Food Chemistry 135 (2012) 770–774

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Zinc incorporation capacity of whey protein nanoparticles prepared with desolvation with ethanol

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ARTICLE INFO

Article history:
Received 30 November 2011
Received in revised form 9 April 2012
Accepted 30 April 2012
Available online 11 May 2012

Keywords:
Whey protein nanoparticles
Zinc incorporation
Desolvation
Ethanol
Particle size analysis
Ion chromatography
Storage stability

ABSTRACT

Whey protein isolate (WPI) nanoparticles were prepared using ethanol desolvation, and their capacity to incorporate $ZnCl_2$ was analysed. Desolvation was carried out at pH 9 and the volume of added ethanol was 0–3 times the volume of protein solution. The desolvated solutions were dispersed in acidified water (pH 3) immediately after desolvation. The size of the WPI nanoparticles increased with the volume ratio of ethanol:water used, as well as with the amount of $ZnCl_2$. The nanoparticles showed high incorporation efficiencies, and remained stable after 30 days of storage at 22 °C. The amount of zinc incorporated in the WPI particle suspensions was within the range of daily zinc requirements for healthy adults.

1. Introduction

Zinc is a trace mineral essential for human growth and development. According to Maret and Sandstead (2006), the required daily intake of zinc is around 2–3 mg for healthy adults. This value is based on the relatively low amounts of zinc that are contained in and lost from the body and the high half-life of this ion. Zinc deficiency might be as high as 40% of the whole population, worldwide, possibly due to the low bioavailability and insufficient consumption of foods that contain zinc. Zinc deficiency is linked to the incidence or severity of impaired cognitive function, persistent diarrhoea, lower respiratory infections, malaria in childhood; and low birth weight (Gibson & Ferguson, 1998). Mild zinc deficiencies are likely to be quite widespread in certain groups of the population that might be more vulnerable than other groups, such as infants and young children, pregnant and lactating women, and individuals chronically on low zinc intakes or diets with poor zinc availability (Krebs, 2000).

Due to the difficulties in implementing a supplementation programme, especially in developing countries (Salgueiro et al., 2002) and the necessity of daily administration of zinc supplements (Gibson & Ferguson, 1998), increasing the zinc content in certain food products could be an alternative means to resolve the low intake of zinc, since excess amounts (i.e., supplementation) are excreted by the body to regulate zinc homeostasis (Krebs, 2000). It must also be stressed that excess zinc intake might have adverse effects, although exposure to excess zinc from foods is quite unusual and unlikely (Salgueiro et al., 2002). Fortification of food or water is effective since the coverage upon the fortification of staple foods can be very high. This facilitates the consumption of target nutrients within the regular diet (Salgueiro et al., 2002). Therefore, nutritional intervention can be effective in lowering the prevalence of zinc deficiency.

In most cases, zinc fortification of foods is characterised by changes in sensory attributes (flavour) or precipitation (Salgueiro et al., 2002). There are various compounds that can be used in fortifying foods, and their solubility and stability at gastric pH affect their bioavailability. For example, zinc oxide is inexpensive and does not cause adverse sensory perception, but due to its insolubility at gastric pH, its bioavailability is low (Gibson & Ferguson, 1998). On the other hand, more soluble salts tend to impart negative sensory attributes. It might be possible to use encapsulated zinc compounds in order to prevent the sensory defects and increase bioavailability. Therefore, the development of delivery tools for food products that can encapsulate and release zinc in a controlled manner would be desirable.

Metal ions can bind to functional groups of proteins, such as imidazole, sulphydryl, carboxyl, amino and peptide groups (Ocak, 2010). The present work explores the use of whey proteins as

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http://dx.doi.org/10.1016/j.foodchem.2012.04.146

appropriate encapsulants for zinc. Shi, Zhou, and Gunasekaran (2008) prepared nanocomposites of zinc oxide and denatured whey protein isolate (WPI). Zinc oxide was added to the WPI solution at pH 8, and the mixture was stirred for 24 h at 40 °C. It was reported that, under these conditions, the complexes formed increased the bioavailability of zinc oxide in the intestine, due to the protection provided by protein aggregates during the gastric stage.

The objective of this work was to evaluate the capacity of WPI nanoparticles to encapsulate zinc ions. Ko and Gunasekaran (2006) prepared β-lactoglobulin nanoparticles using a desolvation technique with acetone and glutaraldehyde cross-linking. Recent studies have shown that WPI nanoparticles can also be obtained using desolvation and that, using ethanol, the particles obtained are stable at acidic pH (pH 3) (Gülseren, Fang, & Corredig, 2012a). It was hypothesised that, the desolvation method may increase the capabilities of WPI to incorporate minerals. For this reason, the formation of WPI nanoparticles containing $ZnCl_2$ was explored, and the encapsulation efficiency and stability of these nanoparticles evaluated over time. The present results may suggest novel means to incorporate minerals in food products; for example, they may enable the manufacture of acidic dairy drinks containing nutritionally significant amounts of minerals.

2. Materials and methods

2.1. Sample preparation

A whey protein isolate (WPI) (Power Pro, Land O'Lakes Dairy Proteins Group, St. Paul, MN, USA) solution was prepared in MilliQ water. The protein sample was used without further purification. Sodium azide was added to all protein solutions as a bacteriostatic (0.02%) (Fisher Scientific, Mississagua, ON, Canada). The pH of the solution was adjusted to pH 9 using concentrated NaOH (2 M), and it was kept refrigerated overnight. Protein solutions were filtered through 0.45 μm PVDF syringe filters (Millipore, Millex HV, Fisher Sci.) and mixed with zinc stock solutions (0.25 M $ZnCl_2$) (⩾99.995%, Sigma–Aldrich, St. Louis, MO, USA, Catalogue No.: 429430) to final WPI and $ZnCl_2$ concentrations of 5% and 1–10 mM, respectively. The pH was then rapidly adjusted to 9 and the solution was stirred for 60 min. Soon afterwards, the mixtures (2 ml) were desolvated using ethanol, at a rate of addition of ethanol of 1 ml min^{-1}. The final volume ratios of ethanol:water ranged between 0 and 3 times the volume of protein solution, and will be indicated as 0–3× EtOH, throughout the work.

The desolvated stocks were immediately diluted 1:100 in water, and acidified with HCl to pH 3. HCl was used to avoid any contamination or interference of the acid during mineral analysis. The ethanol concentrations in the diluted suspensions were roughly 0.5% and 0.75% for 1× and 3× EtOH desolvated stocks, respectively.

2.2. Particle size analysis

Immediately after dilution, particle size analysis was carried out without any further dilution. In addition, changes in particle size were also measured as a function of time during storage at 22 °C. The particle size distribution of the diluted WPI nanoparticle suspensions (1:100, pH 3) that were prepared with varying amounts of $ZnCl_2$ (1–10 mM) were determined using dynamic light scattering (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). All the particle size distributions reported here were determined on the basis of volume frequency.

2.3. Encapsulation efficiency

The WPI suspensions, after dilution at pH 3, were centrifuged at 125,000*g* for 90 min at 25 °C (Beckman Coulter LE-80K ultracentrifuge with a 45 Ti rotor, Beckman Coulter Inc., Mississagua, ON, Canada). The supernatants were carefully decanted and filtered through 0.22 μm syringe filters (Millipore, Fisher Sci.). The filtered supernatants were used in the zinc analysis. The encapsulated zinc amounts were calculated as the difference between the total zinc added and the zinc recovered in the filtered supernatants. Control solutions of $ZnCl_2$, centrifuged under the same conditions, showed a loss of approximately 2.4% and no detectable losses of $ZnCl_2$ in the 10 and 5 mM solutions, respectively. The amount of residual soluble protein after nanoparticle preparation was also measured by centrifuging at 125,000*g* as above. Protein concentration was determined using a Bradford protein assay kit (Biorad, Mississagua, ON, Canada).

To determine the extent of binding in a control, non-desolvated WPI solution containing 5 or 10 mM zinc ions, after dilution of the solution 1:100, pH 3, the free zinc ions were collected using a centrifugal ultrafiltration method provided with 10 kDa filters (2 h at 5000*g*, Avanti J-E centrifuge, with a JA-25.50 rotor, Beckman Coulter, Inc.).

2.4. Zinc analysis by ion chromatography

The amount of zinc ion was analysed using ion chromatography (Rahimi-Yazdi, Ferrer, & Corredig, 2010) (861 advanced compact IC, Metrohm ion analysis, Metrohm Ltd., Herisau, Switzerland). The assembly was controlled with a sample processor (advanced 838, IC Net 2.3, Metrohm Ltd.). The column (Metrosep C2 150/40, 7 μm silica, Metrohom Ltd.) was kept at 30 °C at all times during equilibration and analysis.

The sample preparation was carried out in line with a dialysis unit (833 IC liquid handling dialysis unit, Metrohm Ltd.). The dialysis membrane consisted of a 0.2 μm cellulose acetate membrane (Fisher Sci.) and the membrane was replaced every run. The samples and the zinc standards were placed in polypropylene sample vials. Before sample injection, at least two blank and six standard solutions with different concentrations of zinc (concentrations from 0–10 mg/l) were loaded. Zinc standard was purchased from Fluka (Catalogue No.: 02584) (10 g/l of Zn^{2+} in approximately 0.5 M HNO_3) and was used as certified reference material to prepare external standard solutions.

The samples and acceptor were transferred by the peristaltic pump to the dialysis unit at a flow rate of 1.27 ml/min in 5.5 min using 2 mM nitric acid as carrier. After dialysis, the cations were transferred to the stationary acceptor solution and then injected into the column. Acceptor solution was 2 mM nitric acid solution. Mineral ions were transferred from the sample (i.e., donor) solution to the acceptor solution during the dialysis procedure. This technique allowed a complete recovery of the cations and exclusion of other particles and impurities. The system was calibrated using the external zinc standards. The samples were injected in a 20 μl sample loop for analysis. Before running, the separation column was pre-equilibrated with the eluent for at least 60 min. The elution buffer was 2.5 mM oxalic acid delivered at a flow rate of 0.9 ml min^{-1}. The operating pressure was <8.5 MPa (generally 8.3 MPa) at all times.

2.5. Storage stability

Aliquots (250 ml) of the diluted WPI suspensions (1:100, pH 3) were stored at room temperature (22 °C) for 30 days to evaluate the changes in particle size and zinc release. For zinc analysis, the suspensions were centrifuged and filtered as described above.

2.6. Statistical analysis

All the measurements throughout the study were carried out at least in triplicates. The mean values and standard deviations are reported.