and +86%, respectively; Table 6), but the probability of this correlation being close to 100% was very low. Therefore, fat and protein explain a large proportion of the genetic variability observed in cheese yield, but about one fourthwith the remainder of the genetic variance depends on other factors. As expected given the moderate negative genetic correlation between milk yield and quality, the genetic correlation between the test-day milk yield of the cow and the cheese yield of the milk was low and unfavorable (-29%). The probability of this correlation being lower than 0% (negative correlation) was 86% (data not shown).

The major factors that affected the cheese yield, beyond the milk composition, were the recoveries of individual nutrients (especially protein and fat) and the ability of

the curd to retain water (Fagan et al., 2007). These data cannot be obtained with the procedure described by Othmane et al. (2002a), which uses a 10-mL milk sample. In contrast, the procedure described by Cologna et al. (2009) using 500 mL, which was improved by Cipolat-Gotet et al. (2012b) for use on 1,500 mL and applied in the present paper, allows researchers to analyze milk, whey and curd, and determine the complete nutrient balance between milk, whey, and cheese. As outlined by Cipolat-Gotet et al. (2012b), some studies (De Marchi et al., 2008; Martin et al., 2009) compared different breeds and found that the differences observed in average cheese yield are greater than the differences in the fat and protein (casein) contents of the milk. Furthermore, the genetic variants of milk protein fractions can influence the cheese yield. Walsh et al. (1998) found that milk from cows homozygous for the B variant of κ-casein generated a significantly greater cheese yield than milk from cows homozygous for the A variant, even after correcting for milk composition.

Thus, based on previous findings and the present work, it is evident that water retention in curd and the individual nutrient recoveries from milk play important roles in explaining cheese yield variation (both between and within breeds) even after the data have been corrected for the fat and protein contents.

GENETIC CONTRIBUTIONS OF TOTAL SOLIDS AND WATER TO CHEESE YIELD

Similar to previous reports using similar conditions (Martin et al., 2009; Verdier-Metz et al., 2001), total solids represented 48% of the fresh curd after brining, meaning that water contributed slightly more to the cheese yield (7.8% vs. 7.2%, respectively; Table 1). CY_{WATER} was also characterized by a higher phenotypic coefficient of variation with respect to the CY_{SOLIDS} (16.3 vs. 12.8%, respectively).

The genetic variability of CY_{SOLIDS} was appreciable from the economic point of view, as the genetic standard deviation was 0.40 percentage points (5.5% of the average). The equivalent figure for CY_{WATER} was 0.44 percentage points (5.6% of the average). The two components are comparable to the genetic variation of cheese yield (genetic standard deviation, 0.74 percentage points; 4.9% of the average). From Table 3, it can be seen that CY_{SOLIDS} and CY_{WATER} have a high genetic correlation with each other (+87%), and thus both are very highly correlated with their sum (+97% and +98%, respectively). We may therefore conclude that the genetic improvement of one component will cause a large increase in the other and an even higher increase in the fresh cheese yield, and a genetic increase of these last traits will cause an equivalent high genetic improvement in milk total solids and water retention in the curd after brining.

The contributions of individual environmental variation to CY_{SOLIDS} and CY_{WATER} were higher than the genetic contributions, especially for CY_{WATER} (residual standard deviations 0.66 and 0.81 percentage points, respectively). As a result, the intra-herd heritability of CY_{SOLIDS} was almost identical to that of CY_{CURD} (Table 2), while that of CY_{WATER} was slightly lower (26.3% vs. 22.4%, respectively).

A large difference was found between CY_{SOLIDS} and CY_{WATER} in the effect of herd/test-date, which showed standard deviations of 0.40 and 0.78 percentage points, respectively. The combined effect of the genetic, residual and herd/test-date effects on phenotypic variation explains why the CY_{SOLIDS} estimate of across-herd heritability was slightly larger than that of CY_{CURD}, while the estimate obtained for CY_{WATER} was much smaller (20.6% vs. 13.0%, respectively). However, future work will be required to determine whether this relatively high effect of herd/test-date on water retention by curd

was due to a moderate reproducibility of the model-cheese production procedure or to a larger effect of some environmental condition (barns, feeding, milking procedures, etc.).

In contrast to the genetic correlations, CY_{SOLIDS} and CY_{WATER} showed a moderate positive phenotypic correlation with each other (+37%, Table 3), but high phenotypic correlations with their sum (+86% and +86% with CY_{CURD}, respectively).

The total solid cheese yield showed genetic correlations with the fat, protein and casein contents of milk (+97%, +93%, and +92%, respectively), which were even greater than the high correlations showed by CY_{CURD}; conversely, they showed a greater negative correlation with daily milk yield (-47%). While these results could perhaps be expected, it was somewhat unexpected that CY_{WATER} showed genetic correlations with milk traits similar to those obtained for CY_{CURD} (high and positive with milk contents and low and negative with milk production).

It also worth noting that the across-herd and intra-herd heritability estimates of CY_{SOLIDS} were both much higher than those estimated for milk fat content and almost identical to those obtained for milk protein content (Table 2).

PROTEINS: GENETICS OF CURD RECOVERY AND WHEY LOSSES

The coagulation and syneresis processes that characterize cheese-making are strongly dependent on milk proteins (Emmons et al., 2003). Proteins and fats are the main components of curd, and the losses of protein in whey reduce cheese yield (Hallen et al., 2009).

Almost all of the selection indices used for the genetic improvement of dairy breeds around the world include the protein and fat contents of milk as predictor traits for the cheese value of the milk (VanRaden, 2004). The implicit assumption is that the recovery of protein and fat is constant. However, Aleandri et al. (1989) found that the

curd, salted-curd and Parmesan cheese yields were linearly related to fat content, but curvilinearly related to the protein content of the vat. Our descriptive statistics (Table 1) show that the average recovery of milk protein in curd after brining was almost identical to the average casein index (78.08% vs. 78.05%, respectively). However, the average recovery of milk protein in curd was characterized by an appreciable variability coefficient (3.1%) in the present work. Furthermore, Cipolat-Gotet et al. (2012b) showed that $REC_{PROTEIN}$ is heavily influenced by herd/test-date, parity and days in milk, and discussed the results of previous studies showing that the recovery of casein in cheese can be substantially lower than 100% (Bynum and Olson, 1982; Ikonen et al., 1999; Summer et al., 2003; Malacarne et al., 2006).

As shown in Table 2, an important part of this variability is of genetic origin. In fact, $REC_{PROTEIN}$ had a genetic standard deviation equal to 1.42 percentage points, which is higher than the genetic variability often found for casein index. The residual individual variability was on the same order as the additive genetic variability, so the intra-herd heritability of $REC_{PROTEIN}$ was very high (close to 50%; Table 2). The herd/test-date component was slightly lower than the genetic and residual variances, yielding an across-herd heritability of 35.3% for $REC_{PROTEIN}$.

It is evident that including the protein content of milk in the selection indices for dairy breeds is useful for the indirect selection of higher cheese yield, whereas the inclusion of casein content would not be likely to significantly improve the results of this indirect selection because the casein and protein contents of milk generally show a genetic correlation close to unity (Samoré et al., 2007, and 2012). Furthermore, the present study shows that the genetic correlations of casein with all of the individual cheese yield and nutrient recovery measures were very similar to those found for crude protein. Only $REC_{PROTEIN}$ and/or REC_{CASEIN}, which were shown to be independent of

the protein and casein contents of milk, could potentially contribute new information to support the genetic improvement of animals for increased cheese yield. Unlike the other tested measures of cheese yield and nutrient content, $REC_{PROTEIN}$ seems to be favorably correlated, from the genetic point of view, with a cow's productivity (median +27%, with an 84% probability of being > 0). As the direct measurement of $REC_{PROTEIN}$ is not feasible in practice, new research should focus on calibrating indirect prediction equations. Recent achievements in the improvement of milk coagulation properties have shown the potential for using medium infra-red spectrometry (Dal Zotto et al., 2008; De Marchi et al., 2009; Cecchinato et al., 2009), candidate genes (Glantz et al., 2011; Cecchinato et al., 2012c and 2012d) and genome-wide approaches (Tyrisevä et al., 2008; Glantz et al., 2012). The effect of different protein fractions and/or their genetic variants on cheese yield have been analyzed in some phenotypic studies (Alipanah and Kalasnikova, 2007; Zambrano Burbano et al., 2010; Bonfatti et al., 2011), but their effects on $REC_{PROTEIN}$ have not yet been quantified on a genetic basis.

FAT: GENETICS OF CURD RECOVERY AND WHEY LOSSES

The fat recovery in the curd was close to 90% on average, which can be considered normal for industrial cheese-making (Kefford et al., 1995), and had a coefficient of variation greater than that of $REC_{PROTEIN}$ (4.0% vs. 3.1%, respectively). Proteins are more important than fat in the processes of coagulation and syneresis, but the recovery of fat in the curd or its loss in the whey (which are influenced by coagulation and syneresis) are important to the final cheese yield (Fagan et al., 2007). Cipolat-Gotet et al. (2012b) showed that REC_{FAT} is heavily influenced by herd/test-date and days in milk, and discussed the effects of other relevant phenotypic and technological causes of variation.

In the context of individual factors related to the genetics of the cow, Mistry et al. (2002) found that the higher cheddar cheese yield obtained from the milk of Brown Swiss versus Holstein cows was due to superior fat recovery (94.55% vs. 90.85%, respectively), whereas the protein recovery was similar between the two breeds. Similar results were obtained by Malacarne et al. (2006) when using milk from the same breeds to produce Parmigiano-Reggiano cheese; the authors attributed this difference in fat whey loss mainly to the superior coagulation properties of Brown Swiss breed. Also Alipanah and Kalashnikova (2007) used a small-scale trial to show that the superior cheese yield of milk obtained from cows expressing AB and BB k-casein is mainly due to differences in $REC_{PROTEIN}$ and (especially) REC_{FAT}.

As shown in Table 2, REC_{FAT} had an additive genetic standard deviation similar to that of $REC_{PROTEIN}$ (1.34 vs. 1.42 percentage points, respectively), but showed a much greater variability due to individual residual and herd/test-date effects. As a result, the intra-herd and across-herd heritabilities of REC_{FAT} (20.8% and 14.1%, respectively) were low to moderate, and only about 40% of the corresponding estimates of $REC_{PROTEIN}$. Notably, the heritability estimates for REC_{FAT} were greater than those for the fat content of milk itself (Table 2). Furthermore, REC_{FAT} was genetically influenced by the milk protein and casein contents (median of marginal posterior densities, +40% and +39%, respectively, both with 93% probability of being > 0) but seemed unfavorably related to fat content (median -19%; with a 74% probability of being < 0) and daily milk yield (median -20%, with a 71% probability of being < 0).

Interestingly, the recovery rates of the two major component of cheese were phenotypically independent and their genetic correlation was positive but moderate (+32%; Table 4). In addition, the phenotypic and genetic correlations of $REC_{PROTEIN}$

and REC_{FAT} with the three studied measures of cheese yield were all low to moderately positive (13% to 58%; Table 5).

Similar to $REC_{PROTEIN}$, REC_{FAT} could also play an important role in the potential genetic improvement of cheese yield. Thus future studies should seek means for the direct measurement (practically unfeasible) or indirect prediction (to be studied) of REC_{FAT} through milk recording or genome-wide approaches.

TOTAL SOLIDS AND ENERGY: GENETICS OF CURD RECOVERY AND WHEY LOSSES

Although only about half of the total solids present in milk are retained in cheese (Verdier et al., 1995; Kefford et al., 1995; Verdier-Metz et al., 1998), the energy content of cheese represents about two thirds that of whole milk (Table 1). The recoveries of total solids and energy are more variable than those of their major constituents, and are influenced by herd/test-date and by days in milk (Cipolat-Gotet et al. 2012b).

With respect to the recoveries of the major individual components of cheese, REC_{SOLIDS} and REC_{ENERGY} had similar genetic variability estimates, an intermediate herd/test-date effect and a residual individual variance similar to that of REC_{FAT} (Table 2). The heritability estimates were intermediate compared to those of the protein and fat recoveries and similar to those of the three cheese yields.

As expected, REC_{SOLIDS} and REC_{ENERGY} were strictly correlated with each other, both phenotypically and genetically (Table 4). They were highly correlated with REC_{FAT} (+55% to +70%) and moderately correlated with $REC_{PROTEIN}$ (+22% to +61%). Their high genetic correlations with the fat, protein and casein contents of milk (58% to 84%) reflect the dilution of the relative content of lactose and minerals (which are almost completely lost in the whey) by fat and protein. Both REC_{SOLIDS} and REC_{ENERGY}

showed very high genetic correlations with the three cheese yields (+83% to +97%), high phenotypic correlations with CY_{CURD} and CY_{SOLIDS} (+66% to +93%), and moderate phenotypic correlations with CY_{WATER} (+40% and +33%, respectively).

CONCLUSION

We herein describe the first estimation of genetic parameters of cheese yield in a cattle population, as assessed through individual model-cheese fabrication. The heritability of cheese yield was much greater than that of milk yield and milk fat content, and similar to that of milk protein content.

The cheese yield, which was expressed in terms of curd weight after brining as a percentage of the weight of milk processed, was composed almost equally of retained total solids and water. The cheese yield expressed as total solids per 100 kg milk exhibited heritability estimates very close to those of the fresh cheese yield, and the amount of water retained in the curd after brining (per 100 kg milk) was heritable, albeit to a slightly smaller degree. Moreover, the retention of water in the curd showed a high genetic correlation with the retention of solids, whereas their phenotypic correlation was moderate.

In almost all selection indices used around the world for the genetic improvement of cattle populations, cheese yield is indirectly selected by including the major cheese components of milk: protein and fat. This implicitly assumes that protein and fat are the major determinants of cheese yield, and that their recovery from milk to cheese is approximately constant and is not genetically controlled.

Instead, the present study shows that fat and protein have high genetic correlations with cheese yield, but these values are significantly lower than 100%, indicating that there is room for further genetic improvement of cheese yield. This study

also shows that there is phenotypic variability of the protein and fat recoveries in the curd, and that the cow's genetics are important to this variability. The heritability of protein recovery is high, while that of fat recovery is moderate; both are greater than the heritability estimates for their respective contents in milk and milk yield, whereas their genetic correlations are low or moderate. These two traits are moderately correlated with each other and highly correlated with the curd recoveries of total solids and energy of milk, which were highly correlated with the studied measures of cheese yields.

These results demonstrate the existence of an economically important genetic variability in cheese yield; this does not depend solely on the fat and protein contents of the milk, but rather relies on the ability of the coagulum to retain the highest possible amount of protein, fat and water. This interesting genetic variability seems ripe for possible exploitation. However, as it does not seem feasible to directly measure these aspects at the population level, further research should focus on indirect prediction (i.e., through mid-infrared spectral analysis of milk), the study of individual genes (candidate gene approach), and/or genome-wide scans.

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TABLES AND FIGURES

Table 1. Descriptive statistics of individual cheese yield (weight of fresh curd, curd solids and curd water as percentage of weight of milk processed), milk components recovery (protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed), single test-day milk yield and composition

Trait	N	Mean	SD	P5	P95
Cheese yield					
CY_{CURD}, %	1,162	14.97	1.86	12.03	18.12
CY_{SOLIDS}, %	1,153	7.18	0.92	5.75	8.73
CY_{WATER}, %	1,156	7.77	1.27	5.84	9.90
Nutrient recovery					
$REC_{PROTEIN}$, %	1,158	78.08	2.43	73.90	81.96
REC_{FAT}, %	1,143	89.79	3.55	82.67	94.41
REC_{SOLIDS}, %	1,157	51.88	3.52	46.01	57.64
REC_{ENERGY}, %	1,144	67.19	3.29	61.78	72.42
Production traits					
Milk Yield, kg/d	1,153	24.62	7.81	12.60	38.10
DIM, d	1,167	179.46	110.70	25.00	392.00
Milk quality traits					
Fat, %	1,163	4.21	0.72	3.13	5.40
Protein, %	1,163	3.69	0.42	3.02	4.38
Casein, %	1,163	2.88	0.32	2.37	3.40

[1]P5 = 5th percentile; P95 = 95th percentile.

Table 2. Features of marginal posterior densities of additive genetic (σ^2_A), herd/test-date (σ^2_H), and residual (σ^2_E), variances, and across-herd (h^2_{AH}), and intra-herd (h^2_{IH}) heritabilities for the investigated traits in Brown Swiss cows.

	σ^2_A		σ^2_H		σ^2_E		h^2_{AH}		h^2_{IH}	
Trait	Median	HPD95%	Median	HPD95%	Median	HPD95%	Median	HPD95%	Median	HPD95%
Cheese yields										
CY_{CURD}, %	0.546	0.25; 0.94	0.891	0.63; 1.30	1.501	1.18; 1.79	0.185	0.09; 0.39	0.267	0.13; 0.44
CY_{SOLIDS}, %	0.157	0.06; 0.29	0.158	0.10; 0.24	0.441	0.33; 0.53	0.206	0.09; 0.40	0.263	0.11; 0.46
CY_{WATER}, %	0.192	0.08; 0.35	0.603	0.43; 0.86	0.664	0.53; 0.78	0.130	0.05; 0.23	0.224	0.09; 0.39
Nutrient recoveries										
$REC_{PROTEIN}$, %	2.010	1.16; 3.04	1.543	1.07; 2.25	2.094	1.32; 2.82	0.353	0.21; 0.52	0.490	0.29; 0.69
REC_{FAT}, %	1.809	0.71; 3.49	4.010	2.78; 5.80	6.900	5.50; 8.12	0.141	0.05; 0.27	0.208	0.08; 0.38
REC_{SOLIDS}, %	2.403	1.21; 4.32	2.274	1.52; 3.41	6.363	4.84; 7.57	0.216	0.11; 0.37	0.274	0.14; 0.46
REC_{ENERGY}, %	1.989	0.87; 3.74	2.203	1.47; 3.29	6.574	5.15; 7.74	0.184	0.08; 0.33	0.232	0.10; 0.41
Production traits										
Milk Yield, kg/d	4.124	1.51; 8.76	23.360	16.67; 33.11	18.475	14.79; 21.48	0.089	0.03; 0.19	0.182	0.07; 0.37
Milk composition										
Fat, %	0.051	0.01; 0.11	0.101	0.06; 0.15	0.368	0.31; 0.41	0.096	0.02; 0.21	0.122	0.03; 0.26
Protein, %	0.022	0.01; 0.04	0.022	0.01; 0.03	0.058	0.04; 0.07	0.218	0.11; 0.37	0.279	0.13; 0.47
Casein, %	0.013	0.01; 0.02	0.014	0.01; 0.03	0.034	0.03; 0.04	0.216	0.10; 0.38	0.282	0.13; 0.47

[1]Median = median of the marginal posterior density of the parameter; HPD95% = lower and upper bound of the 95% highest posterior density region

Table 3. Phenotypic (r_p), additive genetic (r_g), and residual (r_e) correlations among individual cheese yield (weight of fresh curd, curd solids and curd water as percentage of weight of milk processed)[1]

Trait	r_p	r_g	r_e
CY_{CURD} with:			
CY_{SOLIDS}	0.86 (0.83; 0.88)	0.97 (0.86; 0.99)	0.81 (0.77; 0.85)
CY_{WATER}	0.86 (0.83; 0.88)	0.98 (0.86; 0.99)	0.81 (0.77; 0.85)
CY_{SOLIDS} with:			
CY_{WATER}	0.37 (0.28; 0.44)	0.87 (0.59; 0.98)	0.31 (0.16; 0.42)

[1]Median of the marginal posterior density of the parameter (HPD95% = lower and upper bounds of the 95% highest posterior density region).

Table 4. Phenotypic (r_p), additive genetic (r_g), and residual (r_e) correlations among milk components recovery (protein, fat, total solids, and energy of the curd as percentage of the protein, fat, total solids, and energy of the milk processed) [1]

Trait	r_p	r_g	r_e
$REC_{PROTEIN}$ with:			
REC_{FAT}	-0.02 (-0.11; 0.06)	0.32 (-0.12; 0.72)	-0.07 (-0.28; 0.13)
REC_{SOLIDS}	0.22 (0.14; 0.30)	0.42 (0.01; 0.73)	0.22 (-0.01; 0.43)
REC_{ENERGY}	0.26 (0.18; 0.34)	0.61 (0.21; 0.85)	0.23 (0.01; 040)
REC_{FAT} with:			
REC_{SOLIDS}	0.55 (0.49; 0.61)	0.65 (0.22; 0.88)	0.54 (0.41; 0.64)
REC_{ENERGY}	0.68 (0.65; 0.72)	0.70 (0.29; 0.89)	0.67 (0.57; 0.75)
REC_{SOLIDS} with:			
REC_{ENERGY}	0.93 (0.92; 0.94)	0.96 (0.90; 0.99)	0.93 (0.90; 0.94)

[1]Median of the marginal posterior density of the parameter (HPD95% = lower and upper bounds of the 95% highest posterior density region).

Table 5. Phenotypic (r_p), additive genetic (r_g), and residual (r_e) correlations between individual cheese yield (weight of fresh curd, curd solids and curd water as percentage of weight of milk processed) and milk components recovery (protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed)[1]

	CY_{CURD}			CY_{SOLIDS}			CY_{WATER}		
Trait	r_p	r_g	r_e	r_p	r_g	r_e	r_p	r_g	r_e
$REC_{PROTEIN}$	0.29 (0.20; 0.37)	0.58 (0.13; 0.87)	0.29 (0.15; 0.42)	0.38 (0.30; 0.45)	0.34 (-0.20; 0.69)	0.41 (0.28; 0.54)	0.13 (0.02; 0.22)	0.50 (-0.06; 0.89)	0.16 (0.01; 0.30)
REC_{FAT}	0.39 (0.31; 0.46)	0.34 (-0.06; 0.64)	0.49 (0.31; 0.67)	0.22 (0.13; 0.29)	0.37 (-0.04; 0.67)	0.28 (0.06; 0.46)	0.40 (0.31; 0.47)	0.38 (-0.06; 0.72)	0.43 (0.25; 0.60)
REC_{SOLIDS}	0.75 (0.71; 0.79)	0.97 (0.87; 0.99)	0.72 (0.64; 0.77)	0.93 (0.3; 0.94)	0.91 (0.81; 0.96)	0.95 (0.93; 0.97)	0.40 (0.32; 0.48)	0.88 (0.58; 0.98)	0.33 (0.17; 0.44)
REC_{ENERGY}	0.66 (0.61; 070)	0.92 (0.73; 0.99)	0.64 (0.55; 0.71)	0.88 (0.8; 0.89)	0.87 (0.71; 0.94)	0.90 (0.87; 0.93)	0.33 (0.24 0.41)	0.83 (0.45; 0.97)	0.26 (0.10; 0.38)

[1]Median of the marginal posterior density of the parameter (HPD95% = lower and upper bounds of the 95% highest posterior density region)

Table 6. Additive genetic (r_g) and phenotypic (r_p) correlations between individual cheese yield (weight of fresh curd, curd solids and curd water as percentage of weight of milk processed), milk components recovery (protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed), with milk yield and milk composition[1]

	Milk yield, kg/d		Fat, %		Protein, %		Casein, %	
Trait	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
CY_{CURD}	-0.29 (-0.70; 0.25)	0.02 (-0.08; 0.12)	0.88 (0.58; 0.98)	0.51 (0.44; 0.57)	0.87 (0.63; 0.98)	0.62 (0.56; 0.67)	0.86 (0.62; 0.97)	0.65 (0.59; 0.69)
CY_{SOLIDS}	-0.47 (-0.86; 0.11)	0.007 (-0.08; 0.10)	0.97 (0.78; 0.99)	0.76 (0.72; 0.79)	0.93 (0.67; 0.99)	0.58 (0.52; 0.62)	0.92 (0.66; 0.99)	0.60 (0.54; 0.64)
CY_{WATER}	-0.23 (-0.77; 0.41)	0.01 (-0.11; 0.13)	0.84 (0.36; 0.99)	0.18 (0.09; 0.26)	0.87 (0.57; 0.99)	0.45 (0.37; 0.52)	0.86 (0.55; 0.98)	0.48 (0.39; 0.54)
$REC_{PROTEIN}$	0.27 (-0.28; 0.78)	0.09 (-0.01; 0.19	0.21 (-0.41; 0.61)	0.09 (0.007; 0.17)	0.03 (-0.37; 0.40)	0.02 (-0.07; 0.10)	0.06 (-0.36; 0.42)	0.05 (-0.03; 0.14)
REC_{FAT}	-0.20 (-0.76; 0.42)	0.15 (0.05; 0.26)	-0.19 (-0.71; 0.44)	0.04 (-0.04, 0.12)	0.40 (-0.11; 0.79)	0.19 (0.10; 0.27)	0.39 (-0.13; 0.78)	0.20 (0.11; 0.28)
REC_{SOLIDS}	-0.25 (-0.70; 0.39)	0.05 (-0.03; 0.15)	0.76 (0.31; 0.96)	0.63 (0.58; 0.67)	0.84 (0.55; 0.98)	0.53 (0.46; 0.58)	0.80 (0.49; 0.96)	0.53 (0.47; 0.58)
REC_{ENERGY}	-0.21 (-0.71; 0.54)	0.08 (-0.08; 0.17)	0.58 (0.04; 0.88)	0.59 (0.53; 0.64)	0.82 (0.47; 0.98)	0.37 (0.30; 0.47)	0.79 (0.42; 0.97)	0.38 (0.31; 0.44)

[1]Median of the marginal posterior density of the parameter (HPD95% = lower and upper bounds of the 95% highest posterior density region).

V CONTRIBUTION

FACTORS AFFECTING THE VARIATION OF TEXTURE, PHYSICO-CHEMICAL AND SENSORY TRAITS FROM AN INDIVIDUAL MODEL CHEESE-MANUFACTURING PROCESS

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ABSTRACT

Cheese chemical composition, physical traits and sensory properties were assessed in a dairy cattle population using an individual model-cheese production procedure. A total of 1,224 Brown Swiss cows from 83 herds of the Alpine province of Trento were sampled once. From each cow, 1,500 ml of raw milk was used for the cheese-making. The composition and physical traits of individual maturated cheese (2 months of ripening) were analysed individually. A sensory panel was made up and trained for the sensory assessing of individual cheese. The assessed traits exhibited almost a normal distribution (expect for the cheese salt). The average cheese quality values ±SD were: protein (%) 26.83±4.01, fat (%) 38.04±4.06, salt (%) 2.04±0.07, total solids (%) 80.06±4.63, pH 5.17±0.17, L* 58.99±6.10, a* 2.07±0.53, b* 7.63±2.83, SI* 7.97±2.61, MSF (N) 35.52±16.98. The average sensory properties values ±SD were: smell 3.06±0.36, flavor 3.33±0.36, salt 3.23±0.44, sour 2.51±0.55, elasticity 2.48±0.71, firmness 4.97±0.72, moisture 2.79±0.48. All traits were highly influenced by herd/test date and days in milk (not significant for: colour traits b* and SI; sensory properties: flavor) of the cow. Chemical composition of matured cheese were influenced by the fat:protein ratio of milk. Only few traits were influenced by order of parity, while milk production resulted not influencing any variable considered in this study. Comparison among sensory properties indicated that only texture indices were highly correlated and exhibited an high relationship with MSF. The described results provide new insight into the variability and relationship among quality traits of cheese. Additional research on this topic is needed, especially in terms of estimating genetic parameters for the described traits and of assessing methods for their indirect prediction.

Key words: individual cheese quality, physical traits, sensory properties

INTRODUCTION

Sensory analysis of cheese and, in general, of dairy products, represents the last step for the quality evaluation (Drake, 2007). The characteristics and the quality of maturated cheese depend upon many factors linked, on one hand, to the quality (chemical and microbiological) of milk, and, on the other to the cheese-making technology process (Verdiez-Metz et al., 1998). Flavor and texture of cheese could be influenced by its chemical composition, retention of milk components in cheese and cheese yield (Green and Grandison, 1993). When sensory properties are evaluated on cheese produced by the same process, milk quality represents an important variability factor that assumes more importance for the production of Protected Designation of Origin (PDO) cheeses because of the relevance of regulations and restrictions on the modifications of raw milk during the cheese-making process.

Normally, sensory analysis is conducted on cheese produced in a cheese-making plant (milk from one or more herds). Few studies investigated the relationship between sensory traits and the aspects that influence milk quality such as species (Ha et al., 1991; Kondyli and Katsiari, 2001), breed (Verdier et al., 1995; Verdier-Metz et al., 1998; Martin et al., 2009), feeding (Verdier et al., 1995; Verdier-Metz et al., 1998) and herd management (Coulon et al., 2004; Martin et al., 2009).

No previous study focused on the variability of sensory traits of cheese produced at animal level especially because of the many manual steps required to produce an high number of model cheese from individual milk samples (Cipolat-Gotet et al., 2012b). This research approach could be fundamental to: 1) evaluate how the effect of physiological factors like the moment of lactation, the state of health (i.e. somatic cells) and order of parity, could influence sensory properties of cheese; 2) estimate genetic parameters of sensory properties and their genetic correlations with milk quality traits.

To study the relationship between sensory traits and the animal is fundamental to assess a cheese-making procedure and a ripening process at individual level. Cipolat-Gotet et al. (2012b) proposed an individual model cheese-manufacturing to assess factors affecting variation of different measures of cheese yield and nutrient recovery and Bittante et al. (2012) estimated genetic parameters of these traits and their genetic relationships with milk yield and composition but no previous study has investigate the variability of sensory properties of cheese produced at individual level. Therefore, the aims of the present study were: 1) to propose an individual model cheese that include the sensory evaluation of cheese produced; 2), to evaluate the variability of components, physical and sensory properties of cheese at individual level; and 3) to investigate several sources of variation for components, physical and sensory properties of cheese using milk from individual cows.

MATERIALS AND METHODS

DATA COLLECTION

Individual milk samples were obtained from 1,224 Brown Swiss cows (sampled once, 15 cows per day with few exceptions) reared in 83 dairy herds located in Trento province. The present study is part of a multi-phase project named Cowability-Cowplus. Details of samples collection and storage were specifically described by Cipolat-Gotet et al. (2012a) and Cecchinato et al. (2012). All samples were analyzed and processed the following morning, within 20 h from collection. Information about cows, herds and pedigrees were obtained from Superbrown Consortium of Trento (Trento, Italy) and from the Italian Brown Swiss Breeders Association (ANARB, Bussolengo, Verona, Italy).

LABORATORY ANALYSIS

Milk samples were analyzed for chemical composition (fat, protein, casein and total solids) using a FT6000 (Foss, Hillerød, Denmark). Somatic cell score (SCC) was determined using a Fossomatic FC counter (Foss, Hillerød, Denmark) and log-tranformed to SCS (Ali and Shook, 1980). The milk pH values were obtained using a Crison 25 electrode (Crison, Barcelona, Spain).

Micro cheese-making sessions (15 individual samples per day) were conducted processing 1500 mL per sample in accord to the protocol set up by Cipolat-Gotet et al. (2012b). After the cheese-making process, cheeses were left to ripen at 15°C and 85% UR (relative humidity) for 2 months. Cheese samples were turned and cleaned from mould using a saline solution at 7, 14, 28 and 42 days from the processing. After 2 months of ripening, cheese samples were weighted and cheese yield (CY_{60D}, %) was expressed as the ratio between the quantity (g) of cheese produced from 1500 ml of milk. Chemical components (fat, protein, salt and total solids) of the cheese were determined using a FoodScan (Foss, Hillerød, Denmark). The acidity of cheese was expressed as pH and measured (3 measurement per sample averaged before data analysis) using Crison 25 electrode (Crison, Barcelona, Spain). Color determination was carried out on cheese samples (3 consecutive readings averaged before data analysis) using a Minolta colorimeter (CM-508c, D65 illuminant and 10° observer, Konica-Minolta Sensing Inc., Ramsey, NJ) and expressed in terms of lightness (L*), redness (a*), and yellowness (b*) and saturation index (SI) according with CIELAB (1976). Cheese hardness ,expressed as maximum shear force (MSF; N), was assessed using a TA-HDi Texture Analyzer (Stable Macro System, London, UK) with a Warner-Bratzler shear attachment (10 N load cell, 2 mm/s crosshead speed) on cylindrical cheese sample

(diameter of 1 cm; 3 repeated measures per sample). Results were interpreted by means of texture expert software (Joseph, 1979).

SENSORY EVALUATION

The panel was made up of 14 technicians of the Department of Agriculture, Food, Natural resources, Animals and Environment (DAFNAE; University of Padova). They were previously selected and trained in the sensory characterization of cheese produced from 1500 mL (Cipolat-Gotet et al., 2012b). For each day of sensory analysis (83 days), 15 cheeses were evaluated by 6 assessors randomly selected from the pool of panel. Testing-day was completed over two sessions, with eight samples for the first and seven samples for the latter. Cheese samples (2 pieces per sample) were presented on a Petri plate and water was supplied to wash the mouth between samples. All sensory analysis were held at mid-morning in a sensory room. The protocol-scorecard comprised 7 sensory terms that describe: one smell term (intensity), one flavor term (intensity), two taste terms (intensity of salt and sour) and three texture (Foegeding and Drake, 2007) terms (elasticity, firmness and moisture). They were used to test the cheese samples on a 15-points scale (0-7 scale considering the half point) where 0 represented absence and 7 represented maximum perception of the attribute under evaluation.

STATISTICAL ANALYSIS

Statistical models were fit using a GLM procedure (SAS Inst. Inc., Cary, NC).). Physico-chemical traits of cheese were analyzed as continuous traits according to the following linear model:

$$y_{ijklmn} = \mu + HTD_i + DIM_j + parity_k + vat_l + MY_m + e_{ijklmn},$$

where y_{ijklmn} is the observed trait (fat, protein, salt, total solids, pH, L*, a*, b* and shear force); μ is the overall intercept of the model; HTD_i (herd/test date) is the fixed effect of the *i*th herd-sampling date (i = 1 to 85); DIM_j is the fixed effect of the *j*th class of days in milk (j = 1 to 10; class 1: < 30 days, class 2: 30 to 60 days, class 3: 61 to 90 days; class 4: 91 to 120 days; class 5: 121 to 150 days; class 6: 151 to 180 days; class 7: 181 to 210 days; class 8: 211 to 240 days; class 9: 241 to 300 days; class 10: > 300 days); $parity_k$ is the fixed effect of the *k*th parity of the cow (k = 1 to 5 or more); vat_l is the fixed effect of the *l*th number of the vat (l = 1 to 15); MY_m (Milk yield, kg/d) is the fixed effect of the *m*th class of milk yield (m = 1 to 7; class 1: < 14.48 kg/d; class 2: 14.48 to 18.43 kg/d; class 3: 18.44 to 22.37 kg/d; class 4: 22.38 to 26.31 kg/d; class 5: 26.32 to 30.26 kg/d; class 6: 30.27 to 34.20 kg/d; class 7: > 34.20 kg/d); and e_{ijklmn} is the residual random error term ~ N (0, σ^2_e).

Prior the statistical analysis, sensory variables were standardized and scaled for each assessor to unit variance and zero centre, i.e. each variable was forced to 0 mean and variance equal to 1 (Næs, 1990). Standardized sensory attributes were analyzed as continuous traits according to the following linear model:

$$y_{ijklmn} = \mu + HTD_i + DIM_j + parity_k + OP_l + MY_m + e_{ijklmn},$$

where y_{ijklmn} is the observed trait (smell, flavor, salt, sour, elasticity, firmness and moisture); μ is the overall intercept of the model; HTD_i (herd/test date) is the fixed effect of the *i*th herd-sampling date (i = 1 to 85); DIM_j is the fixed effect of the *j*th class of days in milk (j = 1 to 10; class 1: < 30 days, class 2: 30 to 60 days, class 3: 61 to 90 days; class 4: 91 to 120 days; class 5: 121 to 150 days; class 6: 151 to 180 days; class 7: 181 to 210 days; class 8: 211 to 240 days; class 9: 241 to 300 days; class 10: > 300 days); $parity_k$ is the fixed effect of the *k*th parity of the cow (k = 1 to 5 or more); OP_l is the fixed effect of the *l*th number of the order of presentation of cheese samples to the

assessors (l = 1 to 15); MY_m (Milk yield, kg/d) is the fixed effect of the *m*th class of milk yield (m = 1 to 7; class 1: < 14.48 kg/d; class 2: 14.48 to 18.43 kg/d; class 3: 18.44 to 22.37 kg/d; class 4: 22.38 to 26.31 kg/d; class 5: 26.32 to 30.26 kg/d; class 6: 30.27 to 34.20 kg/d; class 7: > 34.20 kg/d); and e_{ijklmn} is the residual random error term ~ N (0, σ^2_e).

RESULTS

CHARACTERISTICS OF INDIVIDUAL CHEESES

Mean and standard deviation values of investigated traits (one cheese represented an individual sampled cow) are reported in Table 1. The lactating dairy cows (mean values for DIM and order of parity of 179 and 2.54, respectively) produced on average 24.34 kg/d with a protein, casein, fat, lactose, total solids content; and SCS of 3.75, 2.88, 4.38, 4.77, 13.89% and 2.98, respectively (data not shown). Singular component values of individual cheeses after two months of ripening were 26.83, 38.04 and 2.04% for protein, fat and salt content, respectively, contributing to a cheese yield of 8.73% with a total solid content of about 80%. As expected for the milk, fat content and SCS showed the highest coefficient of variation (20.47% and 62%, respectively) whereas, in the cheese, protein was more variable (CV = 14.94%) then the other components.

Concerning physical traits, colour analysis indicated that individual cheese samples tended to light-yellow showing L* , a*, b* and SI traits of 58.99, -2.07, 7.63 and 7.97, respectively. The hardness of cheese resulted high (35.52 N) and comparable with commercial types of cheese presenting a long ripening time. All of these traits (Table 1) were almost normally distributed with kurtosis and skewness values close to zero, except the cheese salt content (data not shown).

Table 2 reports descriptive statistics for attributes of sensory analysis, Figure 1 and 2 exhibited the corresponding distribution of individual observation, while Pearson product-moment correlations among these standardized traits are given in Table 3. The intensity of smell and flavor exhibited similar mean and variance values with 3.06±0.36 and 3.33±0.36, respectively. The correlation between these two traits assumed a medium value (0.40; $P < 0.001$). Values of skewness and kurtosis close to 0 emphasized the normal distribution of smell and flavor intensity attributes (Figure 1a and Figure 1b). For the taste traits, assessors felt higher values of salt (3.23) than sour (2.51) even if the latter was more variable showing a CV of 21.86%. Although its distribution can be defined as normal, sour attribute exhibited an high value of skewness (Figure 1d) explainable by values of cheeses acidity closer to 0 than for the other sensory traits. Salt was quite correlated with sour attribute (0.40; $P < 0.001$) and the correlations of both taste attributes with smell and flavor intensity were positive, medium and significant (Table 3).

As confirmed by the high content of cheese total solids (Table 1), all of texture traits pointed out high hardness of the cheese samples with 4.97 of firmness, 2.48 of elasticity and 2.79 of moisture attribute. Elasticity showed the highest variability than the other sensory traits with a CV of 28.80%. In general, texture attributes showed a normal distribution (Figure 2) although elasticity presented a skewness value slightly over 0 while firmness skewness was slightly below 0 explainable by the high hardness of the cheese samples. As expected, texture traits were highly correlated: firmness were negatively correlated with elasticity and moisture attributes showing a correlation value of -0.81 ($P < 0.001$) for both, while elasticity was positively correlated with moisture (0.77; $P < 0.001$). Texture and the other sensory attributes were low correlated presenting values from -0.18 to 0.17.

FACTORS AFFECTING VARIATION OF CHEESE QUALITY

Table 4 and 5 presents how the sensory attributes are correlated with milk quality and cheese quality traits, respectively. Smell was not or low correlated with all milk components, while flavor showed a negative correlation with milk lactose content and pH (-0.26, -0.17, respectively; $P < 0.001$). Flavor attribute was also not correlated with any component of cheese while smell presented a low correlation with cheese total solids (-0.09; $P < 0.001$). Salt and sour taste attributes were negative correlated with lactose showing values from -0.40 to -0.28. Salt was positive correlated with milk casein (0.20; $P < 0.001$) and protein (0.28; $P < 0.001$) and this relationship was found with cheese protein (0.21; $P < 0.001$). As expected, sour attribute was negative correlated with pH of cheese (-0.45; $P < 0.001$) although the relationship with pH of milk was less accentuated (-0.23; $P < 0.001$).

In general, the correlations between texture attributes and milk components showed high values. Casein resulted positive correlated with elasticity (0.30 $P < 0.001$) and moisture (0.34 $P < 0.001$) while showed a negative relationship with firmness (-0.32; $P < 0.001$). High values of correlation were also found when texture attributes were correlated with milk total solids (0.31, -0.42 and 0.43 with elasticity, firmness, and moisture, respectively; $P < 0.001$). Moving to the correlations with cheese quality traits, elasticity, firmness and moisture resulted highly positive correlated (-0.70; 0.69; -0.64; $P < 0.001$) with total solids showing an opposite relationship than what found in the case of texture attributes-milk total solids correlation. As expected, MSF of cheese samples was positive correlated (0.61; $P < 0.001$) with firmness while negative related with the other texture attributes.

Table 6 shows the importance of the effects included in the linear model explaining the variability of the cheese quality traits. The coefficient of determination

presented medium values for all the traits ranging from 0.29 (cheese salt) to 0.58 (pH). In general, herd/test date resulted significant for all the traits ($P < 0.001$), but days in milk effect showed higher f-values when cheese components were tested as dependent variables. The maximum differences of the least squares means from the 83 herds were 11.47% for protein, 9.80% for fat, 15.52% for total solids 0.64 for pH 20.66 for L*, 1.29 for a*, 7.76 for b*, 8.96 for SI and 53.11 N for MSF (data not shown). For cheese protein, total solids and MSF, lactation effect (DIM) presented a similar trend to the daily milk yield showing higher values with the peak of milk production and a decrease with the prolonging of lactation; for cheese fat an opposite trend was found (Figure 3). Parity resulted significant when tested on cheese total solids (P < 0.001), L ($P < 0.05$), b* $P < 0.05$), SI ($P < 0.05$), and MSF ($P < 0.05$) without showing any trend between cows presenting different order of parity. MY effect resulted not significant for all the considered traits (except for cheese protein; $P < 0.05$) underlining that the milk production did not affect the composition and the physical traits of cheese. The source of variation directly associated with the cheese-making procedure and the ripening process, did not significantly affected any trait, except the cheese total solids ($P < 0.01$), empathizing an acceptable reproducibility of the entire process (from milk collection to the analysis of ripened cheeses).

In Table 7 are summarized the result from ANOVA obtained testing the effects of herd/test day, days in milk, parity, order of cheese presentation and milk production on sensory standardized attributes. Herd/test day was an important source of variation for all the sensory traits ($P < 0.001$). The maximum differences of the least squares means from the 83 herds were 1.29 for smell, 1.31 for flavor, 1.37 for salt, 2.29 for sour, 2.12 for elasticity, 2.41 for firmness and 1.65 for moisture (data not shown). Days in milk resulted significant for smell trait exhibiting a decrease till the middle of lactation

and a subsequent increase (Figure 4a). Also the texture attributes were influenced by DIM ($P < 0.001$) and presented a trend during lactation in agreement with the results found for total solids and MSF during lactation: the firmness attribute was higher at the beginning (peak of daily milk production) of lactation showing a decrease till the end; the other two texture traits presented an opposite trend (Figure 4b). The order of parity resulted significant for salt ($P < 0.01$), elasticity ($P < 0.01$) and moisture ($P < 0.01$): it was found a trend just for the salt attribute, observing higher salt values with older cows (data not shown).

The order of presentation of cheese to the assessors during the sensory test was an important effect especially for smell and flavor ($P < 0.001$): in the figure it is possible to observe that the first cheese presented to the assessors assumed higher values for the intensity of smell and flavor while the score of these two attributes was more constant during the testing (Figure 5).

Daily milk yield was not significant for all the sensory traits considered in the present work.

DISCUSSION

CHEMICAL COMPONENTS OF CHEESE

The average value of CY_{60D} obtained from mature (two months of ripening) individual cheese was of 8.73% starting from values of fresh curd 15% (Cipolat-Gotet et al., 2012b). This CY is comparable to that obtained for cheeses with long ripening (more than 12 months) such as Parmiggiano Reggiano produced using milk of Brown Swiss cows (Malacarne et al., 2006). This breed, compared to Holstein-Friesian, is characterized to produce milk presenting high fat, protein and casein contents, good

coagulation properties which results in an high CY (Malacarne et al., 2006; De Marchi et al., 2008; Cecchinato et al., 2011).

In the present study, protein, fat salt and total solids of model cheeses produced by raw unskimmed individual milk samples averaged 26.38%, 38.04%, 2.04% and 80.06%, respectively. Days in milk was the most important source of variation ($P < 0.001$). Cheese protein and fat showed an opposite trend on the lactation (Figure 3a) where, at the peak of milk production, cheese protein assumed the highest values while cheese fat the lowest. The percentage difference between the highest and the lowest (end of lactation) values were 13.80% and 10.25% for protein and fat, respectively. This results could be explain by the different fat-protein ratio of milk during the lactation: this ratio assumed values of 1.23 at 90 days from the calving while 1.11 at the end of lactation. Auldist et al. (1996), reported lower values of protein and fat of cheese produced using milk of late-lactating cows compared to the results obtained using milk of early-lactating cows. Coulon et al. (1998), studying the effects of days in milk on quality of Saint-Nectaire-type cheese, divided the entire lactation in four periods and found higher values of cheese fat in the second period (145 days) of lactation. Cheese total solids presented the same trend of fat during lactation with higher values between 45 and 90 days from calving: the percentage difference of higher values than the worst was almost 5% showing less differences than the fat probably due to the opposite trend of cheese protein. For cheese salt, higher values were found at the end of lactation.

Parity factor resulted significant only in the case of cheese total solids without showing any evident trend. Although order of parity was not significant for cheese protein and fat, it was found that the older cows produced cheese with lower content of protein and higher content of fat than the younger (data not shown). Milk production (MY) milk did not presented any effect on the quality of cheese; the inclusion of this

factor in the linear model produced only a halving of F-values for DIM (data not shown).

Moving from the discussion of animal effects, Herd/test date resulted significant for all the cheese components ($P < 0.001$). This factor pointed out high differences (%) between the best herd and the worst, showing values of 36.25% 22.81% and 17.73% for protein, fat and total solids, respectively. Many studies evaluated factors (summarized in the herd effect) affecting the chemical composition of cheese or the strictly related milk quality, such as the herd size (Allore et al., 1997), feeding regime (Verdier-Metz et al., 1998 and 2000), season (Malacarne et al., 2003) and herd management (Coulon et al., 2004; Martin et al., 2009).

The no-significance vat factor (except for totals solids; $P < 0.01$) underlines the good reproducibility of the method proposed (even after ripening of cheese) and joins the good results presented by Cipolat-Gotet et al. (2012b) on the CY and nutrient recoveries of milk cheese in the curd.

PHYSICAL TRAITS OF CHEESE

To our knowledge, there are no studies that have investigate on physical traits of individual cheeses from dairy cows with an high number of observations. Acidity, evaluated measuring pH averaged 5.17, the colour was, as expected, near to yellow (b* = 7.63) showing relatively high values of lightness (L* = 58.99) while hardness of cheese resulted high (MSF = 35.52 N). Herd/test date was the most important source of variation for all traits ($P < 0.001$). The differences (%) between herds showed higher and lower values for MSF (80.63%) and pH (29.15%), respectively, while medium values for the colour. Most of the studies compared results of cheese physical traits considering factors related to feeding system (Verdier-Metz et al., 1998 and 2000) and

breed (De Marchi et al., 2008; Martin et al., 2009). De Marchi et al. (2008), comparing results in three types of cheeses obtained with milk of Holstein Friesian and Brown Swiss cows, found conflicting results for pH and for hardness (MSF, N) of cheese. In a study that compare Holstein Friesian and Montbéliarde (Martin et al., 2009), it was reported lower values of cheese pH for the latter while for the colour traits breed factor was not significant.

Lactation stage (DIM) was another important factor affecting variability of physical traits. This factor was not already studied at individual level but selecting group of cows on the basis of days in milk. Auldist et al. (1996), in a study to assess the effect of somatic cell count (SCC) and the stage of lactation, divided four in groups the cows by the content of milk SCC (high or low) and the moment of lactation (early or late). They found opposite results with higher values of pH in early-lactating cows with low milk SCC while lower values with high milk SCC. Also in the study of Coulon et al. (1998), the effect of stage of lactation was studied on cheese physical traits dividing the pool of cows in 4 groups by the moment of lactation. They reported constant values of pH during lactation with an increase in the last period (298 days). In the present study, pH assumed higher values in the middle of lactation while lower at the beginning and at the end. The lactation stage resulted significant just for L* and b* without showing and continuous trends. Despite this, it has to be highlighted that the variability (%) for b* of least square means for DIM was 18.99 (data not shown).

Figure 3b presented the least square means of MSF for stage of lactation ($P < 0.001$). This trait showed a similar trend similar to the dry matter present in the cheese. This was confirmed by the correlations between these two traits (0.56; $P < 0.001$). The production of individual cheeses individual using the same sampling of milk, the same cheese-making procedure, the same timing and characteristics of cheese ripening has as

obvious consequence a great variability in terms of CY. This implies a different quantity of cheese dry matter and then a different hardness.

Apparently the order of parity did not influence cheese physical traits. This factor was significant for L* ($P < 0.05$), b* ($P < 0.05$) and MSF ($P < 0.05$) but the differences of these traits between cows presenting different order of parity were very low and negligible.

As for chemical components, daily milk production factor was not significant for any physical traits.

SENSORY PROPERTIES OF CHEESE

As for physico-chemical traits, there are no studies that have evaluated sensory properties tested on individual cheeses produced using milk of dairy cows. The herd/test date showed higher f-values and significance than the other factors included in the linear model. When sensory traits were tested, the herd/test date included not only the cheese-making session factor but also the day of the sensory analysis. The differences between opposite values of least square means highlighted an high variability. The relationship between sensory properties and herd was studied especially considering the feeding system (Verdier-Metz et al.; 1998; Buchin et al., 1999; Verdier-Metz et al., 2000) while in one study was related with the number of milking per day (Martin et al., 2009). In the present study characteristics (feeding regime, geographical location, size) of herds were collected and these will be related to the sensory properties in our following studies.

The stage of lactation resulted significant for smell ($P < 0.01$), salt ($P < 0.05$) and sour ($P < 0.001$) intensity and for all the texture properties ($P < 0.001$). in the present study, flavour was not affected by factors related to the animal (DIM, order of

parity and MY) and to the herd. In Figure 4a it is possible to observe that in the middle of lactation smell of cheese assumed lower values. This results are similar to those obtained by Coulon et al., (1998) in which the smell was lower in cheese from the second group of cows (145 days). It's difficult to compare the results of this study with what found by Auldist et al. (1996) because the divided the cows in only two group (related to the lactation).however they reported higher values of flavour in the early-milking cows. For the sour intensity trait it was observed lower values in middle of lactation (data not shown): the same was found by Coulon et al. (1998).

Least square means of texture properties for DIM were plotted in Figure 4b. as expected, firmness property showed a similar trend with cheese total solids and MSF. In fact, the correlation between firmness and MSF was high and positive (0.61; Table 5). The higher values of firmness were obtained for cows in the last part of lactation were the CY_{60D} was the worst. Elasticity and Moisture showed opposite trend than the firmness presenting lower values at the end of lactation. The opposite trend of these two traits was expected observing the correlations between firmness and elasticity and moisture, respectively (-0.81 for both). Coulon et al. (1998) reported opposite values for firmness than the present study, whit higher values at 145 days from calving.

Order of parity factor resulted significant for just for salt ($P < 0.01$) elasticity ($P < 0.01$) and moisture ($P < 0.01$). It was observed higher values of cheese salt with older cows while for the other traits any trend was found.

As it's important to consider the effect of the assessor on sensory assess of results, it is equally fundamental to consider the order in which cheeses are presented for the tasting to understand what is the effect of the saturation of sensory properties (and eventually remove it).

In the present study smell and flavour were especially affected ($P < 0.001$) by order of presentation. In both traits, after the first cheese presented, the evaluations of are more constant during all the tasting session. In figure 5 are given the least square means of smell and flavour for the order of presentation effect: it is possible to observe how the first cheese was evaluated for the smell and the flavor assigning higher values compared to the subsequent samples. For the other sensory traits, order of presentation was significant but it was not found a clear trend to evaluate this effect.

CONCLUSIONS

In conclusion, we have described a method for evaluating the quality of the cheese and sensory properties at the individual level. The results have allowed us to assess in particular the effects of the animal (as stage of lactation and parity of order) on quality traits and sensory properties of the cheese using 1.500 mL of milk per sample.

For cheese chemical components we can conclude that:

- Herd/test date and days in milk were the most important sources of variation.
- Total solids of cheese depends on fat-protein ratio which determinate the chemical composition of cheese because influence the inclusion protein and fat in the cheese (opposite trend during the lactation of a cow).
- Order of parity resulted significant just for total solids.
- Milk yield did not affected chemical composition of cheese.

For cheese physical traits we can conclude that:

- Herd/test date and days in milk were the most important sources of variation.

- When the quality of individual milk is evaluated, it has to consider that hardness of cheese is strictly related to the cheese yield when the processing and the ripening are the same.
- Order of parity did not affected physical traits of cheese.
- Milk yield was not significant for any variable considered.

For cheese sensory properties we can conclude that:

- Herd/test date and days in milk were the most important sources of variation.
- Texture properties are related to the CY and its composition.
- Order of presentation has to be included in the statistical model when sensory properties are analysed.
- Milk yield was not significant for any variable considered.

Furthermore, the results highlighted the need for more investigations on this topic, such as to analyse the genetic aspects of these traits and and to propose measures indirect measure of prediction.

TABLES AND FIGURES

Table 1. Descriptive statistics of physico-chemical traits for cheese at 2 months of ripening[1].

Trait[2]	N	Mean	SD	P5	P95
CY_{60D}, %	1,224	8.73	1.12	6.99	10.61
Protein, %	1,080	26.83	4.01	20.01	32.85
Fat, %	1,072	38.04	4.06	31.70	45.18
Salt, %	1,086	2.04	0.07	1.90	2.12
Total solids, %	1,077	80.06	4.63	71.84	86.91
pH	1,216	5.17	0.17	4.87	5.45
L^*	1,198	58.99	6.10	49.81	69.33
a^*	1,200	-2.07	0.53	-2.91	-1.14
b^*	1,200	7.63	2.83	3.47	12.64
SI	1,197	7.97	2.61	4.21	12.73
MSF, N	1,203	35.52	16.98	13.08	68.08

[1]P5 = 5th percentile; P95 = 95th percentile.
[2]CY_{60D} = cheese yield after 60 days of ripening; L* = lightness; a* = redness; b* = yellowness.

Table 2. Descriptive statistics of sensory traits[1].

Trait	N	Mean	SD	P5	P95
Smell	1,219	3.06	0.36	2.50	3.70
Flavor	1,221	3.33	0.36	2.75	3.92
Taste					
- Salt	1,211	3.23	0.44	2.58	4.00
- Sour	1,211	2.51	0.55	1.75	3.58
Texture					
- Elasticity	1,222	2.48	0.71	1.33	3.75
- Firmness	1,219	4.97	0.72	3.70	6.10
- Moisture	1,222	2.79	0.48	2.00	3.58

[1]P5 = 5th percentile; P95 = 95th percentile.

Table 3. Pearson product-moment correlations among sensory traits.

	Flavor	Salt	Sour	Elasticity	Firmness	Moisture
Smell	0.40^{***}	0.13^{***}	0.09^{**}	0.16^{***}	-0.17^{***}	0.10^{***}
Flavor		0.43^{***}	0.39^{***}	-0.02^{ns}	-0.09^{**}	0.09^{**}
Salt			0.40^{***}	-0.18^{***}	0.01^{ns}	-0.02^{ns}
Sour				-0.06^{*}	-0.12^{***}	0.13^{***}
Elasticity					-0.81^{***}	0.77^{***}
Firmness						-0.81^{***}

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ^{ns} not significant.

Table 4. Pearson product-moment correlations between sensory attributes and quality milk traits.

	Smell	Flavor	Salt	Sour	Elasticity	Firmness	Moisture
Casein, %	0.02^{ns}	0.02^{ns}	-0.03^{ns}	0.20^{***}	0.30^{***}	-0.32^{***}	0.34^{***}
Protein, %	0.05^{ns}	0.09^{**}	0.07^{*}	0.28^{***}	0.21^{***}	-0.26^{***}	0.28^{***}
Fat, %	0.05^{ns}	0.10^{***}	0.07^{*}	0.21^{***}	0.22^{***}	-0.35^{***}	0.35^{***}
Lactose, %	-0.08^{**}	-0.26^{***}	-0.40^{***}	-0.28^{***}	0.28^{***}	-0.13^{***}	0.14^{***}
Total solids, %	0.05^{ns}	0.08^{*}	0.03^{ns}	0.25^{***}	0.31^{***}	-0.42^{***}	0.43^{***}
SCS, units	0.07^{*}	0.10^{***}	0.16^{***}	0.06^{*}	-0.08^{*}	0.04^{ns}	-0.09^{**}
pH	-0.02^{ns}	-0.17^{***}	-0.16^{***}	-0.23^{***}	0.04^{ns}	0.01^{ns}	-0.07^{*}

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ^{ns} not significant.

Table 5. Pearson product-moment correlations between sensory attributes and physico-chemical parameters1 for cheese at 2 months of ripening.

	Smell	Flavor	Salt	Sour	Elasticity	Firmness	Moisture
Protein, %	-0.03^{ns}	0.05^{ns}	0.06^{ns}	0.21***	0.19***	-0.17***	0.15***
Fat, %	0.05^{ns}	0.02^{ns}	0.03^{ns}	0.001^{ns}	-0.15***	0.06^{ns}	-0.06^{ns}
Salt, %	0.02^{ns}	0.02^{ns}	0.05^{ns}	0.13***	0.21***	-0.19***	0.16***
Total solids, %	-0.09**	-0.02^{ns}	0.05^{ns}	-0.12***	-0.70***	0.69***	-0.64***
pH	0.09**	-0.09**	-0.10**	-0.45***	0.05^{ns}	0.12***	-0.13***
L*	0.15***	0.04^{ns}	0.07*	0.09**	0.16***	-0.20***	0.16***
a*	0.09**	0.11***	0.17***	0.17***	0.02^{ns}	-0.10**	0.13***
b*	0.11***	0.10***	0.11***	0.03^{ns}	0.12***	-0.14***	0.15***
SI	0.10***	0.10***	0.11***	0.02^{ns}	0.12***	-0.14***	0.14***
MSF, N	-0.07*	-0.10***	-0.01^{ns}	-0.18***	-0.54***	0.61***	-0.56***

*P < 0.05; **P < 0.01; ***P < 0.001; ^{ns} not significant.

Table 6. Results from ANOVA (F-value and significance) for physico-chemical traits of individual model cheese.

Trait[1]	EFFECT						
	R^2	RMSE[2]	HTD	DIM	Parity	Vat	MY
Protein, %	0.41	3.25	5.16^{***}	9.55^{***}	0.94^{ns}	1.08^{ns}	2.19^{*}
Fat, %	0.36	3.41	4.20^{***}	8.75^{***}	1.23^{ns}	0.82^{ns}	2.01^{ns}
Salt, %	0.29	0.06	2.71^{***}	8.58^{***}	0.66^{ns}	0.56^{ns}	1.19^{ns}
Total solids, %	0.52	3.38	10.46^{***}	16.22^{***}	5.66^{***}	2.23^{**}	0.69^{ns}
pH	0.58	0.12	15.62^{***}	3.00^{***}	1.47^{ns}	1.14^{ns}	1.16^{ns}
L^{*}	0.40	4.97	7.37^{***}	2.68^{**}	2.96^{*}	1.54^{ns}	1.71^{ns}
a^{*}	0.37	0.43	6.21^{***}	3.31^{***}	1.26^{ns}	0.94^{ns}	1.07^{ns}
b^{*}	0.41	2.24	8.13^{***}	1.82^{ns}	3.20^{*}	0.99^{ns}	0.18^{ns}
SI	0.40	2.10	7.81^{***}	1.73^{ns}	3.13^{*}	0.97^{ns}	0.22^{ns}
MSF, N	0.39	14.13	6.45^{***}	3.83^{***}	2.66^{*}	1.12^{ns}	0.89^{ns}

*P < 0.05; **P < 0.01; ***P < 0.001; ^{ns} not significant.
[1]L* = lightness; a* = redness; b* = yellowness.
[2]RMSE = Root means square error.

Table 7. Results from ANOVA (F-value and significance) for sensory attributes.

Trait	EFFECT						
	R^2	RMSE[1]	HTD	DIM	Parity	OP[2]	MY
Smell	0.43	0.43	5.93^{***}	2.78^{**}	0.56^{ns}	16.32^{***}	1.13^{ns}
Flavor	0.31	0.54	4.71^{***}	1.44^{ns}	0.18^{ns}	3.46^{***}	1.24^{ns}
Salt	0.37	0.45	6.27^{***}	2.26^{*}	3.37^{**}	1.76^{*}	0.89^{ns}
Sour	0.47	0.48	9.39^{***}	4.00^{***}	0.52^{ns}	2.22^{**}	1.67^{ns}
Elasticity	0.50	0.57	10.67^{***}	3.50^{***}	4.06^{**}	2.09^{*}	0.70^{ns}
Firmness	0.39	0.67	6.92^{***}	4.09^{***}	2.18^{ns}	2.39^{**}	0.39^{ns}
Moisture	0.29	0.63	3.78^{***}	3.63^{***}	3.89^{**}	1.86^{*}	0.78^{ns}

*P < 0.05; **P < 0.01; ***P < 0.001; ^{ns} not significant.
[1]RMSE = Root means square error.
[2]OP = order of cheese samples presentation to the panelists.

Figure1. Distribution of individual smell [a], flavor [b], salt [c], and sour [d] sensory attributes.

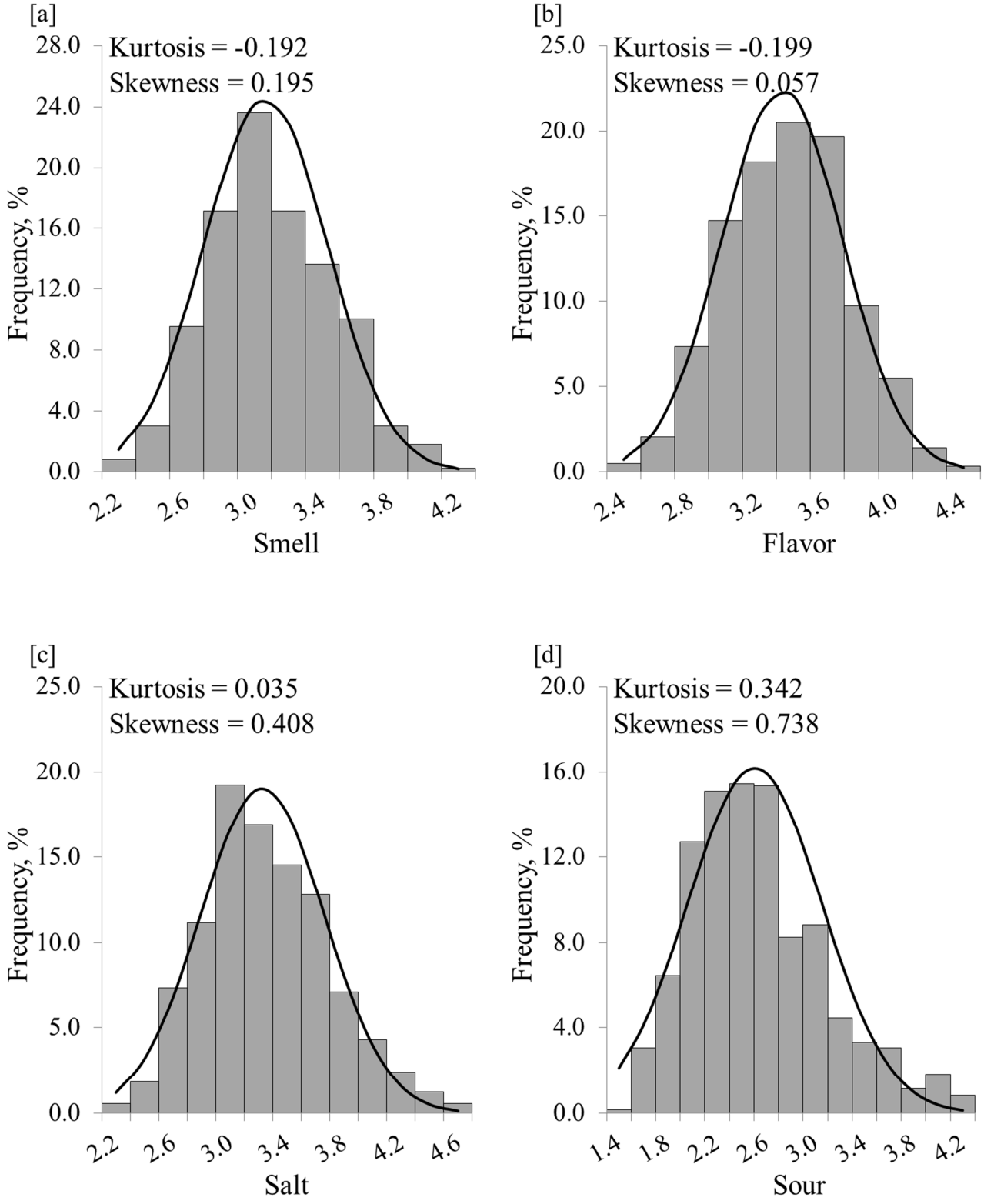


Figure2. Distribution of individual, elasticity [a], firmness [b] and moisture [c] sensory attributes.

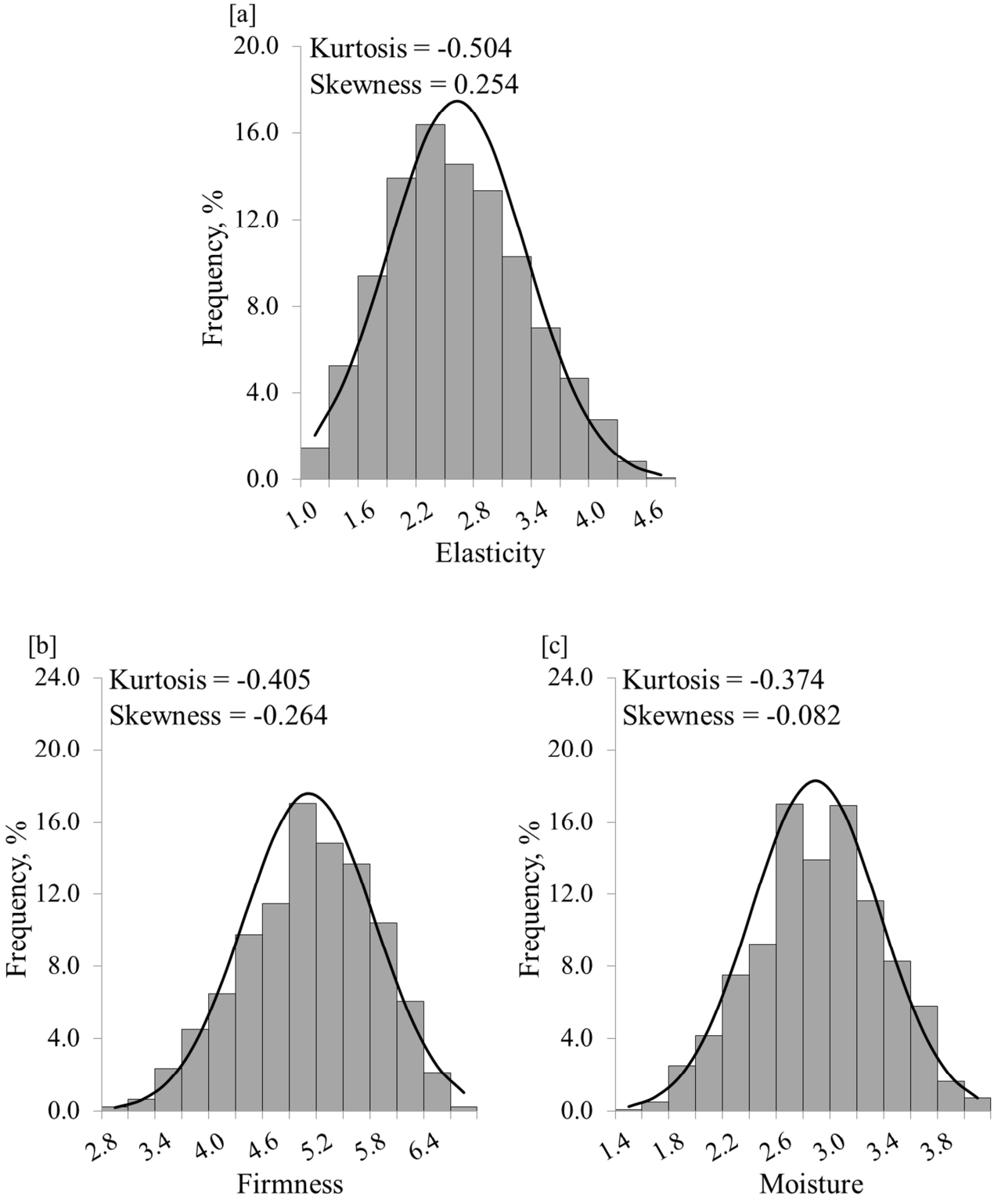


Figure 3. Least square means of cheese protein and fat [a], and cheese total solids and MSF(maximum shear force) [b] over days in milk.

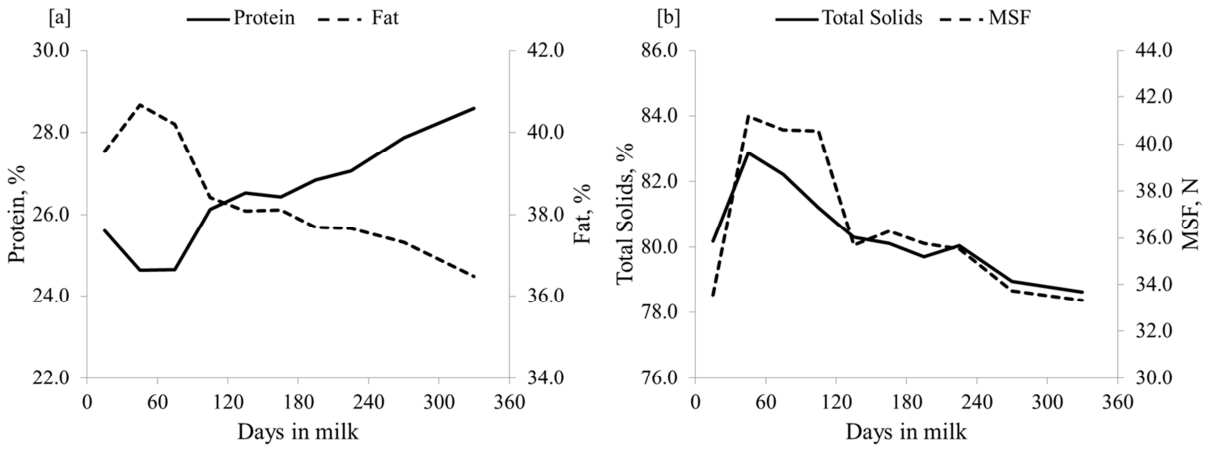


Figure 4. Least square means of smell [a], and firmness, elasticity and moisture [b] over days in milk.

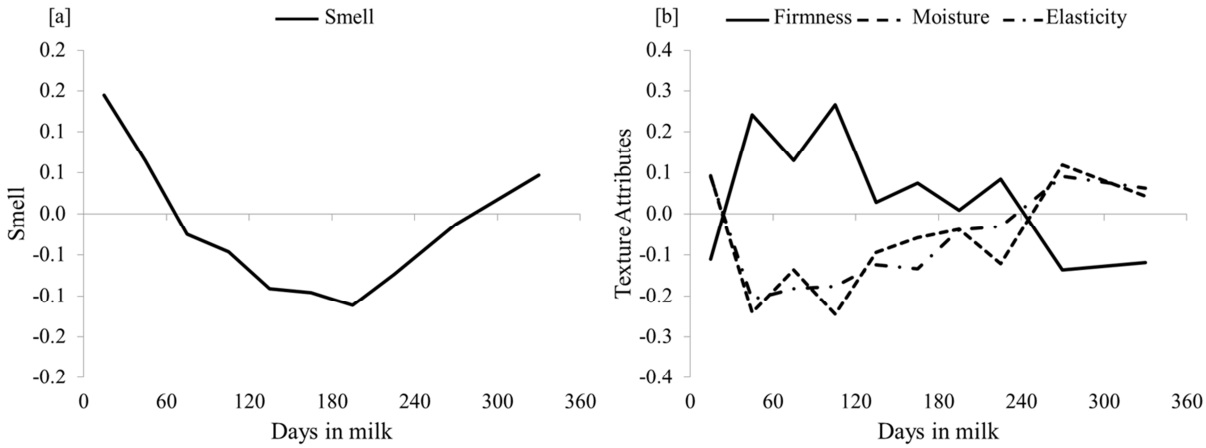
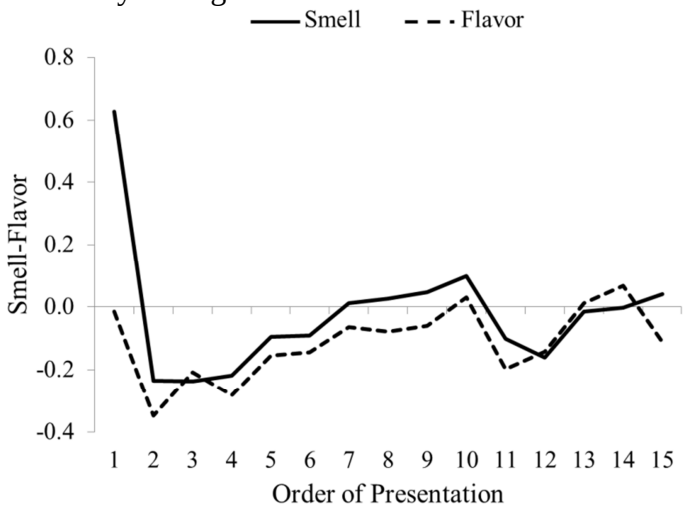


Figure 5. Least square means of smell and flavor attributes over order of presentation of cheeses during the sensory testing.



GENERAL CONCLUSIONS

The main objective of this thesis was to investigate the variability of some “new phenotypes” related to the technological properties of individual milk of Brown Swiss cows.

The comparison of MCPs has shown phenotypic and genetic differences between the measurements of the two instruments (mechanical and optical). The optical instrument could be used in future to assess new traits, especially on the early phases of clotting in which the progress of the process is not visible. The increase of the time-duration analysis (from 30 to 90 minutes) appeared to be a viable and simple solution for the presence of NC samples and allowed to 1) estimate genetic parameters for k_{20}, a trait usually not included in previous genetic studies but of considerable practical importance; 2) estimate a_{45} (phenotypically and genetically) although further investigations are needed to better understand the meaning of the trait. For the MCP as for the milk quality traits, lactation stage resulted the most important factor of variation. In Table 1 and 2 are reported the trends (positive or negative compare to the average of traits) of all quality parameters determined in this experimental project for days in milk and order of parity, respectively.

The proposed individual model cheese-making processing resulted repeatable and has allowed, for the first time, to assess the variability of the individual traits for cheese yield and nutrients recoveries in cow's milk. Lactation stage, as for MCPs, resulted highly influencing these traits (Table 1).

Results demonstrated the existence of an important phenotypic and genetic variability in cheese yield and nutrients recoveries; variations in cheese yield does not depend solely on the fat and protein contents of the milk, but also by the coagulum ability to retain matter (protein, fat and water) useful to compose the cheese. On the

basis of this assumption, it will be necessary to propose new prediction formulas considering the no-costant recovery of individual milk component in the curd.

Further studies are needed to investigate on the genetic results obtained for cheese yields and nutrients recoveries assessing the association of individual genes (candidate gene approach) to genetic variability of these traits.

A phenotypic analysis on cheese quality (chemical, physical and sensory traits) was conducted. Generally, these traits exhibited great variability and highly influenced by the lactation stage. On the basis of phenotypes traits collected it will be possible to carry out a genetic analysis.

Finally, for the large amount of work required in the laboratory to assess all these traits for 1,271 dairy cows it will be important to propose a routine analysis (indirect measures on milk) in order to carry out a study at population level.

Table 1. Phenotypic trend for all investigated traits, expressed as deviation (positive, negative or equal) from the mean trait within class of days in milk

	Days in milk				
Trait	5-60	60-120	120-180	180-240	240-300
Milk Yield, kg/d	0	+	0	-	--
Milk Components					
- Recovered in curd[1]	--	-	0	0	+
- Not recoverd in curd[2]	0	0	0	0	0
MCP[3]	++	+	0	0	+
Cheese Yields	++	-	+	+	++
Nutrients Recoveries					
- Protein, %	0	0	0	0	0
- Fat, %	++	+	0	0	0
- Solids and Energy, %	++	-	+	+	++
Cheese					
- Protein, %	0	-	+	++	+++
- Fat, %	++	+++	+	0	-
Cheese sensory properties					
- Smell	++	+	-	-	+
- Flavor	-	-	-	0	+
- Salt	0	-	0	+	++
- Sour	0	-	-	+	++
- Elasticity	+++	--	-	0	++
- Firmness	---	++	+	-	--
- Moisture	+++	--	-	0	++

[1] milk protein and fat
[2] milk lactose
[3]MCPs measured with Formagrpah (Foss Eletric, Hillerød, Denmark)

Table 2. Phenotypic trend for all investigated traits, expressed as deviation (positive, negative or equal) from the mean trait within class of order of parity.

	Order of Parity			
Trait	1	2	3	4
Milk Components				
- Casein	0	0	-	-
- Fat	-	+	-	--
- Lactose	+	.	-	-
MCP[1]				
- RCT, min	0	--	0	+
Cheese Yields	+	+	-	-
Nutrients Recoveries				
- Protein, %	++	+	0	-

[1] MCPs measured with Formagraph (Foss Eletric, Hillerød, Denmark)

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