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Influence of calcium depletion on iron-binding properties of milk

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ABSTRACT

We investigated the effects of calcium depletion on the binding of iron in milk. A weakly acidic cation-exchange resin was used to remove 3 different levels (18–22, 50–55, and 68–72%) of calcium from milk. Five levels of iron (5, 10, 15, 20, and 25 m*M*) were added to each of these calcium-depleted milks (CDM) and the resultant milks were analyzed for particle size, microstructure, and the distribution of protein and minerals between the colloidal and soluble phases. The depletion of calcium affected the distribution of protein and minerals in normal milk. Iron added to normal milk and low-CDM (~20% calcium depletion) bound mainly to the colloidal phase (material sedimented at 100,000 × *g* for 1 h at 20°C), with little effect on the integrity of the casein micelles. Depletion of ~70% of the calcium from milk resulted in almost complete disintegration of the casein micelles, as indicated by all the protein remaining in the soluble phase upon ultracentrifugation. Addition of up to ~20 m*M* iron to high CDM resulted in the formation of small fibrous structures that remained in the soluble phase of milk. It appeared that the iron bound to soluble (nonsedimentable) caseins in high-CDM. We observed a decrease in the aqueous phosphorus content of all milks upon iron addition, irrespective of their calcium content. We considered the interaction between aqueous phosphorus and added iron to be responsible for the high iron-binding capacity of the proteins in milk. The soluble protein-iron complexes formed in high-CDM (~70% calcium depletion) could be used as an effective iron fortificant for a range of food products because of their good solubility characteristics.
Key words: iron, casein micelle, calcium, milk, phosphorus

INTRODUCTION

Anemia is a global epidemic, with over 1.6 billion people being affected worldwide (McLean et al., 2009). Nutritional iron deficiency is considered to be a contributory factor in at least 50% of these cases. The fortification of food with iron is considered to be a long-term strategy for solving this problem. However, iron is also considered to be the most difficult micronutrient for food fortification, because of its reactivity with food components (Richard, 1999). The direct addition of iron salts into food products may result in rancidity because of lipid oxidation (Hurrell, 2002). Chelated forms of iron have thus emerged as a convenient choice for iron fortification, as these forms have reduced ability to interact with the food components. Early studies were aimed at fortifying milk with iron (Douglas et al., 1981), whereas subsequent work looked at the iron-binding ability of milk proteins (Demott and Dincer, 1976; Gaucheron et al., 1997; Hekmat and McMahon, 1998).

The iron-binding ability of milk proteins, especially caseins, has been recognized for over 50 yr. King et al. (1959) reported that the added iron bound mainly to the serum portion of the milk and later research demonstrated that it bound mainly to the caseins in skim milk (Carmichael et al., 1975; Raouche et al., 2009a). Caseins bind >90% of the iron added to skim milk (Demott and Dincer, 1976). The phosphorylated serine residues are the primary sites for iron binding and are frequently present in clusters of 2, 3, or 4 along the casein chain length. This clustering of the phosphoserine residues, aided by the rheomorphic character of caseins, is responsible for the high metal-binding capacity of caseins. Thus, caseins act as multidentate ligands for binding iron via coordination bonds with oxygen of the phosphorylated residues (Hegenauer et al., 1979c; Bernos et al., 1997). The binding of iron at low concentration (~1.5 m*M*) to the proteins in skim milk is not affected by pH (4.8–6.7) and ionic strength, which has been demonstrated by the successful fortification of products such as yogurt and cheese (Zhang and Mahoney, 1989; Hekmat and McMahon, 1998). The addition of iron to milk alters the distribution of minerals between the soluble and colloidal phases, with a prominent reduction in inorganic phosphorus in the soluble phase (Gaucheron et al., 1997; Raouche et al., 2009b).

At its normal pH, the calcium content of milk can be altered by exchanging the calcium ions with counterions on an ion-exchange resin. Ion exchange of multiva-

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lent cations (especially calcium) with monovalent ions (sodium, potassium) has been performed to improve the functionality and the stability of milk products (Lyman et al., 1933; Burgess, 1982; Bhaskar, 2010) and to manufacture novel products (Bhaskar, 2010). Up to 80% of the calcium in milk can be depleted using this technique (Ranjith et al., 1999). The depletion of calcium from milk induces changes to the structural and physicochemical properties of the milk (Burgess, 1982; Lin et al., 2006; Grimley et al., 2010), but the effects on the iron-binding properties are not known. The present work explores the effects of calcium depletion on the binding of iron to milk proteins. Furthermore, the distribution of minerals between the soluble and colloidal phases of milk is explained.

MATERIALS AND METHODS

Materials

Ultrapure class Type I water, as described by the International Organization for Standardization (ISO, 1987) with a resistivity of 18.2 MΩ·cm, was used for the rinsing of glassware, the dilution of reagents, and the preparation of reconstituted milk. Analytical reagent-grade sodium hydroxide pellets and ferric chloride hexahydrate (>99% pure) were purchased from Sigma Aldrich (Auckland, New Zealand). Individual mineral standards for the analysis of potassium, calcium, magnesium, phosphorus, and iron were prepared using 1,000 ± 4 mg/L standards purchased from Fluka Analytical (Auckland, New Zealand). Low-heat skim milk powder was purchased from Fonterra Co-operative Group Ltd. (Auckland, New Zealand).

Iron Fortification Process

Low-heat skim milk powder was reconstituted in deionized water (11% wt/wt) at 38 to 42°C for 2 h and was kept overnight at 5°C to ensure complete hydration of the protein. Sodium azide at 0.02% (wt/wt) was added to the milk as a preservative. An industrial-grade, weakly acidic cation-exchange resin in H^+ form (Amberlite IRC86, Fluka Analytical, Lyon, France) was converted to the K^+ form by contacting it with 3 bed volumes of 1 *M* potassium hydroxide for 45 min, until the wet volume of the resin increased by 80% as per the manufacturer's specification. The converted resin was washed with at least 20 bed volumes of deionized water until the pH of the wash water was ~7. A single batch of resin was converted and used for all experiments in our study. Milks with different levels of calcium depletion were obtained using a batch ion-exchange process (5–10°C). As a spectrophotometric analysis of calcium was not feasible during the ion-exchange process, the calcium depletion from normal milk was monitored by a complexometric titration method using Patton and Reeder's indicator (Patton and Reeder, 1956). The pH of all the calcium-depleted milks (**CDM**) was readjusted to pH 6.8 using 1 *M* sodium hydroxide solution, followed by volume adjustment using deionized water. The values for minerals reported herein represent their respective contents in the pH-readjusted samples. Iron addition to the CDM was performed after overnight chilled storage and adjustment of the pH to 6.8. Freshly prepared 0.5 *M* ferric chloride solution was added dropwise to the vigorously stirred milks (3–6°C), with the pH being maintained at ~6.8 using 1 *M* sodium hydroxide. The iron-fortified milks were stirred for 30 min at ~5°C and were stored overnight in a chiller (4–7°C).

Protein and Mineral Partitioning

All milks were partitioned into sedimentable and nonsedimentable (soluble) phases by ultracentrifugation at 100,000 × *g* for 1 h at 20°C. The supernatant was collected by inverting the ultracentrifuged tubes. Values for minerals in the nonsedimentable phase were corrected for the excluded volume effect, as proposed by Davies and White (1960). The permeate was obtained from the supernatant after ultrafiltration using 10,000 molecular weight cut-off (regenerated cellulose) membranes (Amicon Ultra, Millipore) at 3,000 × *g* for 30 min. The proportionate distribution of minerals was determined by analyzing the total content in the sample, the nonsedimentable phase, and the permeate. A constant correction factor of 0.96 was applied to the minerals estimated in the ultrafiltration permeate.

Cations (iron, calcium, magnesium, potassium, and sodium) were analyzed using a single flame atomic absorption spectrophotometer (GBC Scientific Equipment, Hampshire, IL) following wet digestion of the milks with concentrated sulfuric and nitric acids (Marshall, 2010). Typically, a 2-g sample was digested in a 125-mL Erlenmeyer conical flask and transferred with sufficient rinsing to a volumetric flask. The wet-digested samples were then diluted approximately 50 to 200 times in deionized water depending on the cation to be analyzed. All cations, except calcium, were analyzed using the air-acetylene flame. Calcium was analyzed using the oxidizing air-nitrous oxide flame; 5,000 mg/L of lanthanum trichloride was included as the releasing agent. Phosphorus was determined by the molybdenum blue method, as described by the International Standards Organization (ISO, 2006). The protein content was determined by the semi-Kjeldahl method using a factor of 6.38 for converting total nitrogen content to protein content.