Interactions of Iron, Protein and Orthophosphate in Milk Systems

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With the blessings of my parents

I dedicate this thesis to my

Wife (Ketki) and Son (Aayush)

Abstract

Although iron deficiency is the largest nutritional disorder affecting the human population, few food products in daily use are fortified with adequate (25% of daily requirements per serving) concentrations of iron. The interaction of iron with food components and the consequential deteriorative effects on colour and taste thwart its use as a fortificant of choice. Milk proteins, especially caseins, are a class of metallo-protein which chelate iron and prevent its interaction with food matrix. However, addition of high levels of iron to protein solutions causes precipitation.

As iron and calcium bind to similar sites on the proteins in milk, the effect of calcium depletion on the iron-binding properties of the milk proteins was examined. A weakly acidic cation-exchange resin was used to remove three different levels i.e. 18–22, 50–55 and 68–72% of calcium from milk, designated as low, medium and high CDM respectively. The depletion of calcium from milk by ion exchange affected its physico-chemical properties, with the extent being highly dependent on the level of calcium depletion. The integrity of the casein micelles was retained at up to ~ 20% calcium depletion from normal milk, but there was substantial disintegration of the casein micelles at calcium depletion levels of > 50%.

Five levels of iron (5, 10, 15, 20 and 25 mM) were added to each of these calcium-depleted milks (CDM) and the resultant milks were analysed for particle size, microstructure and the distribution of protein and minerals between the colloidal and soluble phases. The properties of the milks with different calcium contents were variably affected by the addition of iron. In both normal milk and the low CDM, the majority (> 90%) of the added iron bound to caseins within the casein micelles,

minimally affecting the hydrodynamic diameter, ζ-potential and protein distribution. Protein solubility was adversely affected in the medium CDM, whereas most of the protein and the added iron were associated with the non-sedimentable phase in the high CDM. The high concentration (~ 20 mM) of ferric iron in the non-sedimentable phase of the high CDM (~ 70% calcium depletion) presented a distinct advantage of the calcium depletion process over traditional processes for the iron fortification of milk systems. However, a reduction in aqueous phosphorus, in proportion to added iron, was observed in all milk systems. The addition of orthophosphate prevented aggregation and promoted the formation of small fibrous structure particles in calcium-depleted milk by reducing inter-particle iron bridging. In contrast, added orthophosphate promoted the formation larger unstable aggregates in calcium-depleted milk upon calcium re-addition. The differing effects of added orthophosphate on the iron and calcium-induced aggregation of proteins in calcium-depleted milk were related to the binding characteristic of respective cations. The inclusion of iron in the calcium-restored milk generated substantially smaller aggregates, which were promoted by the addition of orthophosphate. The presence of iron probably blocked the polymerisation pathway required for casein micelle structure formation but not the interaction of calcium with caseins.

The effect of orthophosphate addition on the iron-induced aggregation of caseins in sodium caseinate was examined. The binding of iron at greater than equimolar concentration of organic phosphorus on caseins resulted in the precipitation of both the iron and caseins. In the presence of orthophosphate, however, higher concentrations of iron could be added to sodium caseinate solution (~ 6.9% by weight of casein) with little sedimentation of protein. The presence of

orthophosphate prevented iron-induced casein precipitation in sodium caseinate solutions and also improved the solubility of iron. The formation of small aggregates upon iron addition to sodium caseinate solution containing orthophosphate was responsible for the high solubility of casein-iron complexes. The ^{31}P-NMR study, SDS-PAGE and size exclusion chromatography analysis of the soluble casein-iron complexes revealed that a cluster of inorganic ferric phosphate was stabilised by caseins. The concentration of iron bound by caseins in the soluble form was at least 5 to 10 times higher than in previous studies, thus creating an opportunity to develop an iron ingredient for food fortification.

The effect of exchanging the sequence of adding iron and orthophosphate to sodium caseinate solutions was examined. Surprisingly, the casein-iron precipitates (formed upon > 5 mM iron addition to sodium caseinate solutions) were redispersed upon orthophosphate addition. The added orthophosphate adsorbed onto the casein-iron precipitate. This adsorption displaced a part of the organic phosphorus (contributed by caseins) bound to iron and increased the surface negative charge on the casein-iron precipitate, which probably led to the redispersion of caseins and iron. The adsorption of orthophosphate occured on ferric hydroxide formed in the absence of casein, but does not solubilise iron, suggesting that the presence of caseins was critical for redispersion of the precipitate. Precipitate with higher protein content could redisperse greater concentrations of iron, while requiring lower orthophosphate content. Optimally four moles of iron could be solubilised by one mole of casein in these experiments. The adsorption of orthophosphate onto protein-iron complexes and its consequent solubilisation is a novel finding with a potential to create a new iron fortificant.

Overall, this research highlights the important role that orthophosphate plays in the binding of iron to milk proteins. The soluble protein–iron complexes created in this work could be used to fortify liquid food products. Moreover, two processes involving entirely different mechanisms for the formation of soluble protein–iron complexes have been proposed, which has opened up a number of avenues for further research. The products and processes as an outcome of this research are protected by a patent, namely “Micronutrient fortification process and its usage”, issued by the New Zealand Patent Office on 24 June 2014.

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