**Calcium binding capacity of caseinglycomacropeptide in milk**

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ABSTRACT

Caseinglycomacropeptide (CMP) is the 64 C-terminal aminoacid residue of κ-casein formed by cleavage of this protein by chimosin during cheese manufacture and released into the whey. This peptide has proven beneficial biological and physiological properties and, actually, it is purified at a commercial scale. Previous studies demonstrated that the oral administration of CMP to mice significantly enhanced the content of calcium in femur. Also, other investigations showed that CMP bound calcium in aqueous solutions. Thus, it is hypothesized that if calcium is bound to the CMP in a food, its absorption at the intestinal level could increase. In this work, we explore whether the calcium binding capacity of CMP is maintained when the peptide is added to milk. It should be considered that in milk, calcium is in equilibrium between the micellar (or colloidal) and continuous (or serum) phases. In serum, it is mainly present in free form or associated with citrate and, to a lesser extent, with inorganic phosphate, chloride, and α-lactoalbumin. In the colloidal phase, calcium is present as colloidal calcium phosphate bound to casein micelles. Hence, this delicate mineral balance should be analyzed when adding the CMP, since these changes could significantly affect the quality and stability of milk. Milk samples were reconstituted to 10% w/w from low heat treatment and high quality skim milk powder. Then, CMP was dispersed in a concentration range between 0.4 and 0.8% w/w. The separation of micellar and serum phases was obtained by centrifugation of reconstituted milk samples at 20,000g for 2 h at 25 °C. The following parameters were determined in milk: pH, protein concentration by Bradford method, phosphorus using the standard molecular absorption spectrometry method, total calcium by absorption spectrometry, ionic calcium by calcium ion selective electrode and conductivity. The parameters determined in serum phase were protein concentration, phosphorus, and total calcium. Micellar calcium and phosphorus concentration were determined as the difference between the total and serum minerals. The conductivity of CMP aqueous solutions was measured and was subtracted from the corresponding conductivity of milk+CMP samples to obtain the effective conductivity. Total calcium concentration did not change in serum or micellar phases. Phosphorus concentration in milk increased with the addition of CMP and part of the added phosphorus migrated into the micellar phase. Effective conductivity and ionic calcium concentrations in milk decreased significantly with the addition of CMP. Averages values obtained for the different CMP concentrations (0, 0.4, 0.6 and 0.8% w/w) were 5.45±0.01, 4.93±0.07, 4.92±0.01 and 4.78±0.06 mS cm-1 for effective conductivity and 2.27±0.02, 1.87±0.07, 1.80±0.04 and 1.68±0.03 mmol L-1 for ionic calcium concentrations. Therefore, it could be inferred that CMP bound some of the ionic calcium naturally present in milk. The rest of the parameters analyzed were slightly modified. It is concluded that the addition of CMP to milk reduce the natural ionic calcium concentration without declining its physicochemical quality.

Keywords: milk, caseinglycomacropeptide, calcium binding capacity