**Study on zinc ions binding to the individual casein fractions: *αS1*-, *β*- and *κ-*casein**

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The colloidal behaviour of various caseins forms during the metal binding process is crucial for the further modulation of their stability during the dairy technological processes [1]. The reducing of the repulsive forces responsible for the micelles stability, it is possible to induce aggregation of protein. In the consequence, the texture and structure of dairy products are created [2], [3]. The destabilization of caseins is the common process used in e.g. making a cheese – the milk is coagulated by using chymosin in the presence of calcium and at acidic conditions [4]. Furthermore, the aggregation of caseins seems to be metal-dependent. Metal ions can act as both, promoters or inhibitors, of the protein aggregation process [5], [6]. In the case of caseins, the most discussed is the influence of calcium on this process [7], [8], [9]. Choi, Horne and Lucey [7] have demonstrated that removing of insoluble Ca2+ from casein micelles strongly influnce on rennet gelation properties. The lack of calcium ions have significantly reduced the repulsion forces between phosphoserine residues of caseins, and consequently, the weaker gels were formed. Dalgleish [2], have shown thte Ca2+ caused the aggregation of casein micelles by decreasing their zeta potential. The removal of Ca2+ have increased the repulsion forces as the phosphoserines are negatively charged and the calcium has been neutrailiising this. In the consequence, the gels were found to be weaker. Calcium ions preferably bind to oxygen-containing ligands such as e.g., carboxyl group, but also oxygens of asparagine (Asn), glutamine (Gln), or serine (Ser) [10]. Another important issue, crucial for casein-metal binding is the phosprylation occuring in their amino acid sequence and the ability of phosphorylated serine residues to bind calcium ions. The amount of Ca2+ is important in forming stable micelles [11], [12]. Also zinc might play an important role in casein stabilization through binding to the phosphoserines. Based on the literature, zinc are able to be bound to casein phopsphopeptides (CCP) containing the phosphoserine residues [13], [14], [15]. Singh, Flynn and Fox [14] have observed that *α*S1-casein aggregation was observed with the increasing Zn2+ concentration. Therefore, the aggregation of the all tested in this study casein fractions might be also zinc-dependent, as it follows from the experimental data we received in the present study. In contrast to calcium, Zn2+ ions are able to bind aditionally to sulfur and nitrogen [16]. On the other hand, both zinc and calcium, might exist in the aqua complexes forms, which might influence the stability of biocolloids. This process can described by the bridging effect – divalent aqua complexes screen the charges that make up the electrostatic forces, bind to negatively charged amino acids groups by displacing water molecules from the Stern-Helmholtz layer and, in the consequence, the system charge is increased [17], [18], [19], [20], [21]. As an exaple, in aqueous solutions, zinc forms stable aqua complexes [Zn(H2O)6]2+. Polarization of water molecules by the central zinc ion causes proton dissociation and results in formation of an aqua-hydroxo complex [Zn(OH)(H2O)5]+, which can be further transformed to [Zn(OH)4]2− [17], [16]. Finally, the formation of differently-charged species and binding to the protein might modulate the surface charge of metal-protein complexes. It is in a good correlation with results of Alhazmi et al [20] who have examined the zeta potential of bovine serum albumin after the divalent metal ion (Cu2+) exposure. They have observed that protein dispersion stability depends on the coordination capacity of the metal ions. What is more, metal-protein binding involves the formation of intermolecular bridges between the Me2+ and protein molecules. Another example can be work of Chakraborty and Basak [22] work, who have investigated the influence of the Zn2+ ions on the structure and stability of bovine caseins. At the highest concentration of the added metal, the aggregation and precipitation of protein occurred. Based on our results, due to the various availability of carboxyl groups on the individual caseins surface, the binding of zinc also occur in different manner. It is strongly connected with the generating of stable Zn*αS1*CN complexes. It has a great reflection in the experimental FTIR-ATR analysis results, which was the crucial step in the proper understanding of the described phenomenom.

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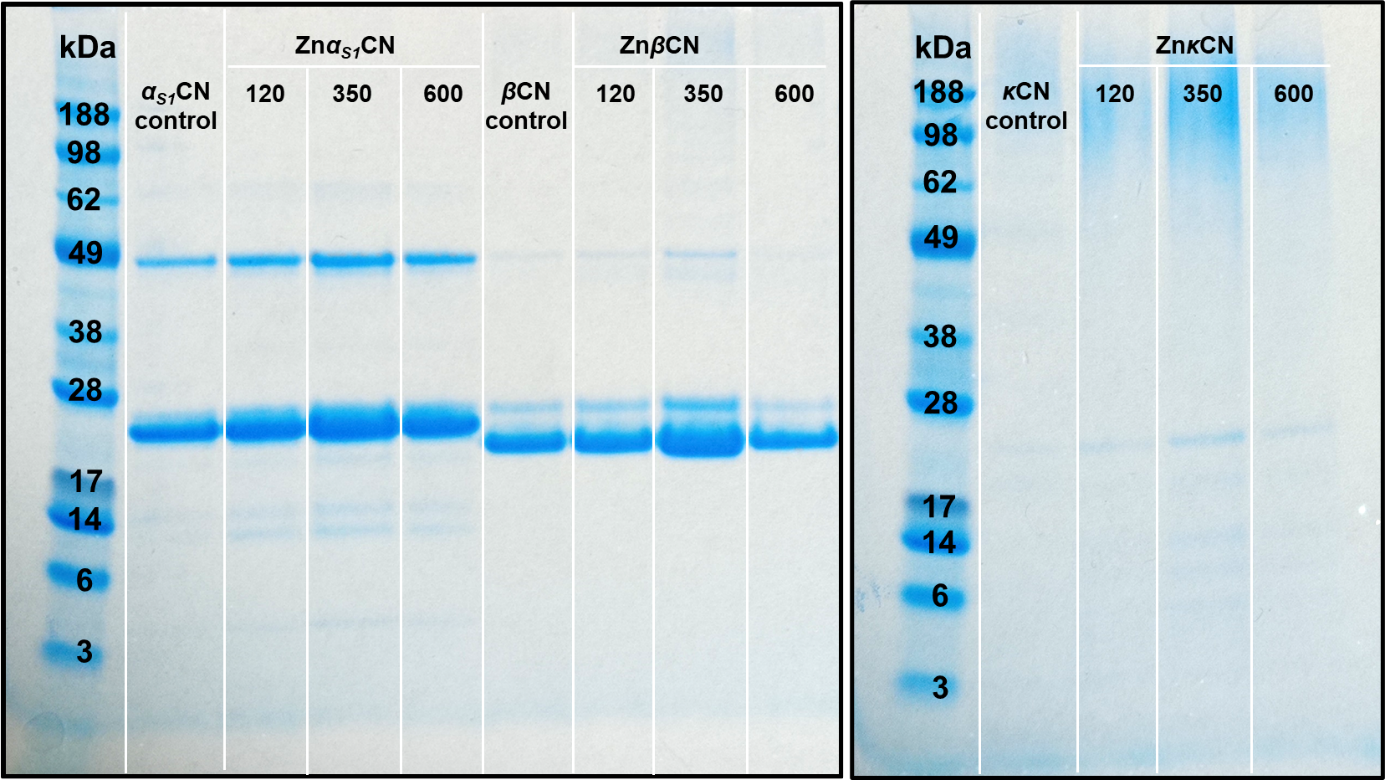
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**Fig. S1.** Electrophoretic separation of casein fractions and their complexes with zinc ions.