Food Research International 66 (2014) 344–355

Contents lists available at ScienceDirect

Food Research International

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

Review

Physical effects upon whey protein aggregation for nano-coating production

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A R T I C L E I N F O

Article history:
Received 5 July 2014
Accepted 26 September 2014
Available online 12 October 2014

Keywords:
Whey proteins
Denaturation
Aggregation
Nanostructures
Molecular interaction
Moderate electric fields

A B S T R A C T

Production of edible nanostructures constitutes a major challenge in food nanotechnology, and has attracted a great deal of interest from several research fields — including (but not limited to) food packaging. Furthermore, whey proteins are increasingly used as nutritional and functional ingredients owing to their important biological, physical and chemical functionalities. Besides their technological and functional characteristics, whey proteins are generally recognized as safe (GRAS). Denaturation and aggregation kinetics behavior of such proteins are of particular relevance toward manufacture of novel nanostructures possessing a number of potential uses. When these processes are properly engineered and controlled, whey proteins may form nanostructures useful as carriers of bioactive compounds (e.g. antimicrobials, antioxidants and nutraceuticals). This review discusses the latest advances in nano-scale phenomena involved in protein thermal aggregation aiming at formation of bio-based nano-coating networks. The extent of aggregation is dependent upon a balance between molecular interactions and environmental factors; therefore, the impact of these conditions is addressed in a critical manner. A particular emphasis is given to the effect of temperature as long as being one of the most critical variables. The application of moderate electric fields (MEF), an emergent approach, as such or combined with conventional heating is considered as it may inhibit/prevent excessive denaturation and aggregation of whey proteins — thus opening new perspectives for development of innovative protein nanostructures (i.e. nano-coatings). A better understanding of the mechanism(s) involved in whey protein denaturation and aggregation is crucial as it conveys information relevant to select methods for manipulating interactions between molecules, and thus control their functional properties in tailor-made applications in the food industry.

Contents

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http://dx.doi.org/10.1016/j.foodres.2014.09.036

1. Introduction

Nanotechnology is a fast emerging field involving design and application of structures or materials where shape and size, at the nanometer scale, are critically controlled (Bouwmeester et al., 2009; Chau, Wu, & Yen, 2007). The properties of materials at this level can be quite different from conventional-sized materials of the same compound. This behavior arises from the large surface area-to-volume ratio typically found in nano-materials, but also to the influence of physical and chemical interactions between materials at the nano-scale that play a significant role on the overall properties of those systems (Kaya-Celiker & Mallikarjunan, 2012). These factors may enhance properties, such as strength and reactivity, thus providing different or new functionalities to existing products: enable dispersion of water-insoluble additives (e.g. carotenoids, phytosterols, fatty acids and natural antioxidants), increase stability, allow specific delivery and controlled release of bioactive compounds, and improve adhesion to and absorption rates through cells (Chaudhry, Watkins, & Castle, 2010; Chen, Remondetto, & Subirade, 2006). Consequently, assessment of physical and chemical properties is relevant to anticipate possible associated hazards (Bouwmeester et al., 2009).

Application of nanotechnology in the food industry has been receiving increasing attention from the scientific community, and has mainly focused on development of nano-sized ingredients, supplements and additives, as well as nanostructures as carriers of bioactive compounds (e.g. antimicrobials, antioxidants and nutraceuticals) or for incorporation in food packaging (to improve their barrier and mechanical properties) (Chaudhry et al., 2008; Kane & Stroock, 2007; Weiss, Takhistov, & McClements, 2006).

Nano-structured systems (e.g. nanoliposomes, nanoemulsions, nanohydrogels, nanotubes, nanofibrils and nano-coatings) are usually characterized according to main material used in manufacture (e.g. polysaccharides, proteins and lipids); production method (e.g. bottom-up or top-down); predominant bonds involved (e.g. covalent or non-covalent); main system properties (e.g. mechanical and optical properties); and associated overall free energy (thermodynamically versus kinetically stable systems) (Acosta, 2009; Gutierrez et al., 2008; Lesmes & McClements, 2009).

Two different building strategies have been used for production of nano-structured systems: 1) “top down” approach, in which nano-level structures are generated by breaking up bulk materials via milling, nanolithography or precision engineering; and 2) “bottom up” approach, allowing nanostructures to be built from individual atoms or molecules that are capable of self-assembling (Hartgerink, Granja, Milligan, & Ghadiri, 1996; Moraru et al., 2003; Reches & Gazit, 2003).

Many synthetic polymers (e.g. polyacrylamide, polyamides, polyphenylesters and polyurethanes) have been successfully used as delivery systems in the biomedical and pharmaceutical areas (Reis, Neufeld, Ribeiro, & Veiga, 2006). However, these polymers cannot be utilized in food applications that require GRAS (generally recognized as safe) ingredients. Therefore, a major challenge in this area is replacement of non-food-grade materials by bio-based alternatives.

Food biomaterials, specifically polysaccharides (e.g. alginate, carrageenan, pectin, dextran and chitosan), proteins (e.g. zein and whey proteins) and lipids (e.g. medium chain triglycerides, tristearin and corn oil) are reasonable possibilities to address that challenge, since they are biodegradable, food-grade and non-toxic, while allowing also novel functionalities and applications (Acosta, 2009; Morris, 2010; Subirade & Chen, 2008). However, the use of these polymers has problems associated with their performance and processing, besides cost that are common to most biodegradable food-grade polymers (Garcia, Forbe, & Gonzalez, 2010). The application of nanotechnology to these polymers may open up new possibilities to improve not only some of their physical limitations, but also their cost-price-efficiency.

Whey protein-based ingredients are widely used in formulated foods because they are by-products from the cheese industry produced to large extents, relatively inexpensive, classified as GRAS materials and having a high nutritional value. Various articles highlight their biological (e.g. digestibility, amino acid profile, high biological value and sensory characteristics) and functional (e.g. emulsification, gelation, foaming and water binding capacity) properties, as well as their application as ingredients in food formulations (Bryant & McClements, 1998; Clark & Ross-Murphy, 1987; Dickinson, 2003; Madureira, Pereira, Gomes, Pintado, & Xavier Malcata, 2007; Walstra, 2003). Among said functional properties, gelation is particularly interesting. Gels of diverse mechanical and microstructural properties can be formed by controlling assembly of protein molecular chains, thus offering the possibility to developing GRAS biocompatible carriers for oral administration of sensitive nutraceuticals in a wide variety of foods.

Nanostructured systems based on whey proteins (e.g. nano-coatings) are interesting because, in addition to their gelling ability, they can be easily prepared, and their size distribution can be effectively monitored. These proteins have also the ability to conjugate nutrients via either primary amino groups, or ionic and hydrophobic binding (Chen et al., 2006). Moreover, several changes can be induced in the whey protein matrix that allow formation of complexes with other biopolymers, chiefly polysaccharides, as starting point for several nanosystems.

Coatings are thin layers of edible material (e.g. whey proteins) directly applied on food aimed at improving surface properties (e.g. appearance, adhesion and wear resistance), while playing an important role upon the preservation of physicochemical and nutritional features of food products, and associated shelf life. A nano-coating is produced through a controlled process at the nano-level, and may significantly enhance the ability of a coating to improve surface properties or even allow new functionalities.

This review will give a crucial insight into the main factors (i.e. temperature, pH, ionic strength, protein concentration and presence of an external electric field) affecting denaturation and aggregation of whey proteins, and therefore into the molecular interactions involved in formation and stabilization of the nano-coatings formed thereof. Understanding of these mechanisms and their mutual relationships is fundamental to control and design structures with intended functionalities. The advantages of application of new approaches, such as moderate electrical fields to control the extent of those processes (denaturation and aggregation), and therefore the size of nanostructures are also addressed. Moreover, several applications of nano-coatings in the food industry are discussed.

2. Whey protein systems

The actual and potential use of milk proteins as food ingredients has been a popular topic of research over the past 40 years. Milk and dairy products have numerous advantages over competitors when used as ingredients: they are colorless, have a bland taste, are rather stable to processing and are essentially free of toxins. As ingredients, dairy products are used mainly because of their unique physicochemical properties (Chobert, 2012). Milk is constituted by two major groups of proteins: caseins that are insoluble, remaining stable as a micellar phase in milk, and whey proteins that are soluble. The casein micelles consist of subunits of the different caseins (i.e. α-s1, α-s2 and ß) held together by calcium phosphate bridges on the inside, surrounded by a layer of 6 casein molecules which helps to stabilize the micelle in solution. Micelles are spherical and have 0.04 to 0.3 μm in diameter, which are much smaller than fat globules (ca. 1 μm in homogenized milk). The casein micelles are porous (allowing the water phase to move freely in and out of the micelle) and stable structures, yet dynamic (i.e. they do not settle out of solution). They can be heated to boiling or cooled, and dried and reconstituted without adverse effects (Holt, Carver, Ecroyd, & Thorn, 2013). Caseins represent 80% (w/w) of all milk proteins and can easily be recovered from skim milk through isoelectric precipitation or rennet-driven coagulation. Both techniques release whey as by-