Available online at www.sciencedirect.com
ScienceDirect
International Dairy Journal 17 (2007) 674–682
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Rheological properties and microstructure during rennet induced coagulation of UF concentrated skim milk

A.O. Karlsson*, R. Ipsen, Y. Ardö

Dairy Technology, Department of Food Science, Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Rolighedsvej 30[5], 1958 Fredriksberg C, Denmark

Received 20 December 2005; accepted 10 August 2006

Abstract

Rennet induced coagulation of ultrafiltrated (UF) skim milk (19.8%, w/w casein) at pH 5.8 was studied and compared with coagulation of unconcentrated skim milk of the same pH. At the same rennet concentration (0.010 International Milk Clotting Units g^{-1}), coagulation occurred at a slower rate in UF skim milk but started at a lower degree of κ-casein hydrolysis compared with the unconcentrated skim milk. Confocal laser scanning micrographs revealed that large aggregates developed in the unconcentrated skim milk during renneting. Following extensive microsyneresis the protein strands were shorter and thinner in gels from UF skim milk. Moreover, during storage up to 60 days (13 °C), the microstructure and the size of the protein strands of the UF gel changed only slightly. Hoelter–Foltmann plots suggested that the coagulation rate was reduced in the UF skim milk due to a high zero shear viscosity of the concentrate compared with the unconcentrated skim milk.

Keywords: Casein gel; Coagulation; Microstructure; Rennet; Rheology; Ultrafiltration

1. Introduction

Membrane processes are used in dairy plants to standardize or increase the protein content in milk and to separate bacteria and spores from milk. Shortly after the introduction of membrane processes for milk, the use of concentrated ultrafiltrated (UF) milk for cheese production was initiated. A liquid pre-cheese (LPC) concept resulted in minimal whey drainage and therefore almost all whey proteins could be incorporated into the cheese and significantly increase the cheese yield (Maubois, Mocqout, & Vassal, 1969). However, cheese made from UF milk and especially cast cheese made using the LPC concept generally have very different sensorial and functional properties compared with cheese from traditional cheese production (Mistry & Maubois, 1993). The incorporation of whey proteins have been suggested to cause many of the differences between cheeses from UF milk and unconcentrated milk (Bech, 1993). Whey proteins act as inert fillers in the casein matrix and increase the water binding of cheese and causes UF cheeses to be softer than traditional cheeses. The presence of whey proteins has also been suggested to reduce the enzymatic proteolysis of caseins during ripening of UF cheeses. UF is hence not used in the production of most cheese varieties. However, some cast-type cheese varieties with a low pH, i.e., Feta and Camembert types, have been accepted by consumers.

The rennet induced coagulation of skim milk constitutes three phases: enzymatic hydrolysis of κ-casein, aggregation of renneted casein micelles and gel development. In the latter phase a three-dimensional protein network develops and micro- and macrosyneresis, i.e., fusion of casein micelles and whey separation, respectively, occurs (Walstra & van Vliet, 1986). With increased casein concentration the coagulation properties of milk change. The coagulation time decreases (Sharma, Mittal, & Hill, 1994), the elasticity of the gel increases (Culioli & Sherman, 1978), the level of hydrolysed κ-casein at the coagulation point is lower (Sharma et al., 1994) and less water and whey proteins are expelled from the gel.

*Corresponding author. Tel.: +45 3528 3253; fax: +45 3528 3190.
E-mail address: aka@kvl.dk (A.O. Karlsson).

0958-6946/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2006.08.002

Results presented by Green, Marshall, and Glover (1981) and Hyldig (1993) show that the protein network coarsens when the casein concentration is increased in casein gels and cheese. However, no physical explanation has been given for this behaviour and the microstructure of milk concentrates has not been studied during coagulation. Since the microstructure of mature casein gels (after syneresis) has been shown to be influenced by the aggregation kinetics (Green, 1990; Wium, Pedersen, & Qvist, 2003) it is important to study the microstructure during coagulation. Especially, coagulation of highly concentrated milk with no whey separation is interesting because this determines the microstructure of cast cheese. This has generally received very little attention, and the changes occurring in the microstructure during longer storage, i.e., months, has not been emphasized.

In the present work we have investigated differences between rennet coagulation of highly concentrated (19.8%, w/w casein) and unconcentrated skim milk. The rheological properties during aggregation and coagulation were related to the enzymatic hydrolysis of κ-casein and the microstructure of developing aggregates in both unconcentrated and concentrated skim milk. Development of microstructure in gels from unconcentrated skim milk after extensive microsyneresis and gels from UF concentrate after storage at 13 °C for up to 60 days were studied and compared.

2. Materials and methods

2.1. Preparation of UF concentrate

The UF concentrate was produced as described by Karlsson, Ipsen, Schrader, and Ardö (2005). The UF process was stopped when the UF concentrate had reached a Brix value of 36.3°, measured using a handheld refractometer (Atago Co., Tokyo, Japan). After production, the UF concentrate was poured into bottles (100 mL), heat treated (62 °C for 30 min), quickly cooled to approximately 4 °C in an ice–water bath and stored in a freezer (−23 °C). The frozen UF concentrate was used within one month. Compared with fresh UF concentrate, the coagulation properties of frozen UF concentrate stored or two months was shown not to be significantly different (results not shown).

Prior to use in experiments, UF concentrate and unconcentrated skim milk was thawed in a water bath (30 °C) for 1 h. Thimerosal (0.02%, w/w; Merck, Darmstadt, Germany) was added as a preservative to all samples to prevent microbial growth. The samples were equilibrated for 24 h at 30 °C before glucono-δ-lactone (GDL; Acros Organics, Geel, Belgium) was added to samples of UF concentrate and unconcentrated skim milk in order to obtain a final pH of 5.8 after 24 h of storage at 30 °C after GDL addition.

2.2. Chemical analysis of unconcentrated skim milk and the UF concentrate

The pH was measured directly using a Knick Portamess (Knick Elektronische Messgeräte, Berlin, Germany) equipped with a Hamilton Tiptrode (Hamilton Instruments, Bonaduz, Switzerland). Total solid contents were determined according to the International Dairy Federation (IDF) standard method (IDF, 1991). Nitrogen was determined using a Kjeltec System 1026 Analyzer (Tecator, Höganäs, Sweden). Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined according to the IDF standard methods (IDF, 1993). The protein content was estimated by multiplying the nitrogen content for casein by 6.36 and whey protein by 6.28 (van Boekel & Ribadeau-Dumas, 1987). Determination of lactose was carried out with a Lactose/D-Galactose Enzymatic BioAnalysis-kit (Scil Diagnostica, Martinsried, Germany) according to the manual of the manufacturer. All chemical analyses were performed at least in duplicate.

Casein has been reported to be totally demineralized from colloidal calcium phosphate at pH 2.7 (Le Graët & Gaucheron, 1999). Thus, by adjusting the pH to the interval 2.2–3.0 using 1 M HCl, all the colloidal calcium was dissolved in the serum phase of unconcentrated skim milk and UF concentrate. At pH 5.8, adjusted by GDL, only a part of the colloidal calcium is dissolved in the serum phase of skim milk and UF concentrate. Rennet (CHY-MAX Extra Liquid, Chr. Hansen A/S, Hørsholm, Denmark) was diluted 10 times and 2.5 μL g^{-1} sample was added to samples 24 h after addition of HCl or GDL. The samples were then stirred for 1.5 min. The samples coagulated in 24 h (30 °C) and the serum phase could be separated from the gels by ultracentrifugation ($100000 \times g$ for 60 min at 30 °C) using a Beckman L8-70M Ultracentrifuge with a SW28 rotor (both from Beckman Instruments Inc., Palo Alto, CA, USA). Following centrifugation, the supernatant was carefully removed and transferred to plastic tubes, which were placed in the freezer (−23 °C) for later determination of calcium. Prior to analysis the supernatants were thawed in a water bath (20 °C). Two supernatants of every sample were individually prepared.

The calcium concentration was determined using a Perkin Elmer Atomic Absorption Spectrometer (Perkin Elmer, Boston, MA, USA). A calibration curve was created by measuring standard solutions of $CaCl_2$. Supernatant solutions from ultracentrifugation were diluted with double deionized H_2O. Diluted supernatant solutions of samples and standard solutions contained 0.02654 M $LaCl_3$. Dilution of every solution was performed at two independent times to quantify the error of the sample preparation.

2.3. Degree of κ-casein hydrolysis during renneting

Different amounts of rennet [0.010–0.003 International Milk Clotting Units (IMCU) g^{-1}, CHY-MAX Extra