ELSEVIER

Trends in Food Science & Technology 45 (2015) 50–59

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Formation mechanisms, handling and digestibility of food protein nanofibrils

Saina Moayedzadeh[a], Ashkan Madadlou[b,c,d,*] and Asghar Khosrowshahi asl[a]

[a]Department of Food Science and Engineering, College of Agriculture, Urmia University, Urmia, Iran

[b]Department of Food Science and Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[c]Center of Excellence for Application of Modern Technologies for Producing Functional Foods and Drinks (FFDCE), University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[d]Interdisciplinary Research Department of Agricultural and Natural Resources Nanotechnology (IRDANN), University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

(Department of Food Science and Engineering, University College of Agriculture and Natural Resources, University of Tehran, P. O. Box: 31587 77871, Karaj, Iran. Tel.: +98 26 32248804; fax: +98 26 32249453; e-mail: a.madadlou@ut.ac.ir)

Background: Globular proteins including whey proteins, soy proteins and egg white proteins self-assemble into fibrillar structures with several nanometers thickness and several micrometers length by prolonged heating at very acidic conditions. These *in vitro* synthesized fibrils resemble amyloids associated with various neurodegenerative diseases and hence have received special attention by bioscientists. The synthesized fibrils are however of widespread potential for food and colloid sciences applications. Characteristics such as aspect ratio, and persistence and contour lengths of the artificially-synthesized fibrils could be elaborated by appropriate selection and employment of parameters of fibrils formation process.

Scope and approach: The fibrillation process of whey proteins, proposed mechanisms and potent inhibitors of the process, as well as, alternative enzyme-based routes of fibrillation are overviewed in the present article. Then post-formation treatment and applications of globular proteins fibrils and their gastric digestibility behavior are briefly referred followed by representing some future trends in this field.

Key findings and conclusions: The building units of protein fibrils are hydrolysis-generated polypeptides rather than parent intact protein monomers. It was hypothesized that proteins fibrillation is an oxidation-triggered process and may be inhibited by antioxidant agents that suppress the generation of reactive oxygen species. Whey protein-originated fibrils may be exploited in formation of heat-resistant protein-stabilized emulsions and nanoemulsions. However, more effort is required to characterize the interfacial behavior of fibrils in comparison with native and heat-denatured whey proteins. Gastric and intestinal digestion fate of protein nanofibrils is also a hot topic for upcoming research studies.

Keywords: Protein nanofibril; Whey proteins; Amyloid; Mechanism; Reactive oxygen species; Digestibility

Introduction

Heat denaturation of proteins can form a wide variety of structures such as fibrils, flexible strands, branched structures and random aggregate (Bolder, Hendrickx, Sagis, & van der Linden, 2006) depending on the pH value, salt type and concentration, heating conditions and protein concentration (Nicolai, Britten, & Schmitt, 2011), among which, the greatest impact belongs to heat (Pearce, Mackintosh, & Gerrard, 2007). The pH value of protein solution is also of profound influence on the organizational arrangement of super-structures. The architecture and life span of these structures are determined by balance between attractive hydrophobic and repulsive electrostatic interactions. At low ionic strengths and at pH values far from the iso-electric point (pI), electrostatic repulsive forces overcome the attractive interactions resulting in formation

* Corresponding author.

http://dx.doi.org/10.1016/j.tifs.2015.05.005

of fibrillar structures with high aspect ratio (length versus diameter); whilst, at pH values close to pI, spherical microgels (with sub-micron diameters) and random aggregates are formed (Fig. 1) (Bolder, 2007; van der Linden, 2006; van der Linden & Venema, 2007). The stimulant-induced self-assembly of globular food proteins to fibrillar structures has received considerable attention owing to the ability of the fabricated structures to display novel profitable characteristics (van der Linden, 2006). In the assembly process, protein monomers are organized into giant supramolecules in which non-covalent interactions such as hydrogen bonds, and electrostatic, hydrophobic and van der Waals interactions hold the building units together (Bolder, 2007). Fibrils formation may involve covalent linkages (disulfide bridges) in addition to non-covalent interactions depending on pH. Heat treatment at pH 3.35 assembled fibrils not only by hydrogen bonds and hydrophobic interactions but also by limited number of disulfide bridges; whilst, by heating at pH 2.0 merely non-covalent hydrophobic interactions contributed in fibrils formation (Mudgal, Daubert, Clare, & Foegeding, 2010).

The essential characteristics of fibrils

Several types of food proteins such as milk proteins, soy proteins and egg white proteins can assemble *in vitro* at appropriate condition into fibrillar structures with micrometer length and nanometer thickness (Bolder, 2007; van der Linden, 2006). These objects resemble amyloid fibrils that are associated with neurodegenerative diseases like Alzheimer's, Parkinson's, Huntington's and Creutzfeldt–Jakob diseases. Accordingly they are often called "amyloid-like" (Jones & Mezzenga, 2012). The amyloid-like nanofibrils are of scientific interest to investigate their role and/or application in diverse areas such as biomedicine that the inhibition of nanofibrillation is desired, as well as in molecular biology, material science, and food science. In the latter scope, fibrillation is deliberately induced for obtaining purposive functionalities (Zhao, Pan, & Lu, 2008).

The properties of the fibrils depend on length distribution and their aspect ratio. For example, a high aspect ratio may lead to an entangled network at much lower protein concentrations (van der Linden & Venema, 2013). These features propose a variety of applications for protein nanofibrils in food industry including utilization as efficient thickeners, gelators, foaming agents, stabilizers of foams and emulsions, and enzyme immobilization matrices, as well as, for encapsulation purposes and fabrication of biosensors (Nicolai *et al.*, 2011). It is important to control and elaborate the length distribution of nanofibrils. Two length scales are used to characterize fibrils morphology: persistence length (Lp) and contour length (Lc). The former exhibits fibril flexibility, while the latter is fibril's length at maximum extension. When $L_p << L_c$, fibril is flexible; whilst, when $L_p >> L_c$, fibril is rigid. When L_p and L_c have comparable magnitudes fibrils are regarded semi-flexible (Loveday, Rao, Creamer, & Singh, 2009). Mudgal, Daubert, and Foegeding (2009) randomly selected four to five β-lactoglobulin fibrils from different quadrants of transmission electron microscopy (TEM) images and digitized the fibrils along their contours into small segments. Summation of the length of all segments was considered as L_c and the bond angles correlation along the contour was employed for L_p calculation. They found that heating of β-lactoglobulin solution at pH 3.35 yielded more flexible fibrils compared to pH 2.0 which was evidenced with shortened persistence length from 788 nm (at pH 2.0) to 35 nm (at pH 3.35).

vandenAkker, Engel, Velikov, Bonn, and Koenderink (2011) intended to link the mechanical properties of fibrils to their molecular structures, in order to gain appropriate understanding of fibrils assembly process. At low protein concentration, straight fibrils with high persistence length and well-developed β-sheet structures formed while at high protein concentration flexible fibrils with shorter persistence length and high α-helix content generated. The persistence length of fibrils varied from about 3818 nm at 3% β-lactoglobulin concentration to 92 nm at 7.5% β-lactoglobulin concentration. The much longer persistence length of straight fibrils originated from a network by hydrogen linkages in the linear backbone of β-sheet-rich fibrils. Accurate measurement of length distribution of nanofibrils is crucial for fundamental understanding and controlling of the fibrillation process in order to develop efficient therapies against amyloid-contributed diseases. It would also make possible to determine the mechanical characteristics and biological impacts of amyloid-like fibrils generated artificially as potential nano-materials. The Weibull distribution model applied for describing the length of human β_2-microglobulin linear fibrils that had been imaged by a tapping-mode atomic force microscope revealed that fibril fragmentation, a mechanism that shortens fibrils is of significant influence on prions replication and phenotype (Xue, Homans, & Radford, 2009).

It is essential to have an accurate understanding of the formation process and properties of the fibrils and to have ability to control these properties and consequently the functionality of the fibrils for successful application in various products. Herein, the assembly behavior of globular food proteins specially β-lactoglobulin and the properties of the generated fibrils are comprehensively reviewed.

Fibrillation process

Process overview

Beta-lactoglobulin is a globular whey protein with molecular mass of 18400 Da and radius of ~2 nm (Bolder, Vasbinder, Sagis, & van der Linden, 2007). It exists in three genetic variants A, B and C which differ from each other at the subsititutions in their amino acid sequences. The variants A and B contain Gln at residue 59 while variant C contains His. As well, variant A contains respectively Asp and