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# Effect of microparticulated whey proteins on milk coagulation properties

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## ABSTRACT

The enhancement of milk coagulation properties (MCP) and the reuse of whey produced by the dairy industry are of great interest to improve the efficiency of the cheese-making process. Native whey proteins (WP) can be aggregated and denatured to obtain colloidal microparticulated WP (MWP). The objective of this study was to assess the effect of MWP on MCP; namely, rennet coagulation time (RCT), curd-firming time, and curd firmness 30 min after rennet addition. Six concentrations of MWP (vol/vol; 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0%) were added to 3 bulk milk samples (collected and analyzed during 3 d), and a sample without MWP was used as control. Within each day of analysis, 6 replicates of MCP for each treatment were obtained, changing the position of the treatment in the rack. For control samples, 2 replicates per day were performed. In addition to MCP, WP fractions were measured on each treatment during the 3 d of analysis. Milk coagulation properties were measured on 144 samples by using a Formagraph (Foss Electric, Hillerød, Denmark). Increasing the amount of MWP added to milk led to a longer RCT. In particular, significant differences were found between RCT of the control samples (13.5 min) and RCT of samples with 3.0% (14.6 min) or more MWP. A similar trend was observed for curd-firming time, which was shortest in the control samples and longest in samples with 9.0% MWP (21.4 min). No significant differences were detected for curd firmness at 30 min across concentrations of MWP. Adjustments in cheese processing should be made when recycling MWP, in particular during the coagulation process, by prolonging the time of rennet activity before cutting the curd.

**Key words:** microparticulated whey protein, milk coagulation property, dairy industry

## INTRODUCTION

The dairy industry has an important economic role in Italy and a main focus is the transformation of milk into high-quality cheeses. In this context, the evaluation of milk coagulation properties (**MCP**) is valuable to improve milk processing (Cassandro et al., 2008; De Marchi et al., 2008). Indeed, good technological properties of milk have been associated with enhanced cheese yield (Riddell-Lawrence and Hicks, 1989; Pretto et al., 2013). Several instruments can be used to measure MCP (O'Callaghan et al., 2002), and mechanical tools such as the Formagraph (Foss Electric, Hillerød, Denmark) have been widely used to determine MCP. These instruments produce a typical diagram as reported by De Marchi et al. (2009) and provide measurement of rennet coagulation time (**RCT**), curd-firming time, and curd firmness 30 min after rennet addition. Recently, mid-infrared spectroscopy combined with chemometric analysis has been proposed as fast, nondestructive, and cheap technique to predict MCP (De Marchi et al., 2013, 2014; Tiezzi et al., 2013).

At present, improvements in the efficiency of the dairy industry are needed. The recovery of whey protein (**WP**) after cheese-making process is an interesting application. Native WP can be extracted from whey through filtration to obtain WP concentrate (**WPC**), which is further aggregated and denatured through a controlled process to produce colloidal microparticulated WP (**MWP**; Spiegel and Huss, 2002). In addition, the dairy industry faces growing demand for low-fat products; in this context, the incorporation of WPC and MWP as fat replacer into milk to maintain the yield and nutritional value of dairy products can be an efficient solution (Lo and Bastian, 1998). Microparticulated WP has been used to improve overall sensory properties of low-fat dairy products such as ice cream (Yilsay et al., 2006; Karaca et al., 2009), yogurt (Janhøj et al., 2006; Aziznia et al., 2008; Torres et al., 2011), and cheeses (Koca and Metin, 2004; Sahan et al., 2008; Ismail et al., 2011). Substitution of fat with MWP reduces the firmness of low-fat products because of the water-holding capacity of MWP. Excessive addition of WP is likely to interfere with curd formation and adversely affect cheese quality (Guinee et al., 1998).

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The aggregation of MWP is influenced by WP composition, the presence of lactose or κ-CN (Guyomarc'h et al., 2009), heat treatment, pH, and ionic strength conditions (Chen et al., 2006; Nicolai and Durand, 2007; Gulzar et al., 2011). There is a paucity of studies investigating the effect of the addition of WP to milk. Ismail et al. (2011) reported shorter RCT of buffalo milk with added WPC, whereas Guinee et al. (1997) found impaired renneting properties of bovine milk with added WPC. Interest in valorizing whey components and improving MCP during cheese making is high in a dairy industry specializing in cheese production. Therefore, the aim of the present study was to investigate the effect of increasing concentrations of MWP (from 0.0 to 9.0%, vol/vol) on MCP.

## MATERIALS AND METHODS

### Sample Collection and Experimental Design

Three samples of raw bulk milk were collected from 3 farms of the Veneto region (northeast Italy) during 3 sampling dates in October 2012. Milks were stored in a portable refrigerator (4°C) and analyzed within 1 h in the milk quality laboratory of the Breeders Association of Veneto region (ARAV, Padova, Italy) for fat, protein, CN, and lactose contents using a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark).

Microparticulated WP was collected in the Soligo dairy cooperative (Farra di Soligo, Treviso, Italy) at the beginning of the trial, and it was used for all the sessions. On the days of analysis, aliquots of MWP were stored at −20°C. Microparticulated WP was extracted from total skim sweet whey produced by the dairy cooperative in a working day through the ultrafiltration process (Tetrapak International SA, Rubiera, Italy), using a tubular semipermeable polyethersulfone membrane with a surface area of 700 $m^2$, and a cut-off of 10,000 Da (6338 HFK-131; Koch Membrane System, Wilmington, MA) at 10°C. Moreover, the microparticulation process was carried out for 10 min at 95°C by using a shell-and-tube heat exchanger and homogenization at 4,000 kPa following the manufacturer's protocols (Tetrapak International SA).

Microparticulated WP were added at increasing concentrations (1.5, 3.0, 4.5, 6.0, 7.5, and 9.0%; vol/vol) to bulk milk. A sample without MWP (MWP = 0.0%) was used as control. Within each day of analysis, 6 replicates of MCP for each treatment were obtained, changing the position of the treatment in the rack. For the control samples, 2 replicates per day were performed. In addition to MCP, WP fractions were measured on each treatment during the 3 d of analysis.

### Analysis of MWP

Microparticulated whey protein fractions were quantified by reverse-phase (**RP**)-HPLC after solubilization with 6 *M* guanidine hydrochloride (Sigma, St. Louis, MO) for 24 h. Reverse-phase HPLC analysis was carried out in the laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy) using an Agilent 1260 Series chromatograph instrument (Agilent Technologies, Santa Clara, CA), and separation was performed on a reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, Agilent Technologies) with a Poroshell packing (5 μm, 300 Å, 2.1 × 75 mm). Detection was at 214 nm. Gradient elution was carried out with a mixture of 2 solvents: solution A consisted of 0.1% trifluoroacetic acid and 5% acetonitrile in water, and solution B was 0.1% trifluoroacetic acid in acetonitrile. The gradient started with 95% of solution A, after 1 min the gradient was 82% of A, after 2 min it was 70%, and after 5 min A and B were in equilibrium. From 5 to 9 min (end of the run), the gradient was brought back to initial conditions (95% A). Quantified WP were α-LA, β-LG A and β-LG B variants, BSA, lactoferrin (**LF**), caseinomacropeptide (**CMP**), and proteose-peptone (**PP**).

### Analysis of MCP

Aliquots of daily milk and serial percentage of MWP were mixed within each day of the trial and kept at 4°C until the beginning of the analysis. A final volume of 10 mL for each sample was heated to 35°C in 10 min; once 35°C was reached, 200 μL of rennet (Hansen standard 190, Pacovis Amrein AG, Bern, Switzerland) diluted 1.6% with distilled water was added to milk (Pretto et al., 2013). In total, 144 measures of MCP were determined using a Formagraph (Foss Electric). The working principle of the Formagraph is based on the swing of a pendulum immersed in milk and driven by an electromagnetic field. As the cheese milk coagulates, the swing of the pendulum becomes smaller and differences in the electromagnetic field are recorded (O'Callaghan et al., 2002). The output of the instrument consists of 3 measurements of MCP: RCT (min), defined as the interval from the addition of the clotting enzyme to the beginning of coagulation; curd-firming time ($\mathbf{k_{20}}$, min), which is the interval from the beginning of coagulation to the time at which the width of the graph attains 20 mm; and curd firmness ($\mathbf{a_{30}}$, mm), defined as the width of the diagram 30 min after rennet addition.