Food Chemistry 239 (2018) 17–22

Contents lists available at ScienceDirect

Food Chemistry

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

The adsorption of orthophosphate onto casein-iron precipitates

Vikas A. Mittal [a], Ashling Ellis [a,b], Aiqian Ye [a,b], Patrick J.B. Edwards [c], Harjinder Singh [a,b,*]

[a] Riddet Institute, Massey University, Private Bag 11 222, Palmerston North 4474, New Zealand
[b] Massey Institute of Food Science and Technology, Massey University, Private Bag 11 222, Palmerston North 4474, New Zealand
[c] Institute of Fundamental Sciences, Massey University, Private Bag 11 222, Palmerston North 4474, New Zealand

ARTICLE INFO

Article history:
Received 10 February 2017
Received in revised form 1 June 2017
Accepted 6 June 2017
Available online 7 June 2017

Chemical compound studied in this article:
Ferric chloride hexahydrate (PubChem CID: 24380)
Dipotassium hydrogen orthophosphate (PubChem CID: 24450)
Sodium azide (PubChem CID: 33557)
Sodium hydroxide (PubChem CID: 14798)
Hydrochloric acid (PubChem CID: 313)

Keywords:
Casein
Iron
Orthophosphate
Phosphoserine
Solubility
Adsorption

ABSTRACT

This study explored the interactions of orthophosphate with casein-iron precipitates. Casein-iron precipitates were formed by adding ferric chloride at ≥10 mM to sodium caseinate solutions ranging in concentration from 1 to 3% (w/v). The addition of different concentrations of orthophosphate solution to the casein-iron precipitates resulted in gradual adsorption of the orthophosphate, causing re-dispersion of the casein-iron complexes. The interactions of added orthophosphate with iron in the presence and absence of caseins are postulated, and new mechanisms are proposed. The re-dispersed soluble complexes of casein-iron-orthophosphate generated using this process could be used as novel iron fortificants.

1. Introduction

Although iron is the most abundant metal on earth, the deficiency of iron in the human diet is the largest nutritional disorder affecting world population. Iron is important for regular metabolic functions in the human body and its roles include oxygen transport, energy production and immunity (Carpenter & Mahoney, 1992). Fortification of foods with iron has been recommended as a long term strategy for reducing iron deficiency, but it is also recognised that iron is one of the most difficult micronutrients for food fortification (FAO, 1997). This is mainly because iron is a highly reactive metal that interacts with the food components, affecting the taste and colour of the fortified foods (Hegenauer, Saltman, Ludwig, Ripley, & Bajo, 1979). Soluble sources of iron, such as ferrous sulphate, are considered to be the most bioavailable but they are highly reactive within the food matrix, often resulting in protein precipitation and lipid oxidation. Insoluble sources of iron can be used as a source for food fortification due their lower deteriorative effects but these sources cannot be used for fortification of liquid food products, due to sedimentation issues. The chelated forms of iron, e.g. ferrous bis-glycinate, sodium feredetate, have been used for liquid food products, as the chelation of iron with other molecules reduces its interaction with the food components. However, the high cost of these ingredients and instability during processing prevent their use in everyday food products.

Casein proteins in milk are metal-chelating phosphoproteins, due to the presence of highly clustered phosphorylated serine residues. These naturally occurring phosphoproteins act as multidentate ligands that bind multivalent metals, such as calcium, copper, iron and magnesium. Iron can occur in a wide range of oxidation states (from −2 to +6), the most common being the

* Corresponding author at: Riddet Institute, Massey University, Private Bag 11 222, Palmerston North 4474, New Zealand.
E-mail addresses: v.a.mittal@massey.ac.nz (V.A. Mittal), a.ellis@massey.ac.nz (A. Ellis), a.m.ye@massey.ac.nz (A. Ye), p.j.edwards@massey.ac.nz (P.J.B. Edwards), h.singh@massey.ac.nz (H. Singh).

http://dx.doi.org/10.1016/j.foodchem.2017.06.035
0308-8146/

ferrous and ferric forms. In aqueous solution, ferric iron forms insoluble ferric hydroxide at pH > 3.5 (Stumm & Lee, 1960). Ferrous iron is easily oxidised to ferric form in the presence of oxygen commonly encountered in food processing operations (Emery, 1992).

It has been shown that iron in the ferric form will bind more strongly to caseins than if it were in the ferrous state (Raouche, Dobensque, Bot, Lagaude, & Marchessau, 2009). The binding of iron to caseins improves the solubility of ferric iron, but only to a certain extent. Above an equimolar concentration, iron precipitates along with the caseins (Mittal et al., 2016; Sugiarto, Ye, & Singh, 2009). Although the iron-casein complexes can be produced easily, the highest concentration of iron added to casein in a dispersible format is about 1% on w/w basis (Sher, Jacobson, & Vadehra, 2006). Iron-casein complexes containing higher concentration of iron (>1% w/w) have been made by freeze drying the casein precipitate generated upon iron addition (Zhang & Mahoney, 1989). However, these complexes are insoluble in water and hence could not be included in liquid food products. The precipitation of milk protein (especially caseins) at high levels of iron addition is a major drawback in the development of a commercially-viable casein-iron ingredient.

Our previous work has shown that the inclusion of orthophosphate in a mixture of sodium caseinate and iron improves the solubility of the protein when the iron addition concentration is greater than equimolar (Mittal et al., 2016). We showed that the dispersion stability of the casein-iron complex could be affected in numerous ways by the addition of orthophosphate. The interaction of orthophosphate with iron reduced the screening of negative charges on the caseins. The interaction also resulted in the formation of smaller aggregates.

It should be noted that iron has a strong affinity for phosphate and generally forms an insoluble precipitate when added to aqueous solutions containing orthophosphate (Crabb & Moore, 2010; Schwertmann & Cornell, 2000). The casein-iron complex is responsible for the solubility of the final product containing casein-iron-orthophosphate (Mittal, 2014; Mittal et al., 2016). Inorganic orthophosphate interacts with caseins in the presence of iron and the casein-iron complex could also potentially interact further with orthophosphate. This study extends our previous work and further examines the effect of orthophosphate addition on the dispersibility of iron-sodium caseinate complexes.

2. Materials and methods

2.1. Materials

Sodium caseinate (Fonterra Co-operative Group Ltd, New Zealand) was purchased from Davis Trading Co., Palmerston North, New Zealand. Analytical-reagent-grade ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and dipotassium hydrogen orthophosphate (K_2HPO_4) were obtained from Sigma-Aldrich, Sigma-Aldrich, St. Louis, MO, USA). All other chemicals used were analytical grade and were purchased from Merck, Sigma-Aldrich or BDH Chemicals. Standards for the analysis of iron and phosphorus contents were prepared using 1000 ± 4 mg/L standards purchased from Fluka Analytical, New Zealand.

2.2. Adsorption of orthophosphate onto ferric hydroxide

Ferric hydroxide solution was prepared by adding 15 mM iron as 0.5 M $FeCl_3$ to 96 mL of de-ionised water at ~5 °C. The pH of the solution was adjusted to 6.8 using 2 M NaOH and the solution was stirred for 30 min at ~5 °C. Inorganic phosphorus (as K_2HPO_4) was added to this solution at nine different concentrations, ranging from 0 to 2000 mg/kg, and the pH was re-adjusted to 6.8. The volume of all samples was then adjusted to 110 mL using Milli Q water. As ferric hydroxide is insoluble in water, the solutions were allowed to stir for 24 h at room temperature and were then centrifuged at 500*g* for 20 min at 20 °C. The supernatant was filtered through a 0.45 μm syringe filter (Sartorius Stedim, Goettingen, Germany). The adsorption of phosphorus was determined by the difference in the phosphorus contents of the sodium caseinate solution and the filtrate.

2.3. Preparation of protein and iron solutions

The iron solution (0.5 M $FeCl_3$) was added to a sodium caseinate solution (800 g) containing 0.025% sodium azide at ~5 °C, while maintaining the pH at ~6.8. This resulted in precipitation. The suspension was stirred for 30 min at ~5 °C and then divided into portions of 103 g each. Different amounts of orthophosphate (16–64 mM), as required for experiments, were dissolved in 5 mL of water and were added dropwise to vigorously stirred iron-added sodium caseinate mixture solutions. The pH of these solutions was re-adjusted to ~6.8 after phosphorus addition using 1.5 M HCl. The volumes of the samples were adjusted using Milli Q water to compensate for the differences in the amounts of HCl required for pH adjustment. The solutions were then stirred at room temperature for another 30 min and left overnight storage at room temperature. The samples were then centrifuged at 500*g* for 20 min at 20 °C to sediment large protein/iron aggregates. The permeate was obtained by centrifugal ultrafiltration of the supernatant using a 10,000 molecular weight cut-off (regenerated cellulose) membrane (Amicon Ultra-4, Millipore Corporation) at 2000*g* for 20 min.

2.4. Analysis of iron, phosphorus and protein

The iron content in the samples was analysed as described in a previous study (Mittal, Ellis, Ye, Das, & Singh, 2015). Briefly, single flame atomic absorption spectroscopy was used to analyse the iron content in the supernatant of the samples (GBC Scientific Equipment, Hampshire, IL, USA). Wet digestion was performed using concentrated sulphuric acid and nitric acid, as described by Nielsen (2010). All further dilutions (50 or 100 times) were done using Milli Q water.

The phosphorus content (original sample, supernatant and permeate) was determined using the molybdenum blue method (International Organisation for Standairisation, 2006).

The protein content was determined using the micro-Kjeldahl method.

2.5. Turbidity measurements

In order to determine the turbidity of the sodium caseinate samples, a single cell UV–visible spectrophotometer was used (Genesys 10 UV, Thermo Scientific). 3 mL samples were placed in the 1 cm path length of a disposable cuvette (4 mL) and the absorbance was measured at 650 nm. All samples were measured in duplicate. Milli Q water was taken as the reference standard.

3. Results and discussion

3.1. Interaction of orthophosphate with casein iron precipitates

The dispersion of sodium caseinate in water at up to 3% (w/v) protein resulted in transparent solutions with no visible precipitate. When ferric chloride was added to a sodium caseinate solution at a concentration of >5 mM, protein precipitation and sedimentation occurred as had been seen in previous studies