Invited review: Hygienic quality, composition and technological performances of raw milk obtained by robotic milking of cows. *By Hogenboom et al.*

Robotic milking in a stable platform allows the cow to set her own milking schedule, with a milking frequency that typically is 2.5 to 3 times per day. Beside benefits on herd management and cow health, both robotic milking and the increased milking frequency influence some milk characteristics. Changes in fine composition and selected biochemical traits in turn influence milk behavior during processing. Awareness of such changes in milk quality, composition and technological performances is relevant to the dairy industry because of the profound impact these can have on quality, durability and yield of the end products.

Invited review: Hygienic quality, composition and technological performances of raw milk obtained by robotic milking of cows

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ABSTRACT

Automatic milking systems (AMS), first introduced on dairy farms in the 1990s, rapidly spread across many countries. This technology is based on the voluntary milking of dairy cattle in a completely automated process, which relies on computer management, with a substantial average increase in milking frequency. Compared with conventional milking, AMS significantly alters herd management, with important implications on economic, technical and social aspects of farming, on animal physiology, health and wellbeing. These aspects are explored in an extensive body of research. In contrast, the impacts of AMS adoption on milk quality are often overlooked. This review draws together both positive and negative effects of AMS on the milk production chain, particularly emphasizing the variations of hygienic and compositive characteristics of raw milk and their interplay, as compared with milk obtained with conventional milking. Scattered and sometimes conflicting literature exists on whether and how these variations may influence quality and yield of the derived dairy products. Current scientific knowledge on these crucial aspects is thus reviewed, with particular focus on milk technological suitability for being processed into dairy products having the target characteristics in terms of taste, structure, on-storage stability and sustainability. Provided the managing conditions are optimized, AMS allows increased milk production, mostly due to more frequent milking, without compromising the milk characteristics which are crucial to food industry for processing. Nevertheless, specific biochemical aspects related to the changed milking interval, which determines the duration of enzyme activities and bacterial growth in milk, need further research.

Keywords: automatic milking system, milking frequency, milk composition, free fatty acid, cheese

INTRODUCTION

The introduction, during the early 1990s, of automatic milking systems (**AMS**), also called robotic milking systems, undoubtedly represented a major breakthrough in dairy farming techniques. The success of AMS was mainly due to the improved quality of labor and farmer's lifestyle it brought at the dairy farms with respect to conventional milking systems (**CMS**) in a parlor. In the last two decades the number of farms in the world that milk their cows automatically increased dramatically: at the beginning of 2000s it was approximately 1,250 (de Koning and van der Vorst, 2002); at the end of 2010, worldwide over 10,000 commercial farms used one or more AMS to milk their cows (de Koning, 2011). It can be roughly estimated that at present approximately 38,000 units of AMS are installed globally (Hallén-Sandgren and Emanuelson, 2017). The AMS technology is more widely used in Europe than in other countries; according to Barkema et al. (2015), about 25% of dairy farms is using AMS in Denmark, followed by Sweden, Iceland and The Netherlands. In North America the number of AMS is also increasing; in particular, approximately 7% of Canadian dairy farms have already adopted an AMS unit (Tse et al., 2017).

Since the first prototypes were developed, the industry has invested in improving efficiency of equipment for AMS. Fully integrated management solutions are currently available where most of critical points have been overcome by introducing electronic sensors, lasers and data recorders. Basically, cows are individually milked in a box where they enter voluntarily as attracted by feed supplements (Jacobs and Siegford, 2012). Robotic arms perform all the preliminary operations of brushing and sanitizing udder and teats, and stimulating them. Based on a cow's identification tag, the robot adapts milking conditions to her morphological characteristics (height, udder size, teat shape and angle), interval from previous milking, health conditions. Quarter-based in-line milk meters as well as sensors for milk composition monitoring and early mastitis detection have been implemented: they are needed to establish the correct time for automatic cup detachment, adapt feed supplement distribution to the animal production level, identify and automatically separate abnormal milk and produce health alerts. The way the AMS, the cow and the farmer interact is illustrated in Figure 1.

Figure 1

Adoption of AMS has raised a number of questions in various scientific fields, addressed by a substantial number of research papers. Most of these have covered farming and breeding aspects. The present review will principally focus on the most recent insights on the effects of AMS on hygienic quality, composition and technological performances of the obtained cow milk. Despite the relevance to today's dairy industry, which requires the production of milk of excellent quality, these aspects are less addressed by the scientific literature and, most notably, controversial data are sometimes reported. To compile this review, both Scopus (www.scopus.com) and ISI Web of Science (www.webofknowledge.com) databases were used over the range of years from 2000 to 2018. Overall, the number of papers published on the topic has sharply increased during the last few years.

Approximately, 80 documents were recorded in both databases on 2018, including research articles, reviews, book chapters and proceedings papers. The records were mostly from Germany, USA, Netherlands, Denmark, Italy, Canada, and Sweden.

BASIC CONCEPTS AND CONSIDERATIONS

Automatic milking differs from conventional milking in a number of ways, each potentially capable of modifying milk composition and technological properties.

Milking frequency and intervals are determined by the cows individually and may significantly vary from cow to cow, influencing not only milk yield and composition, but also its SCC and bacteriological characteristics (for reviews see Hovinen and Pyörälä, 2011; Jacobs and Siegford, 2012; John et al., 2016).

Furthermore, the absence of human operators makes visual control of the udder and milk appearance for the detection of clinical mastitis impossible, unless suitable dedicated devices are incorporated in the AMS equipment. In addition, the lack of visual check by the milker can impair the effectiveness of teat cleaning before milking. On the other hand, milking is quarter-based: this is more respectful of the physiological characteristics of the udder and helps to prevent overmilking of less productive quarters (Bava et al., 2005).

Another advantage is that AMS equipment allows recording of huge amounts of data on individual cows as well as on herd performances. Data recorded from AMS can be statistically handled to gather information on cow conditions and adopt prompt interventions.

Among the main aspects evaluated in the most recent literature on AMS, besides the effects on milk production and quality, there are economic, social, technical and management issues as it is summarized in Table 1. In addition, many studies have dealt with the effect of the adoption of automated milking on health, reproduction, behavior and welfare of cows. The most recent research frontiers are the genetic selection aspects, including udder morphology and adaptability, linked to AMS diffusion (Carlström et al., 2016), the responses of AMS herds to hot environment and climate change (Mattachini et al., 2017), the environmental sustainability of milk production with AMS (Oudshoorn et al., 2012), and the ethical implications of AMS adoption (Holloway et al., 2014).

EFFECTS OF AMS ON MILK YIELD

Several researches have shown that AMS increases milking frequency, favoring an increase in milk yield in the order of 5 to 10% compared with the fixed-frequency regime of daily milkings of CMS, nowadays typically set at 3 in large farms and 2 in small and medium size farms (Table 2). The effect of increased milking frequencies appears to be more important for multiparous cows than for heifers (Petterson et al., 2011; Bogucki et al., 2017). The average number of daily milkings per cow in AMS is generally included in the range of 2.5 to 3.0 but rather big differences in milking intervals are reported by commercial farms (de Koning, 2011). The positive effects of increased milking frequency on milk yield had already been proven on farms equipped with CMS, well before the introduction of AMS (Friggens and Rasmussen, 2001), generally comparing twice daily milking against thrice daily milking, at regular intervals. Negative feedback mechanisms from milk have been proposed to regulate the activity of secretory cells, *via* either an unidentified protein (FIL -Feedback Inhibitors of Lactation) or serotonin, as quoted by Ferneborg et al. (2017). Hale et al. (2003) demonstrated that increased milking frequencies during early lactation promoted persisting higher milk yields during the whole lactation. Mechanisms of epigenetic control of gene expression were proposed to explain the effects of milking frequency at the beginning of lactation on the proliferation of secretory cells and long-term alteration of mammary functions (Wall and McFadden, 2012). However, with the introduction of AMS, the effects of higher milking frequency on milk yield are more complicated, as milking intervals become variable from cow to cow and even for the same cow daily. Some commercial farms show difficulties to reach satisfactory milking frequencies with AMS: in some cases, a forced-traffic system is adopted to compel cows visiting the milking box before the access to the feed bunk or before returning to the resting area. Both types of forced-traffic solutions can have negative influence on some welfare parameters as they can reduce resting time, feeding time or after-feeding behavior (Melin et al., 2007; Bach et al., 2009). Alternatively, an operator has to fetch cows several times a day to invite them to be milked (Bach et al., 2009). The number of the daily visits to the milking box is also strongly influenced by the composition and palatability of the concentrate fed in the AMS, being the basal mixed part of the diet usually offered *ad libitum* at the feed bunk (Madsen et al., 2010). Moreover, besides milking frequency, other aspects were observed to influence milk production in AMS-equipped farms, including feeding management (Tremblay et al., 2016) or the success rate of teat cup attachment (Gygax et al., 2007). In addition, the effects of AMS on udder health can substantially modify the cows' responses in terms of milk yield due to the negative effect of mastitis and high SCC, as reported by Seegers et al. (2003).

EFFECTS OF AMS ON SOMATIC CELL COUNT OF MILK

A substantial amount of studies dealing with milk quality traits related to AMS have looked at the SCC, an indicator of the possible presence of inflammatory processes in the cow's udder, principally mastitis. Bulk milk having high SCC (usually >300,000 cells mL^{-1}) may be unsuitable for cheese manufacturing and has detrimental effects on quality and sensory properties of finished products (Barbano et al., 2006). In fact, milk with elevated SCC, in comparison with normal SCC milk, has a lower content of casein, due to a reduced synthesis, a lower content of lactose, that partially leaks into blood stream through the damaged blood-milk barrier (Bruckmaier et al., 2004), and an increased content of whey proteins and selected ions due to leakage from blood (Barbano et al., 2006). Although most studies report a depression of milk fat content, it has not been demonstrated so far whether every mastitis case gives a decreased milk fat content (Seegers et al., 2003). The elevated fat content could be the consequence of reduced milk volume. Proteases associated to the somatic cells, including the activators of the plasminogen-plasmin system, are responsible for casein hydrolysis that compromises milk technological performances (Murphy et al., 2016). Furthermore, due to their heat resistance, these proteases are involved in gelation and sedimentation phenomena occurring during storage in drinking milk (Rauh et al., 2014; D'Incecco et al., 2018c) and fermented milk (Fernandes et al., 2007). As previously stated, in the case of AMS there is no visual control by the milker of udder conditions and the characteristics of the milk. This control is carried out automatically through the use of sensors and analyzers that monitor in real time the characteristics of the milk (conductivity, color) and allow the system to make decisions about the need to separate the milk and to produce health alerts as decision support for treatments.

Most studies reported an increase in milk SCC and a worsening of the udder health status after the introduction of AMS; however, after an adaptation period of variable duration, some authors observed a decrease in SCC content. Rasmussen et al. (2002) noticed an initial significant increase of the mean SCC in individual milk from 98 Danish farms but, three months after the change, the number of cows with high SCC slowly dropped. Kruip et al. (2002), analyzing the effect of AMS on fertility and SCC among dairy herds participating in the national Dutch milk recording system, observed significantly (P <0.01) higher SCC after the introduction of robotic milking. de Koning et al. (2003) found a significant (P <0.01) increase of the bulk milk SCC after the introduction of AMS in farms in The Netherlands (262 farms) and Denmark (99 farms), not in Germany (33 farms). In a study on 88 Finnish herds (Hovinen et al., 2009), the proportion of cows at risk for high SCC was larger in AMS than in CMS herds (3.3 vs 2.1%).

The increased frequency of milking with AMS is one of the claimed reasons for the SCC increase. Higher milking frequency can have contrasting effects on udder health as quoted by Hovinen and Pyörälä (2011). On one hand, increased milking frequency allows the drain of bacteria from the udder, reducing the time for bacteria to colonize the quarters. On the other hand, frequent milking provides greater opportunities for bacterial invasion during milking, as teat sphincters remain open after every milking, exposing quarters to environmental bacteria. The irregularity of milking intervals is proposed as an additional factor for explaining high SCC in milk from AMS. Mollenhorst et al. (2011) analyzing the data from 151 AMS farms, concluded that irregular milking intervals can promote high SCC. Milking failures could worsen the situation, especially if the milking interval of the infected quarter becomes longer. Rasmussen (2006) reported an increase in the milking interval of cows with clinical mastitis of about 2 h and the frequency of milking failures increased from 5% to 30% on the day of treatment. Stefanowska et al. (2000), in a study on 12 cows observed that unsuccessful milkings cause milk leakage, a potential risk for poor udder health. The farmers' increased workload during and immediately after AMS installation, resulting in poorer herd management, was indicated as an additional cause for high SCC. One other explanation of detrimental effects of automatic milking on udder condition is attributed to bacterial cross contamination among cows through the milking equipment. In CMS, infected cows are milked as the last, to reduce the risk of cross contamination, whereas in AMS the milking order is generally casual. To overcome this problem, AMS are equipped with back flushing or steaming milking lines between cows but the abatement of pathogens is not always complete (Hovinen and Pyörälä, 2011). In addition, good teat hygiene before milking is not always achieved. The AMS cleans the teats with automatic devices, without the visual control of the milker as in CMS, and no adjustments on the cleaning procedure can be made for individual cows, as pointed out by Dohmen et al. (2010) in a study on 151 Dutch dairy farms with AMS. This can result in an easier entry of the mastogenic bacteria through the teat sphincter during milking.

Other authors observed different situations. Tousova et al. (2014), whose investigation involved 200 Czech Fleckvieh cows milked in CMS and 300 milked in AMS, recorded milk SCC not significantly different between the two systems. Janštová et al. (2011), comparing one farm milking with AMS to 2 using CMS, found milk from AMS to have the lowest SCC, but the difference was significant (P <0.01) only in one case. Similarly, Petrovska and Jonkus (2014) in a comparative study on 40 cows milked in AMS and 71 milked in CMS, registered lower (P <0.05) SCC values in the AMS group at 20 and 50 DIM for primiparous cows, but not for multiparous cows. Berglund et al. (2002), studying two groups of 33 cows, noticed significantly lower SCC values in the quarter strip milk (obtained separately from single quarters) of the cows milked in the AMS, as compared to those milked in the CMS, whereas the composite milks of the two groups were not significantly different. With the possibility of sampling milk from each cow and each quarter individually, AMS supplies an easy way of detecting SCC increases in an early stage. In addition, AMS provides some advantages in terms of preventing the spread of mammary infections: in particular quarter based milking allows a more respectful milking avoiding in particular overmilking of lower producing quarters and reducing the risk of cross contamination among quarters of the same cow through the milking claw (Hogeveen et al., 2001).

Overall, albeit with some exceptions, there has been an evolution through the years towards lower SCC values in milk obtained in AMS, with SCC values matching those of milk obtained in CMS after an adequate adaptation period. This trend is likely due to technical improvement of the AMS equipment and to enhanced operational settings.

EFFECTS OF AMS ON BACTERIOLOGICAL QUALITY OF MILK

Contradictory data are reported also for the effect of AMS on microbiological quality of milk. Most of the studies refer to the total bacterial count (**TBC**), whereas in some cases the variations for single species were considered. On the 28 farms monitored by Klungel et al. (2000), the mean TBC increased from 8,000 cfu mL^{-1} to 19,000 cfu mL^{-1} after introduction of AMS. Concurrently, the incidence of bulk milk samples with TBC >50,000 cfu mL^{-1} increased from 4% to 15% and those with TBC >100,000 cfu mL^{-1} from 1.6% to 6.8%. de Koning et al. (2003), from their investigation on 394 farms located in Denmark, Germany and the Netherlands, reported an increase (P <0.05) of TBC after introduction of AMS, very rapid during the first 45 d, while the increase from 3,800 to

12,400 cfu mL^{-1}, observed by Salovuo et al. (2005) on 3 farms in Finland, was not statistically significant. During their one-year study on 98 Danish farms, Rasmussen et al. (2002) recorded an increase (P <0.01) of spores of anaerobes, attributable to insufficient cleaning of cow's teat surface. Overall, the increments of TBC are principally attributed to problems in the cleaning process of the teats. Teat skin has been reported to be the most important source of milk microbiota, with environmental sources (e.g., herd feces, bedding material and milking equipment) being of secondary relevance (Derakhshani et al., 2018). As previously mentioned, a satisfactory teat sanitation before milking is not always achieved in AMS. In current AMS no methods are implemented to evaluate teat dirtiness and adapt cleaning consequently. Moreover, teat cleaning failures are quite frequent. This interpretation is supported by the data of other authors (Janštová et al., 2011; Tousova et al., 2014) who found an improvement of microbiological quality in milk obtained with AMS ascribed to the implementation of correct milking hygiene practices, including regular teat brushing and milking cup cleaning, as well as frequent sanitation of the milk piping and bulk tanks. Milk from AMS also showed lower counts for psychrotrophic species, *Escherichia coli*, enterococci and *Staphylococcus aureus*. In some cases (Tousova et al., 2014), a very high standard deviation in average TBC of AMS milk was highlighted which reflects the presence of a certain quantity of poor quality milk. At this regard, it has been underlined that, if an AMS is not continuously in use, some residual milk might remain in the system pipelines for some time and its TBC increases. The continuous presence of low milk flow in the pipelines and the uninterrupted supply of warm milk in the milk tank can favor bacteria proliferation.

Overall, although AMS seems to generally worsen microbiological quality of milk, TBC does not depend on milking system only, but is also affected by other parameters, such as barn hygiene, equipment sanitation, frequency of controls on milk and animals, and particularly milking interval, which determines the duration of bacterial multiplication in the teat cistern. Optimization of all of these parameters will certainly increase milk hygiene. On the other hand, a contamination of lactic acid bacteria, to a certain extent, is considered beneficial in milk for selected destinations, such as the manufacturing of raw milk cheeses.

EFFECTS OF AMS ON MILK FREEZING POINT

The freezing point (**FP**) of milk is rather constant since it derives from the osmotic equilibrium between blood and milk. Much research has focused on the increase of milk FP as an indicator of increased water content due to adoption of AMS. This aspect is of concern since a dilution of milk, even of small entity, implies a lower concentration of nutrients and worse technological performances. de Koning et al. (2004) mentioned an increase ($P < 0.05$) by 0.005°C of the FP in AMS milk, with the level remaining substantially higher afterwards, but did not hint at a possible explanation. Klungel et al. (2000) reported an increase of the same entity (from -0.520°C to -0.517°C) of average FP after introduction of AMS, which they attributed to the frequent cleaning and rinsing of the system adding some residual water to the milk. Rasmussen et al. (2002) highlighted the same reason and suggested the adoption of procedures to blow residual water out of the system. Also Janštová et al. (2011) reported a slight increase of the FP in milk of a Czech farm after adoption of AMS but the reasons were not investigated. Salovuo et al. (2005) monitored milk quality traits on the first 3 farms that introduced AMS in Finland. The authors noted that, after an increase ($P < 0.01$) from -0.531°C to -0.518 °C, the FP value slowly decreased until the initial figure was reached after one year. Innocente and Biasutti (2013) obtained fully comparable FP values in milk samples taken repeatedly at AMS equipment from different manufacturers. Contrary to the majority of researchers, Tousova et al. (2014) reported that FP was significantly ($P < 0.01$) lower in milk from 300 cows milked by AMS than in milk from 200 cows milked by CMP. In general, literature data suggest that the increase of milk FP, sometimes observed after introduction of AMS, is attributed to the frequent cleaning and rinsing of the system and, therefore, could be avoided.

EFFECTS OF AMS ON MILK COMPOSITION

Current literature does not report a clear consensus on the effects of milking system on milk composition. It has already been discussed that the increased milking frequency in the AMS is positively correlated with the amount of milk produced daily. In addition, AMS seems to have some detrimental effects on udder health, although the results reported in literature are not always consistent. All these factors may in turn influence the composition of the milk to a different extent and in different ways, thus making the interpretation of results difficult. Minor changes in milk composition can have relevant economic implications in the long term, or when large milk volumes are dealt with, especially for milk destined to cheese production. In spite of this, studies specifically focused on the effects of AMS on the chemical composition of milk are rather few, mostly considering fat and protein content. Abeni et al. (2005b), Janštová et al. (2011), Innocente and Biasutti (2013), and De Marchi et al. (2017), who compared milk samples obtained with AMS and CMS in different-sized herds, at different stages of lactation and at different periods of the year, all found that the milking system does not affect fat, protein, casein, lactose and non-fat solids content significantly. Salovuo et al. (2005) reported an average increase of fat content from 3.85 to 4.20% after introduction of AMS. Although not statistically significant, such an increase was attributed to shorter milking intervals. In contrast to previously referred data, Klungel et al. (2000) and Tousova et al. (2014) found higher content ($P < 0.01$) of fat and protein in AMS milk than in milk from CMS, whereas the relative casein contents remained stable. In a large study, involving 51 farms using AMS and 53 farms using CMS, Johansson et al. (2017) found no difference in fat content between the two groups, whereas the protein content was lower ($P = 0.005$) in AMS milk. In particular, the casein-to-protein ratio was 82.1% in AMS milk and 84.3% in CMS milk ($P = 0.001$), indicating that the former milk would give a lower yield when processed into cheese. The principal component analysis (score plot) of the composition data and selected quality traits (SCC, pH, proteolytic activities) showed that bulk milk samples from AMS herds were more clustered, thus more homogeneous, compared with samples from CMS herds. This study, however, only considered bulk milk samples taken during the indoor period (October). Notably, De Marchi et al. (2017) noticed that, when milk samples were screened based on DIM, the protein content of milk samples from AMS was higher (P <0.05) at the beginning and lower at the end of the lactation compared with CMS milk samples. Ferneborg et al. (2017) compared the composition of milk samples obtained with either 2 or 4 milkings per day and evaluated the possible combined effect of residual milk removal. In no case, significant differences were observed in fat, protein and lactose content with the exception of a higher fat content when the higher milking frequency (4 per day) was combined with residual milk removal. Indeed, the proportion of residual milk increased when 4 daily milkings were used. Interestingly, these authors reported that the fatty acid composition did not change among treatments. It is worth noting that this study was conducted with 4 cows only, although comparable in terms of productivity and DIM. This is a common situation in studies where physiological aspects need to be in-depth evaluated and extra sources of variation must be minimized. Furthermore, accurate collection of samples during milking becomes an essential condition that can only be achieved dealing with very few cows. In an experiment conducted on 18 dairy cows in early-, mid- and late-lactation, milked after 4, 8 and 12 h from previous milking, milk fat content was shown to be function of the degree of udder filling at actual milking: values were higher after short- than after long-milking intervals (Bruckmaier et al., 2001). The same authors reported that, besides milking frequency, other factors may influence milk ejection and residual milk in AMS. In particular, an experiment on 10 Brown cows showed that prolonged time for cup attachment, common in AMS, can result in decreased oxytocin concentration and increased amounts of residual milk. Dutreuil et al. (2016) worked on 6 cows and observed that milk accumulation in the udder increased from 5.4 kg after 4 h up to 23.1 kg after 20 h and then stabilized with a slight increase up to 24.7 kg recorded after 36 h. Except for the extremely short (4 h) or long (36 h) accumulation times considered in the study, prolonging milking interval did not result in a significant increase of fat, lactose, crude protein, casein and non protein nitrogen content of milk. In contrast, the content of soluble protein slowly increased from 5.55 to 6.88 g/kg (P <0.05), likely because of an altered permeability of the mammary epithelia. The increased opening of tight junctions allows a more efficient transfer of protein from blood to milk. The same physiological reason was indicated for the decrease (*P* <0.05) of soluble calcium observed between 11 and 24 h of milk accumulation. Slow moving of soluble calcium to blood might also explain the reported variability of the content of colloidal calcium since the 2 forms are in equilibrium. In contrast to these observations, Abeni et al. (2008) did not detect differences in Na, K and Cl contents of milk obtained from primiparous cows milked with AMS or CMS, although at the fourth week of milking they noted a trend for higher Na and Cl contents in CMS milk. These data would support the preservation of mammary integrity and epithelial permeability by AMS (Herve et al., 2017).

Resuming, the majority of authors agree that the milking system, *per se*, does not significantly affect gross composition of milk. Extremely short or extremely long milking intervals may nevertheless cause variations in milk composition, but independently from the used milking system.

EFFECTS OF AMS ON TECHNOLOGICAL PROPERTIES OF MILK AND QUALITY OF MILK PRODUCTS

Very few reports have addressed the changes in technological characteristics of milk associated with adoption of AMS, despite the relevance of this aspect to dairy industry worldwide. More attention has been paid, instead, to the effects of an increased milking frequency, which is by far the major result when AMS is adopted. Milk intended for cheesemaking must have particular characteristics that guarantee obtaining good quality cheeses with high yield. Protein content and composition, mineral composition, acidity, among others, are crucial for optimal coagulation of milk (Guinee, 2016). Plasmin, the main indigenous protease in milk, has a strong activity on caseins, mostly on β-casein and α_{s2}-casein. This proteolytic activity progressively impairs the integrity of the micelles and therefore their suitability to aggregate and give rise to a compact rennet gel (Srinivasan and Lucey, 2002). Extensive activity of plasmin in milk is particularly detrimental for manufacturing hard and extra-hard cheeses. Plasmin originates from its inactive zymogen plasminogen through a complex activation mechanism (Kelly et al., 2006). In a study including primiparous cows only, Abeni et al.

(2008) found levels of plasmin to be lower (*P* =0.002) in AMS milk than in CMS milk. That difference was attributed to shorter milking interval in the former and, consequently, to a shorter time for plasminogen to be transferred from blood to milk and in parallel for it to be converted into plasmin. Data of Johansson et al. (2017) confirmed a lower (*P* =0.001) total activity of plasminogen and plasmin in milk from AMS herds compared with milk from CMS herds. This hypothesis is in agreement with the lower content of proteose-peptones we observed in milk samples from AMS (540±54 mg/L) than in milk samples from CMS (607±62 mg/L) in a study conducted in the production area of the Protected Designation of Origin (**PDO**) Grana Padano cheese (L. Pellegrino, unpublished data). Innocente and Biasutti (2013) observed a comparable difference between the contents of proteose-peptones of AMS and CMS milk samples collected in the production area of Montasio, another popular Italian PDO cheese. Although this difference was reported not to be statistically significant, it supports a reduced degradation of β-casein by plasmin in AMS milk, being proteose-peptones primary specific breakdown products of such a proteolytic activity (Kelly et al., 2006). Despite this, the authors reported that no differences in milk coagulation properties were observed between the milk obtained by the two systems. Other authors observed an increase of the soluble nitrogen fraction in milk as milking intervals increased (Sapru et al., 1997; Dutreuil et al., 2016). This fraction is not retained in the curd and therefore represents a loss in cheese yield. In this perspective, AMS milk would better perform in cheesemaking than CMS milk. In their study conducted on milk samples from 51 dairy farms using AMS and 53 using CMS, Johansson et al. (2017) observed a significantly higher (*P* =0.001) proteolytic activity in the former in combination with an elevated SCC level and a relatively low plasmin activity. Based on these findings, the authors hypothesized a role played in raw milk by proteases other than plasmin and that could negatively affect stability and sensory properties of the derived dairy products. In a more recent study, individual milk samples were collected over 3 yr from both 4 herds milked by AMS and 4 herds milked using a CMS and analyzed for relevant coagulation traits (De Marchi et al., 2017). The former type of milk showed a longer rennet coagulation time (23.9 vs 22.7 min; *P* <0.05) than the latter, whereas the obtained coagula were not different in strength. The authors hypothesized the slightly lower pH of CMS milk to be more favorable to the chymosin activity. However, these data need to be confirmed by comparing the performance of milk from the two milking systems throughout parallel cheesemaking trials.

Technological traits of milk as well as structure, texture and sensory properties of the derived milk products are strongly influenced by fat. In particular, size and integrity of milk fat globules are likely the most relevant characteristics in modifying the behavior of fat during manufacturing of dairy products (Ong et al., 2010). According to Abeni et al. (2005b), fat globule size ($d_{3,2}$), globular surface area and inter-globular distance were not dependent on milking system itself although there was a positive interaction ($P < 0.05$) between milking system and stage of lactation or sampling period for globule size only. Data of Wiking et al. (2006) indicated that medium-size and large globules increased ($P < 0.05$) when daily milking frequency was increased from 2 to 4 four times in half udder of 11 cows. Recently, Dutreuil et al. (2016) evaluated several compositional and technological traits in milk collected at milking intervals of increasing duration, i.e. by decreasing milking frequency. Milk fat globule size ($d_{3,2}$) decreased from 5.56 to 4.49 μm when milking interval was increased from 4 to 20 h, then it increased again to 5.48 μm for milking interval up to 36 h. The milk fat content had the opposite behavior across the same study, with the highest levels in milk collected at milking intervals of 4 h (62.8 g/kg) and 36 h (57.7 g/kg). In line with previous findings, milk synthesis begins after 4 to 5 h, proceeds at a rather constant rate for 16 to 18 h, and then largely decreases (Dutreil et al., 2016). Accordingly, milks that were milked at 4 h (corresponding to residual milk) and at 36 h respectively both accumulated in the udder for a long time before milking. The presence of larger fat globules in these two milks was likely due to coalescence of small globules taking place over time (Evers, 2004), being this phenomenon even facilitated at the high body temperature of the cow (D’Incecco et al., 2018a). By increasing the milking frequency, as it occurs when AMS is adopted, the incidence of residual milk increases and, therefore, the presence of larger fat globules is expected to increase as well (Wiking et al., 2006). As previously mentioned, factors other than milking frequency, such as time for teat cup attachment, may influence milk ejection and residual milk in AMS (Bruckmaier et al., 2001). Larger fat globules affect the strength of rennet gel positively, especially when the presence of large (average size ranging from 4.5 to 5.4 μm) fat globules was combined with that of casein micelles of relatively small size (164 to 168 nm; Logan et al., 2015). This effect was explained as the result of the role of spatial fillers played by fat globules that better fit the pore size of the casein network. Notably, largest fat globules could have a disrupting effect on the structure of the rennet gel, which impairs curd firmness. Consistently with the observed higher strength of the curd, Cheddar cheese made from milk containing a large proportion of large fat globules was higher in fat and lower in moisture and salt compared to the control cheese, while the protein content was not different (Logan et al., 2015). Overall, yield on a wet basis of the former cheese was lower because of a reduced whey retention. Other authors reported similar trends for Camembert (Michalski et al., 2003), Emmental (Michalski et al., 2004) and Cantal (Martin et al., 2009) cheeses. In their study on the suitability of AMS milk for Montasio cheese manufacturing, Innocente and Biasutti (2013) did not consider the size of fat globules among the studied parameters. However, they showed the moisture content of cheese made with AMS milk to remain slightly lower than that made with CMS milk throughout the 12-mo ripening period suggesting that a curd with higher strength was likely obtained with the former milk. In spite of the concordant results obtained in the above-mentioned studies, the observed differences were often of the same order of the natural variability and therefore not compromising cheese quality.

Abeni et al. (2005b) found that milk obtained with AMS had a lower attitude to natural creaming, i.e. fat separation by gravity, than milk from CMS, although there was an interaction with the milking interval (*P* =0.001). The authors were not able to explain this difference because they did not find significant differences in fat globule size between milk obtained with the two systems. Natural creaming of fat represents a key step in the cheese making process of some traditional long-ripened hard cheeses such as PDO Grana Padano and Parmigiano-Reggiano. In fact, besides lowering the fat-to-casein ratio to the optimum level for the development of the distinctive grainy structure in the cheese, natural creaming of fat allows an effective removal of both spores and cells of clostridia (Caplan et al., 2013; D'Incecco et al., 2015, 2018b). In this respect, natural creaming represents a traditional way of preventing the late blowing defect in these PDO cheeses and, for this purpose, maintaining the native structure of fat globules intact becomes an essential condition. Rising of fat in milk is faster than what expected according to Stokes' law. This is due to the progressive clustering of fat globules mediated by immunoglobulins A or M (Geer and Barbano, 2014; D'Incecco et al., 2018a). Dutreuil et al. (2016) recorded a fat globule size increase when a 36-h accumulation time of milk in the udder was considered. This was assumed to be the result of i) the increase in intramammary pressure causing coalescence of fat globules or ii) fusion of lipid droplets in the cytoplasm before milk secretion. At the same time, it is well known that the sheer stress caused by milk pumping, stirring, or transportation, may easily disrupt the membrane surrounding the fat globules and, consequently, both casein micelles and whey proteins adsorb onto the fat globule surface. These phenomena affect fat globule properties such as size, density, surface charge, and hydrophobicity in different ways, and thus their behavior during gravity separation may vary greatly. The temperature the milk is kept at before natural creaming also affects the behavior of fat globules during creaming (D'Incecco et al., 2018a). Also fatty acid composition is affected by milking frequency, with a smaller proportion of PUFA in milk produced in shorter time intervals (Wiking et al., 2006; Dutreuil et al., 2016).

A broad agreement exists in literature on a higher content ($P < 0.05$) of free fatty acids (**FFA**) in milk from AMS, irrespective of the stage of lactation. Since FFA are usually referred to milk fat content instead of milk volume, their interpretation might sometimes be difficult. More in general, FFA content in milk increases with decreasing intervals between milkings, indicating that milking frequency is more relevant in determining FFA content than the milking system *per se*. Klungel et al.

(2000) reported an increase of FFA from 0.38 to 0.53 meq/100 g fat in bulk milk after switching to AMS. Other authors found higher concentrations of FFA, depending on the studied conditions, that however confirmed this phenomenon (de Koning et al., 2003; Abeni et al., 2005b; Wiking et al., 2006; De Marchi et al., 2017). Wiking et al. (2006) studied the effect of milking frequency by collecting milk from udder halves that were milked 2 *vs* 4 times daily. Just after milking, the FFA content in the two types of milk was the same whereas, after 24 h of storage at 5°C, it was much higher ($P < 0.01$) in the latter. Based on these data, the authors indicated weakness of the fat globule membrane likely to be the cause of the higher FFA content in milk obtained with higher milking frequency. Consistently with the above reported findings, larger fat globules in AMS milk are more easily damaged upon mechanical stress due to continuous pumping and temperature fluctuations in the storage tank. Disruption of the globule membrane allows the milk lipases to access triglycerides inside the globule. Negative impacts of fat globule damage in raw milk on the properties of the derived food products are well documented in literature. High levels of FFA, particularly the short chain acids, impart unpleasant off-flavors to mildly flavored dairy products, such as pasteurized drinking milk or fresh cheeses (Deeth and Fitz-Gerald, 2006) and adversely affect the shelf-life (Barbano et al., 2006). It has been reported that the sensory threshold of FFA in pasteurized milk can be as low as 0.25 meq/L (Santos et al., 2003). The FFA in milk destined to cheese manufacturing are mainly lost in whey (Sapru et al., 1997) with a reduction in cheese yield. Presence of damaged fat globules dramatically affects the structure of cheese (Lopez and Briard-Bion, 2007; Ong et al., 2010; Logan et al., 2015). Relevant information for manufacturers of both cheese and fermented dairy products was reported by Sapru et al. (1997) who suggested that FFA may inhibit the activity of starter cultures. Awareness of these drawbacks is of extreme importance to the dairy industry because storage of raw milk at low temperature for hours before processing is a common practice.

CONCLUSIONS

Like other precision dairy farming technologies, AMS was designed to achieve advantages in terms of farmer labor saving, lifestyle quality improvement and increased milk production without prejudicing animal health and welfare. This review has highlighted how broad the debate on the advantages and disadvantages of AMS is. Although it was first proposed 25 yr ago, recent studies have demonstrated that shifting from CMS to modern AMS represents not only a change in technological hardware but it also involves complex animal-machine interactions, with a drastic modification of the farm- and herd-management processes.

An impressive amount of research has been dedicated to performances, management and sustainability of AMS at farm level. In contrast, effects of AMS on milk characteristics are controversial and there is still a lack of knowledge to fill before this technology could be considered suitable for producing good quality raw milk, whatever its destination. However, it must be underlined that most of the differences recorded between milk obtained by automatic and conventional milking systems are relatively small, and the variability among herds, feeding rations and seasons may exceed them by far. Most of the modifications of milk characteristics documented by the literature were conceivably the carryover of the increased milking frequency related to AMS adoption. Therefore, the impact of the milking system itself on quality and technological traits of milk is hard to recognize. The increased contents of FFA and the decreased content of proteose-peptones are effects most likely related to AMS adoption and due to the shorter residence time of milk in the udder. However, further work to confirm these findings is needed. Due to the number of possible effects, either positive or negative, that usage of AMS might have on the industrial transformation of the derived milk, as we have illustrated in this review, a more integrated approach in further optimizing the equipment and its operating conditions at farm level is certainly advisable.

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Table 1 - Main topics related to the introduction of automatic milking systems addressed by literature

Parameter	Size and duration of the study	Details	References
Economic aspects			
Investments and Profitability	Review article	Budget simulation to model profitability of AMS *vs* CMS in small, medium and large farms	Salfer et al. (2017)
	308 herds, 12 yr (2,071,662 milkings)	Comparison of test-day data from herds milked either in CMS or in AMS	Wade et al. (2004)
	62 farms, 1 yr	Comparison based on real accounting data between farms using CMS or AMS	Bijl et al. (2007)
Operational costs	7 farms, 12 mo	On-farm measurement of daily and seasonal water and electricity consumption of AMS used in pasture-based dairy farms	Shortall et al. (2018)
Social aspects			
Farmer's labor and lifestyle	107 farmers	Survey among farmers who had recently installed an AMS in different countries	Mathijs (2004)
	217 farmers	Survey among farmers concerning perceived changes in housing, farm management and cow health and fertility after transition to AMS	Tse et al. (2017)

Farmer's health and wellbeing	60 farmers, 4 yr	Survey among farmers having long or short, positive or negative, experience with AMS	van Dooren et al. (2010)
Technical aspects			
Equipment	12 farms, 240 cows	Comparison of functional characteristics of 2 AMS from different manufacturers with CMS	Gygax et al. (2007)
Sensors and Electronics	Review article	Overview of sensor systems suitable for dairy health management	Rutten et al. (2013)
	Technology study	Development of a sensor system for a rapid and accurate automatic locating of cow's teats	Azouz et al. (2015)
Management			
Farm/herd management	Review article	Guidelines on diverse aspects of barn design and of farm- and herd management for optimal utilization of AMS	Rodenburg (2017)
Feeding strategies	175 cows (1 herd), 1 mo	Field study on the effect of pre- or post-milking supplementation on daily milk yield	Lyons et al. (2013)
	2 farms, 146 cows, no duration indicated	Study on the effect of different feed deliveries frequency and responses to hot conditions in AMS and CMS herds	Bava et al. (2012)
	168 cows (1 herd), 16 d	Investigation on the effect of providing a small feed reward on voluntary pre-milking waiting time	Scott et al. (2014)
Animal health and welfare			
Physiology and reproduction	3 herds, 415 lactations, no duration indicated	Study on the effects of increased milking frequency on cow's fertility	Kruip et al. (2000)

	42 farmers	Survey among dairy farmers from CMS or AMS dairies on general farm management and on herd reproductive management	Keeper et al. (2017)
	2 herds, 158 cows, 4 mo, 1,252 milk emission curves	Study on milk ejection during automatic milking	Bava et al. (2005)
Animal health	43 herds (45-120 cows each), 18 mo	Study on health parameters before and after installation of AMS	Hillerton et al. (2004)
	128 farms, 6 yr	Study on milk yield and mastitis prevalence on farms that had changed their milking system, housing system, or both	Hovinen et al. (2009)
	36 farms	Identification of farm- and cow-related factors potentially causing lameness	Westin et al. (2016)
	33 infected and 139 healthy quarters, 4 wk	Investigation on the suitability of diverse electrical conductivity-derived indices and algorithms for the early detection of clinical mastitis in cows milked with AMS	Khatun et al. (2017)
Wellbeing and behavior	10+10 heifers, 22 wk	Evaluation of metabolic and psycho-physiological aspects of animal welfare of 2 groups of heifers milked in CMS or in AMS	Abeni et al. (2005a)
	12 herds, 1 yr	Evaluation of milk cortisol concentration (stress indicator) or restlessness behaviour in milk from herds milked in CMS or in free- or partially forced traffic AMS	Gygax et al. (2006, 2008)
	2 farms, 146 cows, 8 mo	Study on cow time budget in AMS and CMS herds with different environmental conditions	Mattachini et al. (2017)

Selection and genetic traits	9 AMS herds and 74 CMS herds, 6 yr	Estimation of genetic parameters for several traits that are important in AMS	Carlström et al. (2016)
Environmental sustainability			
	9 farms using AMS and 9 farms using CMS, 1 yr	Evaluation of several indicators of environmental, social and economic sustainability of AMS use on organic dairy farms	Oudshoorn et al. (2012)
Ethical aspects			
	Discussion paper	Evaluation of new forms of relationships between cows, technologies and dairy farmers in AMS farms	Holloway et al. (2014)

Table 2 - Articles debating the effects of automatic milking systems on milk yield

Studied parameter	Number of cows and breeds	Study duration	Conditions	Results	References
Milking behavior and milk yield of cows milked twice daily in a CMS and milked on voluntary base with AMS	84 in CMS and 44 in AMS, no breed indicated	39 d	All cows in mid-to-late lactation, housed in the same barn and fed the same ration	MF and milk yield of CMS cows were significantly (P <0.05) lower than those of AMS cows	Wagner-Storch and Palmer (2003)
Effects of different MI on milk yield and composition	664 in AMS, 3 breeds (Holstein, Red Dane, Jersey) , 1 herd	4 yr	Cows housed indoors in free-stall barn. Total mixed ration fed *ad libitum*, amounts of concentrates offered in AMS on the basis of the last MI. Minimum MI of 4h	A positive (P <0.05) correlation between MF and milk yield over 24 h was observed, but resulted negative between MF and yield per milking. Negative correlation between MF and fat and protein content	Løvendahl and Chagunda (2011)
Effects of MF on milk yield	280 in AMS, 1 breed (Polish Holstein), 1 herd	12 mo	Cows, kept in a free-stall barn, received partial mixed ration twice a day and were fed concentrate in the AMS	Higher MF for multi-parous cows with respect to heifers. Increasing MF increased milk yield, more for multiparous cows than for heifers	Bogucki et al. (2017)

Milk yield at quarter level as the effect of several parameters, including MF	780 in AMS, 1 breed (Korean Holstein), 7 herds	8 mo	Cows in parities 1 to 4. No limitations in MF; 6 kg/d of concentrate per cow fed *ad libitum*	MF varied from 1 to 6 daily, and significantly (*P* <0.001) affected milk yield, which increased upto 4 milkings/d, dcreasing afterwards	Vijayakumar et al. (2017)
Relationship between MI and milk production rate at quarter level in primiparous and multiparous cows	1280 in AMS 1 breed (Friesian Holstein), 1 herd	6 mo	Minimum MI: 5 h for primiparous cows, 6 h for multiparous. Partial mixed ratio concentrate feeding in the AMS, not balanced on cow production	A significant negative linear relationship was observed between MI and quarter-level milk production rate for all lactation groups, DIM windows, and quarters	Penry et al. (2018)

Figure 1. Conceptual framework for automatic milking system