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Thermal aggregation of whey proteins in the presence of buttermilk concentrate

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ABSTRACT

The objective of this study was to assess the impact of adding buttermilk concentrate to the denaturation and microparticulation process of cheese whey protein concentrate. For this purpose, the two concentrates were mixed and co-denatured (pH 4.6, 90 °C,) and homogenized. The presence of buttermilk significantly increased aggregation yield and decreased water-holding capacity of aggregates up to a buttermilk:whey protein ratio of 75:25. Modification of rheological properties suggests that denatured whey protein interacted with casein. A thiol blocker, *N*-ethylmaleimide, was added before heating to measure the role of disulphide bond formation in the aggregation process. Results showed that both covalent and non-covalent interactions were involved in aggregate formation. Ultrasound treatment was applied during denaturation process and was shown to influence aggregate formation. It appeared that under increased turbulence and cavitation conditions, aggregation yield was increased and water-holding capacity decreased.

1. Introduction

A number of technological approaches have been developed to increase the retention of whey proteins into cheese (Lawrence, 1989, 1993; Lelièvre, 1990). A widely used approach consists in the addition of denatured and aggregated whey proteins to cheese milk. However, one of the most serious challenges is to optimize the size and water-holding capacity of the aggregated whey proteins. Indeed, larger aggregates readily interfere with the casein network and they are poorly retained in cheese curd (Lelièvre, 1990). Such a problem can, however, be circumvented by homogenization of the aggregates, thus facilitating retention (Mignot and Tracard, 1976; Punidadas et al., 1999). The addition of whey proteins into cheese has proved to increase yield but to reduce quality (Lawrence, 1993; Lebeuf et al., 1998; Punidadas et al., 1999). Another problem associated with the incorporation of aggregated proteins is related to their effect on cheese moisture. Excessive cheese moisture is a common defect which limits the amount of whey protein that can be added to cheese milk.

Substituting buttermilk to cheese milk at a concentration over 5–10% to milk has been shown to generate texture defects and high moisture (Joshi et al., 1994). However, other studies have shown that buttermilk could be a valuable product for increasing the moisture content and improve the texture of low fat cheese, essentially because it contains fragments of milk fat globule membranes (MFGM), including phospholipids, and whey proteins (Mistry et al., 1996; Raval and Mistry, 1999; Turcot et al., 2001, 2002).

It was shown by Morin et al. (2008) that the poor coagulation properties of buttermilk were related to cream pasteurization that probably induced important modifications of the MFGM surface during the heating process. Many authors have reported that whey proteins had the potential to interact with κ-casein (κ-CN) (Morr and Josephson, 1968, Sawyer, 1969; Vasbinder and de Kruif, 2003; Donato et al., 2007) and particularly with serum-phase κ-casein after the dissociation of κ-CN from the micelle (Anema, 2008). In addition, Ye et al. (2004a,b) demonstrated that β-lactoglobulin (β-lg) and α-lactalbumin (α-la) associated with the MFGM by thiol–disulphide bonds in heated milk.

In line with these observations, we hypothesized that co-denaturation of cheese whey and buttermilk proteins would induce the formation of aggregates between whey proteins–κ-CN – MFGM by means of SH/SS interchanges as well as by other non-covalent interactions such as ionic, hydrophobic or van der Waals. Also, the aggregation yield and water-holding capacity of the aggregates would be dependent to the ratio between whey and buttermilk, as well as on the availability of free-SH groups and on the possibilities of rearrangements during aggregation process.

N-ethylmaleimide (NEM), a thiol blocker, can be used to prevent or slow down the thiol/disulphide interchange reactions between denatured milk proteins (Hoffman and Van Mil, 1997; Alting et al., 2000). Havea et al. (2004) has shown that gels formed by

Abbreviations: *k*, consistency coefficient; *n*, flow behavior index; WHC, water-holding capacity; NEM, *N*-ethylmaleimide.

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non-covalent interactions were more rigid than those formed by disulphide bonds. We also hypothesized that applying physical treatments that would promote rearrangement of the aggregates during heating and would increase the compactness and reduce the hydration of the aggregates. Ultrasound treatments are known to generate cavitation, turbulence and heat (Patist and Bates, 2008). High intensity ultrasound (20 kHz) generates shear forces that were effective in reducing viscosity of dairy ingredients (Zisu et al., 2009) and in homogenizing fat globule membrane (Ashokkumar et al., 2009; Jambrak et al., 2009).

The use of ultrasound treatments in food processing is considered as an emergent potential alternative to heat or to homogenization treatment (Patist and Bates, 2008). Moreover, under turbulent conditions due to ultrasound treatments, particle mobility is increased, which promotes the formation of aggregates (Walstra, 1983). The challenge remains, however, to control yield aggregation and water-holding capacity (WHC).

The objective of the present work was to characterize the heat-induced aggregation process of whey protein–buttermilk concentrates mixtures with different buttermilk protein content (0%, 25%, 50%, 75% and 100%) on the aggregation yield and water-holding capacity of the aggregates. The effects of blocking free-SH by addition of NEM and applying power ultrasound (20 kHz) during aggregation process were also assessed.

2. Material and methods

2.1. Materials

Fresh Mozzarella cheese whey and buttermilk were obtained in a local cheese factory (L'Ancêtre, Bécancour, QC, Canada). *N*-ethylmaleimide (NEM) was obtained from Alfa Aestar (Ward Hill, MA, USA). All other reagents were from Fisher Scientific (Ottawa, ON, Canada).

2.2. Preparation of whey and buttermilk concentrates

Fresh Mozzarella cheese whey and buttermilk were skimmed by using a pilot scale milk separator (Alfa Laval, Uppsala, Sweden). Bacterial contamination of cheese whey was reduced by microfiltration (TetraPak MSF1, Lund, Sweden) through a 1.4 μm membrane (Membralox®, Bazet, France). Microfiltered whey and buttermilk were concentrated by ultrafiltration (UF) through a 5 kDa membrane (Romicon, Koch Membrane Systems, Wilmington, MA, USA) to a final protein concentration of 9.5% (w/v) in the UF-rententate. The concentrates were frozen to −28 °C until further analysis.

2.3. Heating experiments

Concentrates were mixed at the following buttermilk:whey protein ratios: 0:100; 25:75; 50:50; 75:25 and 100:0. Mixtures (200 mL) were adjusted to pH 4.6 by the slow addition of HCl 1 N and heated from 4 °C to 90 °C in a thermostatically-controlled water bath, under constant stirring for 25 min (including come-up time) at 168 rpm agitation rate. After thermal treatment, mixtures were cooled to 30 °C in an ice bath and homogenized for five passes at 65.5 MPa using an Emulsiflex-C5 (Avestin Canada, ON, Canada). The overall process used for the preparation of aggregates is summarized in Fig. 1.

2.4. Treatments with N-ethylmaleimide (NEM) and power ultrasound

A concentration of 9.5 mM *N*-ethylmaleimide (NEM) was used in accordance with the experiments of Alting et al. (2000) in order to completely block the free thiol groups. NEM (9.5 mM) was added in whey–buttermilk concentrate (9.5% protein concentration) mixtures (200 mL) before heat treatment.

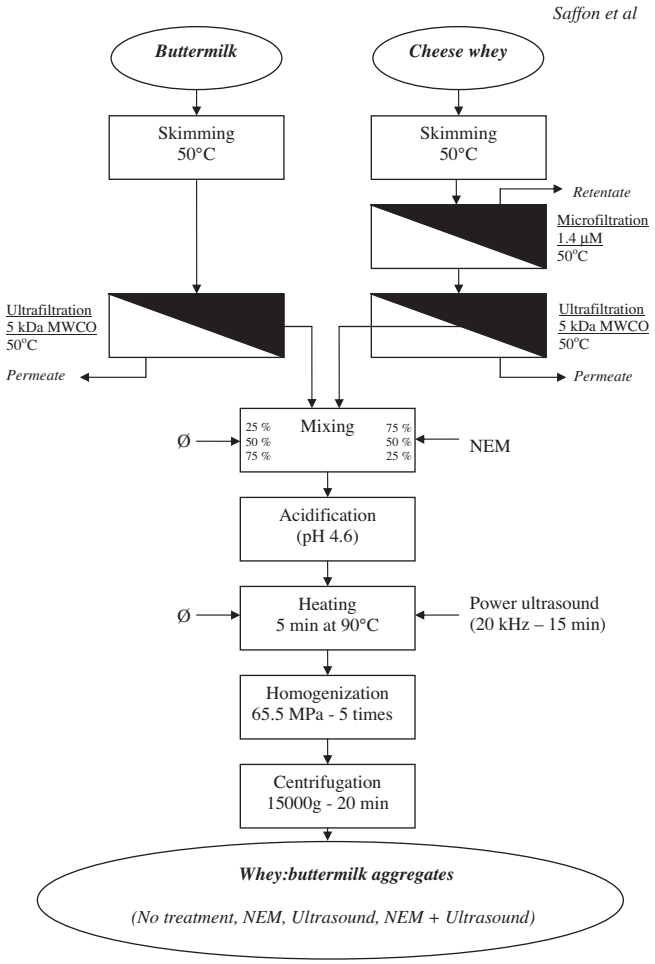

Fig. 1. Experimental procedure used to prepare aggregated buttermilk and whey proteins mixtures.

Power ultrasounds were applied using a constant frequency 20 kHz probe (Virsonic-550, The Virtis Company, Gardiner, NY, USA). The power level was fixed to 275 W. The titanium tip was immersed 2 cm underneath the surface during the treatment. The treatments were applied to the mixtures after 10 min of thermal denaturation, until the end of heating (25 min).

2.5. Analytical methods

2.5.1. Compositional analyses

Total nitrogen content was determined by the Dumas combustion method (IDF, 2002) using a LECO equipment (Protein Analyzer model FP-528 Leco Instruments Ltd., Mississauga, ON, Canada). Nitrogen values were converted into protein values using 6.38 as the nitrogen conversion factor. Fat was determined using the Mojonnier extraction method (IDF, 2008) and lactose by enzymatic method (IDF, 2001). Total solids were obtained by microwave drying (Smart System 5, CEM Corp., Matthews, NC, USA) and ash was measured by incineration in a muffle furnace at 550 °C for 20 h (AOAC, 1990). The overall composition of each mixture as determined experimentally is summarized in Table 1.