Food Chemistry 197 (2016) 233–239

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of calcium chelators on calcium distribution and protein solubility in rennet casein dispersions

Irene McIntyre, Michael O' Sullivan, Dolores O' Riordan *

UCD Institute of Food & Health, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 16 October 2015
Accepted 18 October 2015
Available online 19 October 2015

Keywords:
Rennet casein
Calcium chelating salts
Chelation
Protein solubility

ABSTRACT

This study investigated the effects of calcium chelating salts on calcium-ion activity ($A_{Ca^{++}}$), calcium distribution, and protein solubility in model $CaCl_2$ solutions (50 mmol L^{-1}) or rennet casein dispersions (15 g/100 g). Disodium phosphate and trisodium citrate at concentrations of 10 and 30 mmol L^{-1} and at ratios of 1:0, 2:1, 1:1, 1:2 and 0:1 were added to both systems. The $CaCl_2$ system, despite its simplicity, was a good indicator of chelating salt–calcium interactions in rennet casein dispersions. Adding trisodium citrate either alone or as part of a mixed chelating salt system resulted in high levels of dispersed "chelated" calcium; conversely, disodium phosphate addition resulted in lower levels, while the $A_{Ca^{++}}$ decreased with increasing concentration of both chelating salts. Neither chelating salt produced high levels of soluble protein. Thus calcium chelating salts may play a more subtle role in modulating hydration during manufacture of casein-based matrices than simply solubilising calcium or protein.

1. Introduction

Chelating agents (e.g. citric acid and its derivatives, various phosphates, and salts of EDTA) are known to form complexes with monovalent or polyvalent metal cations e.g. Na^+ or Ca^{++}. Calcium (Ca) and calcium ions (Ca^{++}), can be present in foods in the form of an equilibrium between ionic, soluble non-ionic (i.e. chelates and complexes), and colloidal species e.g. in dairy systems (DeMan, 2013). The Ca^{++} ions are of particular importance, these contribute to the internal stability of casein micelles as they form linkages between protein molecules, either as colloidal calcium phosphate (CCP) or directly bound to caseins (Holt, 1992; Horne, 1998; Schmidt, 1982; Tsioulpas, Lewis, & Grandison, 2007). The addition of calcium chelating agents presumably results in the complexation of Ca present in the soluble phase and, then if sufficient chelating agent is added, the Ca associated with the casein is removed. This process can alter the distribution of Ca^{++} between the soluble and colloidal phases (Udabage, McKinnon, & Augustin, 2000), which can effect the stability (Gaucheron, 2005) and structural integrity of casein micelles (De Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011) and consequently the functionality (e.g. water binding capacity, emulsifying properties, viscosity and solubility) of casein in dairy systems.

Rennet casein is prepared by the enzymatic precipitation of casein from pasteurised skim milk. The high colloidal Ca content retained in the resulting *para*-casein curd after this process is largely responsible for the lack of solubility of rennet casein in water (Ennis, Thornton, & Mulvihill, 2000). The ability of rennet casein to disperse in water is governed by the amount of colloidal Ca (Ca phosphate) that is available to cross-link the amorphous *para*-casein matrix (Fox, Guinee, Cogan, & McSweeney, 2000), and findings of model studies involving dilute suspensions of *para*-casein, have shown that hydration increases as the level of colloidal Ca decreases (Sood, Gaind, & Dewan, 1979). The application of calcium chelating salts (CCS), such as disodium phosphate (DSP) and trisodium citrate (TSC) is thought to result in the solubilisation of colloidal Ca (Ca phosphate) from casein micelles (De Kort et al., 2011) through formation of chelates with Ca^{++}. Ca chelation involves the exchange of the Ca^{++} in the *para*-casein network of rennet casein, for the monovalent cations (e.g. Na^+) of the CCS. This results in the partial hydration of the insoluble *para*-casein and its conversion to a water soluble sodium *para*-caseinate dispersion. In certain applications e.g. processed cheese, the CCS function is not only to chelate Ca, but also to increase pH which further aids in protein dispersion and hydration (El-Bakry, Duggan, O' Riordan, & O' Sullivan, 2011b) by increasing the net negative charge on the caseins. All of the above CCS-induced changes favour a more open reactive *para*-caseinate conformation with enhanced water binding and emulsifying ability (Carić & Kaláb, 1985; Fox et al., 2000). CCS therefore modify the functional properties of rennet casein

* Corresponding author.
E-mail address: dolores.oriordan@ucd.ie (D. O' Riordan).

http://dx.doi.org/10.1016/j.foodchem.2015.10.084
0308-8146/

and, together with the aid of heat and shear, facilitate its use in food applications. The extent and nature of hydration of the protein are critical factors in determining the functional performance of rennet casein during manufacture of processed and analogue cheese and in influencing the end-product functionality (e.g. texture-related and melting properties) of such products.

Some manipulations (e.g. addition of CCS, heat treatment, pH), that one performs during processed and analogue cheese manufacture can influence the degree of casein demineralisation and hydration during processing. While CCS-rennet casein interactions have been studied in dilute systems (Ennis & Mulvihill, 1999; Ennis, O'Sullivan, & Mulvihill, 1998; Ennis et al., 2000; O' Sullivan & Mulvihill, 2001), to our knowledge the interactive effects of the type and concentration of CCS, temperature and pH have not been investigated in detail in concentrated rennet casein systems. Thus, in this study, relationships between all of these parameters are examined in relation to their influence on the functional performance of rennet casein during processed and analogue cheese manufacture. The aim of the study was to investigate the effects of adding CCS, individually or in mixtures, to rennet casein (RC) dispersions. The effects of varying the proportions of different types of CCS used in the mixture, and the total concentration of CCS applied were investigated under conditions (i.e. pH and temperature) simulating industrial processing of processed and analogue cheese. Preliminary studies were carried out using $CaCl_2$ solutions to better elucidate CCS–Ca interactions in a simplified system and to establish if this system could then be used to predict or model CCS behaviour in a more complex Ca containing dispersion. Effects of CCS on Ca-ion activity ($A_{Ca^{++}}$) and Ca distribution in both $CaCl_2$ and RC systems were determined, additionally, the viscosity and protein solubility of RC dispersions with added CCS were studied.

2. Materials and methods

2.1. Materials

Rennet casein powder (Kerrynor™ R190) was purchased from Kerry Ingredients Ltd (Listowel, Co. Kerry, Ireland). CCS: disodium phosphate (DSP) (Albright & Wilson Ltd. Cheshire, England), dipotassium phosphate (DPP), trisodium citrate (TSC), and tripotassium citrate (TPC) (Jungbunzeuer GmbH., Pernhofen, Austria) were anhydrous and of food grade quality. Chemical reagents: Calcium Chloride ($CaCl_2$) anhydrous, Calcium reference standard solution for AAS (1000 mg/L Ca), Potassium Chloride (KCl), Lanthanum trichloride heptahydrate ($LaCl_3 \cdot 7H_2O$), and Nitric acid (HNO_3) were purchased from Sigma Aldrich (Ireland) and were of analytical grade. De-ionized water was prepared in a Milli-Q water purification system.

2.2. Experimental design

The first part of the study investigated the effects of different types of CCS (i.e. DSP, DPP, TSC, and TPC) and their concentrations (0–100 mmol L^{-1}) on the $A_{Ca^{++}}$ in 50 mmol L^{-1} $CaCl_2$ solutions. This calcium concentration was selected, because the casein micelles in concentrated dairy systems have comparable calcium concentrations (De Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2009). Experiments were performed at different pH values (8.0 or 6.7) and heat treatments (50 °C and 70 °C), which reflect the manufacturing conditions for processed and analogue cheese.

In the second part of the study, the effects of CCS (DSP and TSC) on Ca distribution in both $CaCl_2$ solutions (50 mmol L^{-1}) and RC dispersions (15 g/100 g) were investigated. The CCS used in the manufacture of processed and analogue cheese are mainly sodium salts (Carić & Kaláb, 1985; Cavalier-Salou & Cheftel, 1991), most commonly phosphates and citrates. These CCS show desirable performance characteristics when used in processed and analogue cheese manufacture, therefore only sodium based CCS were used for this part of the study. The CCS at concentrations of 10 and 30 mmol L^{-1} and at DSP:TSC ratios of 1:0, 2:1, 1:1, 1:2 and 0:1 were added to both systems and the pH adjusted; the effect of temperature (22 °C, 50 °C and 70 °C) was only investigated in the case of RC dispersions. Subsequently the Ca and protein contents of the dispersed phase obtained after centrifugation were determined. Concentrations of 10 and 30 mmol L^{-1} CCS were selected, because the largest decrease in $A_{Ca^{++}}$ was measured between 0 and 40 mmol L^{-1}. Similar concentrations of CCS were used by Udabage et al. (2000) when investigating mineral and casein equilibria in milk protein systems.

2.3. Sample preparation

Aqueous stock solutions of CCS (100 mmol L^{-1}) were prepared and an aliquot added (with stirring) to $CaCl_2$ solutions or RC dispersions to give the final desired concentration of CCS. The pH of solutions was adjusted using 1 mol L^{-1} NaOH or 1 mol L^{-1} HCl. All pH measurements were carried out on a pH meter (EL20, Mettler Toledo, Schwerzenbach, Switzerland) calibrated with standard solutions at pH 4.0 and pH 7.0; measurements were performed at 25.0 ± 0.1 °C. All pH adjusted samples were stirred (IKA RCT basic, IKA® Werke, GmbH & Co. KG, Germany) for one hour; afterwards, the pH was checked, readjusted if necessary and de-ionized water added to achieve the desired concentrations. RC dispersions were heated to 50 °C or 70 °C using a heated magnetic laboratory stirrer (IKA RCT basic, IKA® Werke, GmbH & Co. KG, Germany). After heating, RC dispersions were allowed to cool to room temperature. The dispersed and insoluble phases were separated by centrifugation (Hettich Rotofix 32 A, Andreas Hettich GmbH & Co. KG. Germany) at 3000*g* for 10 min (IDF, 2002). All samples were prepared and analysed in triplicate.

2.4. Calcium analysis

2.4.1. Calcium-ion activity ($A_{Ca^{++}}$)

The $A_{Ca^{++}}$ in samples was measured with a calcium-ion selective electrode (ISE 25 Ca; Radiometer Analytical, Mendes, France) and a reference electrode ("Red Rod" REF 251; Radiometer Analytical, Mendes, France) fitted to a pH meter (EL20, Mettler Toledo AG, Schwerzenbach, Switzerland). Calibration was performed at ambient temperature with standard solutions containing 0.5, 5.0, or 50 mmol L^{-1} $CaCl_2$ and 80 mmol L^{-1} KCl. Addition of this monovalent background electrolyte was necessary, as it keeps the $A_{Ca^{++}}$ effectively constant in the calibration solutions (De Kort et al., 2009). Preliminary experiments were carried out using 5 $CaCl_2$ standards, selected from the range 0.001–100 mmol L^{-1} to confirm a linear response up to 100 mmol L^{-1}. A calcium-ion activity co-efficient ($y_{Ca^{++}}$) of 0.29 was calculated for the calibration solutions using the formula of Davies (1962) below:

$$\mathrm{Log}(\gamma \mathrm{Ca}^{++}) = -0.5z^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \quad (1)$$

In which z is the charge (valence) on the ion and I refers to the ionic strength of the solution in mol/L. The calculated $y_{Ca^{++}}$ value obtained was comparable to that reported by De Kort et al. (2009). The $A_{Ca^{++}}$ was determined by multiplying the experimental $[Ca^{++}]$ by the activity coefficient of 0.29. The $[Ca^{++}]$ was calculated from the regression equation, derived from the calibration curve. All experiments were carried out under continuous stirring with a laboratory stirrer (IKA RCT basic, IKA® Werke, GmbH & Co. KG, Germany).