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Review

# Functional properties of peptides: From single peptide solutions to a mixture of peptides in food products

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ABSTRACT

This review presents the advances made concerning the ability of peptides to bestow particular functional properties on various matrices including foods. We focus on systems ranging from model solutions in which peptides are rationally designed to orient their structure and to form hydrogels to mixtures of peptides in complex food matrices. In the latter case, peptides are an integral part of food formulations due to their production *in situ* or their addition as an ingredient. Examples of complex matrices such as food products, where mixes of peptides are present as hydrolysates with various physico-chemical properties, focus on the ability of peptides to modulate the texture of foods and their functional properties, including solubility, gelation and even emulsifying and foaming properties. Attempts have been made to establish relationships between the physico-chemical and structural characteristics of peptides and their functional properties.



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## 1. Introduction

Food products have complex structures and variable compositions of macromolecules, including proteins, lipids and carbohydrates, as well as their derived products such as peptides, fatty acids, etc., that change throughout manufacturing and storage. Proteins and peptides largely contribute to the final texture,

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organoleptic properties and health-promoting activities of foods. Peptides can enhance emulsion or foam formation and stabilize them by preventing coalescence or flocculation. They can improve the solubility of proteins, increase the viscosity of a solution, and make gels under appropriate physico-chemical conditions (Caessens, De Jongh, Norde, & Gruppen, 1999; Kilara & Panyam, 2003; Saito, Ogasawara, Chikuni, & Shimizu, 1995). They are also an inherent part of the nutritional value of food through their contribution to the reduction of allergenicity and their various bioactivities, as shown in recent reviews (García, Puchalska, Esteve, & Marina, 2013; Moayedzadeh, Madadlou, & Khosrowshahi asl, 2015; Nongonierma & FitzGerald, 2015).

Due to their intrinsic physical and chemical characteristics, i.e., sequence, size, charge, structure and their interaction with each other and other molecules, peptides are able to form self-assemblies or aggregates and to thus develop new functional properties. Recent advances in this field show the ability of these peptides to form hydrogels, nanotubes and fibers under defined conditions. In these systems, the peptide sequence is tailored to satisfy specific needs (Boyle et al., 2012), the types of interactions involved, the supramolecular structures formed as well as the properties of the solutions or gels, are highly controlled. The aggregation capacity of proteins and peptides may be either enhanced or impaired when they are present in a heterogeneous and highly concentrated medium such as that of food products, in the presence of other molecules (polysaccharides, lipids and other proteins). The concept of molecule confinement, referred to as crowding, has recently been developed in order to understand the modulation of the interactions between peptides and proteins in high concentrations such as those encountered in cell cytosol, which can reach 300–400 g/l, and that can be found in food products.

It clearly appears that such new advances will help us address the question in this review of the extent to which peptides can be an important part of the final functional properties of food products and how this occurs. In this paper, we emphasize: (i) the progress that has been made and existing gaps in our knowledge in order to establish relationships between the structural and functional properties conferred by peptides in various matrices including food products; and (ii) how recent knowledge about the capacity of peptides to self-assemble or aggregate can help us learn more about assembly and functional properties of peptides in complex media such as food.

This review is divided into six sections. Section 1 is devoted to the production of peptides and the method of characterization; Section 2 deals with recent knowledge about peptide self-assembly and their implication in functional properties; Section 3 describes the role of peptides in functional properties when they are mixed with other peptides and proteins and with components other than proteins, i.e., in food products, the subject of Section 4; Section 5 focuses on the concept of crowding that has to be considered as another extrinsic factor capable of exerting deep changes in peptide functional properties under high concentration conditions; and, finally, Section 6 presents the conclusion.

Since much discussion about the definitions of “self-assembly” and “aggregation” exists in the literature, we will consider here that the self-assembly of peptides represents a spontaneous and reversible reaction in contrast to aggregation. In the case of self-assembly, non-covalent bonds such as hydrogen bonds, electrostatic and van der Waals interactions are involved in the peptide assembly (Bouhallab & Croguennec, 2014; Chen, 2005). Concerning aggregation, we will consider that it is an irreversible reaction involving covalent bonds such as disulfide bonds (Bouhallab & Croguennec, 2014; Bouhallab, Riaublanc, & Croguennec, 2011).

## 2. Production of single molecules or mixtures of peptides and the main methods of characterization

Peptides are chains of two to 100 successive amino acids (Bodanszky, 1988) and of higher amino acid residue numbers for proteins, which are connected to each other via covalent bonds between their amino group and their carboxyl group.

Peptides can be chemically synthesized as a single molecule or produced from the parent proteins by enzymatic hydrolysis as a mixture of peptides, referred to as hydrolysates. In the former case, synthesized peptides have a rationally-designed sequence and are well identified (Kyle, Aggeli, Ingham, & McPherson, 2009). The difficulty is the presence of biohazard products that result from synthesis. In the latter case, peptides can be produced in large amounts at a much lower cost. Hydrolysates are increasingly used in food formulations for their particular functional properties. The composition of the hydrolysate is governed by the enzyme specificity, the enzyme:protein ratio, the extent of hydrolysis and the physico-chemical conditions applied (Gauthier, Paquin, Pouliot, & Turgeon, 1993; Kristinsson & Rasco, 2000), as detailed below.

Hydrolysate composition first depends on the enzyme specificity, which determines the number and the size of the peptides produced (Darewicz, Dziuba, & Dziuba, 2006). Commercial enzymes with well-known specificities are available, the most common of which are trypsin, papain, pronase, pepsin, bromelain, alcalase and chymotrypsin (Kumagai, 2012). The *in silico* method based on the known specificity of enzymes now makes it possible to predict the type and the number of peptides that can be theoretically produced from proteins by taking the possible formation of intermediate and/or end products within the hydrolysate into account. The second key factor essential for hydrolysis concerns the protein used as substrate, its nature, its concentration and the extent of its denaturation. For example, the thermal denaturation of whey proteins improved the extent of their hydrolysis by exposing previously inaccessible amino acids in the native protein to cleavage and, as a result, changing the type and the number of peptides produced compared to the native protein (Tavano, 2013). The third parameter is linked to the conditions in which the hydrolysis is performed, i.e., the enzyme:substrate ratio, the pH, the ionic strength, the temperature and the reaction time that can impact the enzyme activity, the accessibility to substrate and eventually change the final composition of the hydrolysate (Amiza, Kong, & Faazaz, 2012; Neklyudov, Ivankin, & Berdutina, 2000; Panyam & Kilara, 1996; Yin et al., 2010).

Therefore, hydrolysate can contain mixtures of native proteins, peptides and amino acids in various amounts depending on the hydrolysis conditions used, which will have a subsequent impact on the final functional properties (Damrongsakkul, Ratanathammapan, Komolpis, & Tanthapanichakoon, 2008; Liu et al., 2014; Shahidi, Han, & Synowiecki, 1995).

Many different techniques are available to monitor and control the production of peptides and to provide additional information. The most frequently used to qualify a hydrolysate is the degree of hydrolysis of proteins (DH), which refers to the proportion of cleaved peptide bonds per total number of bonds existing in the protein, and which varies from 0 to 100% depending on the intact proteins and the complete conversion of protein into amino acids, respectively (Liceaga-Gesualdo & Li-Chan, 1999; Mahmoud, 1994; Panyam & Kilara, 1996). It is usually determined by monitoring the pH or by quantifying the free $NH_2$ groups because of the ease with which this can be done (Spellman, McEvoy, O'Cuinn, & FitzGerald, 2003). Most of the time, the higher the DH is, the smaller and the more soluble the peptides will be (Adler-Nissen, 1976; Ghribi et al., 2015; Jamdar et al., 2010; Quaglia & Orban, 1987). However, it reflects neither the quantity of peptides present in the hydrolysate vs.