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Bioactive Peptides: Synthesis, Properties, and Applications in the Packaging and Preservation of Food

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Abstract: Bioactive peptides are protein fragments which have a positive impact on the functions and conditions of living beings. Peptides have shown several useful properties for human health, including antimicrobial, antifungal, antiviral, and antitumor activities. These compounds are produced by almost all species of life. However, they are produced in limited quantities in nature. As a result, researchers have tried to synthesize bioactive peptides to study their properties and applications in various areas. Among their applications in food preservation, peptides have been incorporated into packaging materials. This review begins with a brief description of the methods used for the synthesis, purification, and characterization of peptides. Also, the main bioproperties and mechanisms of action of peptides are discussed. Finally, some applications of peptides are presented, especially their use in active packaging, their effects on the polymeric matrix, and peptide migration.

Introduction

Food safety is a growing concern of great importance worldwide. Recently, the estimated costs of diseases caused by foodborne pathogens was about $152 billion in the United States (Scharff 2010), and it is estimated that in the United States alone about 47.8 million illness cases, 128000 hospitalizations and 3000 deaths will be caused by foodborne pathogens in 2011.

The consumption of processed foods with chemical preservatives has led to increased consumer concern and the demand for more natural and minimally processed foods. As a result, researchers have shown a growing interest in natural antimicrobial agents such as certain peptides.

Bioactive peptides are defined as specific protein fragments that have a positive impact on the functioning or conditions of living beings, thereby improving their health (Korhonen and Pihlanto 2006). The beneficial effects are attributed to different properties found in peptides such as antimicrobial (Reddy and others 2004; Rajanbabu and Chen 2011), antioxidant (Sarmadi and Ismail 2010), antithrombotic (Wang and Ng 1999), anti-hypertensive (Erdmann and others 2008), and immunomodulatory activities (St Georgiev 1990; Gauthier and others 2006), among others.

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Peptides with antimicrobial properties are used as the first chemical barrier against microbial attack, being synthesized in response to bacterial infections. They are produced by almost all species of life, from microorganisms, plants and animals, to humans (St Georgiev 1990; Hancock and Diamond 2000). In animals, antimicrobial peptides are produced mainly in those tissues exposed to adverse conditions such as skin, eyes, and lungs, which are more likely to be in contact with microorganisms (Zasloff 2002; Papo and Shai 2003).

More than 700 antimicrobial peptides have been reported, showing significant variations with respect to their sequence, length, and structure (Papo and Shai 2003).

Antimicrobial peptides have found many applications, including those in biomedical devices, food processing equipment, and food preservation.

In food preservation, peptides can be incorporated into materials to create antimicrobial packaging (Appendini and Hotchkiss 2002). In this way, antimicrobial packaging plays an important role in maintaining the safety and quality of food, since the aim is to prolong food shelf life and to reduce bacterial growth on the product surface (Soares and others 2009a). This type of active packaging interacts with the product and/or the headspace inside to reduce, inhibit, or retard the growth of microorganisms that may be present (Soares and others 2009b).

This review highlights the main methods of peptide synthesis and noteworthy peptide bioproperties. Also, specific peptide applications in food preservation are reviewed, focusing on their incorporation in polymeric matrices. Finally, the effects of peptide incorporation on packaging characteristics as well as their migration into food are discussed.

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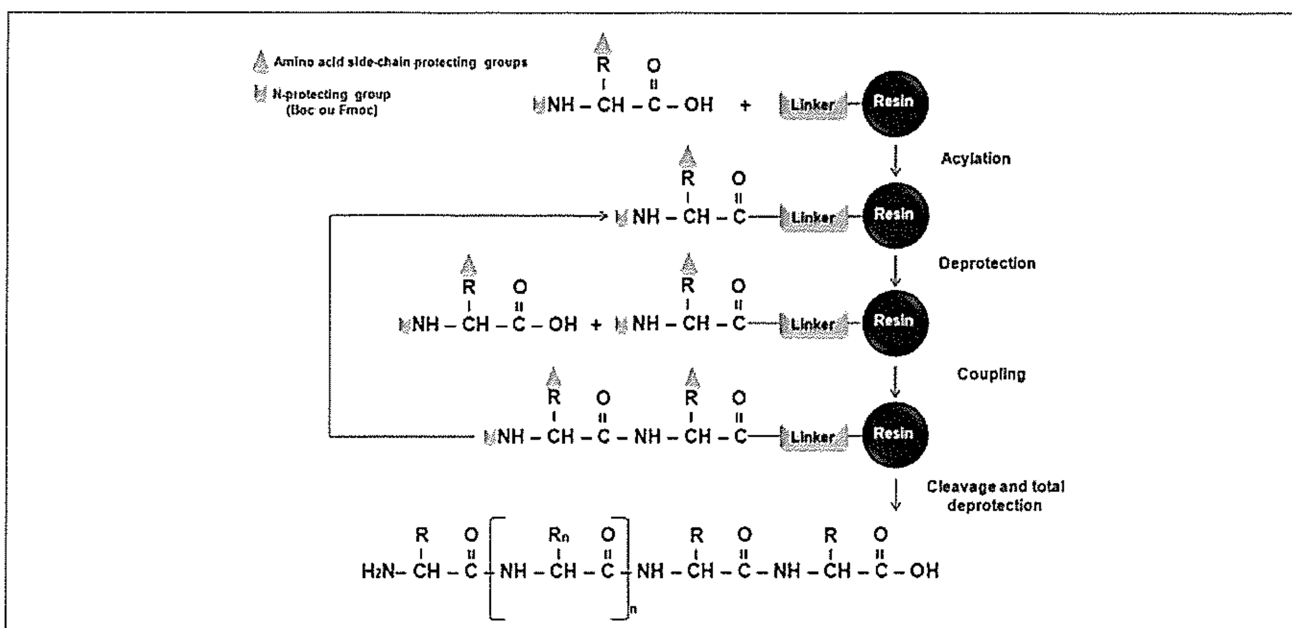

Figure 1–Peptide synthesis in solid phase Adapted with permission from Borgia and Fields (2000). Copyright (2000), Elsevier.

Peptide Synthesis

Peptides are biomolecules that contain between 1 to several dozen of amino acid residues joined by peptide bonds.

The discovery of the different peptide activities has generated enormous interest in this class of compounds and in the methods of isolation, analysis, purification, identification, and quantification. These methods have been systematically studied and improved. However, most sources of natural peptides are poor in these compounds, thus preventing their isolation in sufficient quantities for research.

As a result, there was a growing need to synthesize peptides for application in physiological, chemical, physical, pharmacological, biochemical, and clinical studies.

Total of 3 methods of peptide synthesis have been developed and improved: Chemical synthesis, which uses chemical reagents to mediate peptide bond formation (Andreu and Rivas 2002), enzymatic synthesis, in which the peptide bond formation is catalyzed by enzymes (Bongers and Heimer 1994; Boeriu and others 2010), and the DNA recombinant technology synthesis, based on the use of cloning and ribosomal techniques from biological systems for peptide formation (Sewald and Jakubke 2002).

Chemical synthesis

Research on this synthesis method was first initiated more than 30 y ago. However, the construction of peptides has recently become more accessible due to advances in process efficiency, including the development and use of fast coupling reagents, as well as the minimization of side reactions (Borgia and Fields 2000).

The main aspects of chemical synthesis are protection and activation. Protection strategies are intended to provide chemical selectivity necessary for the construction of a particular peptide sequence. Activation refers to the chemical coupling necessary to ensure quantitative formation of each peptide bond in the sequence (Andreu and Rivas 2002).

In chemical synthesis, chemical reagents are used to activate the carboxylic acid (RCOOH) of the amino acid, which will donate the acyl group (R-CO-) to form the peptide bond. The peptide bond presents a nucleophilic attack of the α-amino group by another amino acid (H_2N-R). In this synthesis, the reactive functional groups that are not directly involved in peptide bond formation receive prior protection (Machado and others 2004). There are 2 types of chemical peptide synthesis, synthesis in solution (classical synthesis) and solid-phase synthesis.

Chemical synthesis in solution is performed with all reagents and reaction products dissolved in the medium (Kent 1988). In comparison, solid-phase synthesis (SPS) is a simple procedure to produce peptides in large quantities on a solid support which remains insoluble in the reaction medium (Shigeri and others 2001). The solid support is a polymeric resin that has a functional group on its surface (linker) that allows it to form stable bonds in the peptide sequence to the reagent used for the de-protection of the N-amino group.

Peptide synthesis in the solid phase generally consists on the acylation of an amino acid to be linked to an insoluble support (resin) via a linker (Figure 1). After that, the protecting group of the N-terminal is removed (the unprotecting step) to allow the next amino acid of the sequence to be attached to the complex "peptide-linker-resin." The unprotecting-coupling cycle is repeated until the desired sequence is complete. Finally, the cleavage reagent is used to separate the complex "peptide-linker-resin." This reagent should also remove the protecting groups of side chains that are stable to unprotecting conditions of the N-terminal group (Borgia and Fields 2000).

Peptide chemical synthesis can use 2 protocols, Boc (tert-butyloxycarbonyl) and Fmoc (9-fluorenylmethyloxycarbonyl), named according to the type of protector of the reactive group of the amino acids (N-terminal) involved in the synthesis.

The first protocol employs the tert-butyloxycarbonyl (Boc) group for N-amino protection. This protocol is based on gradual differences in their sensitivity to acids. Thus, the Boc group is typically removed with trifluoroacetic acid (TFA), while the protecting groups of the lateral chains (ester, ether, and urethane derivatives based on benzyl alcohol) are specifically designed to be stable to repeated cycles of Boc removal and are removed only with

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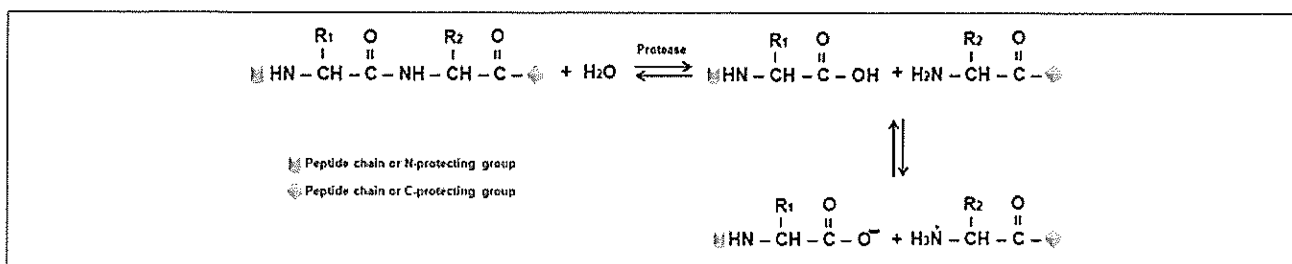

Figure 2–Enzymatic peptides synthesis by the reverse hydrolysis reaction.

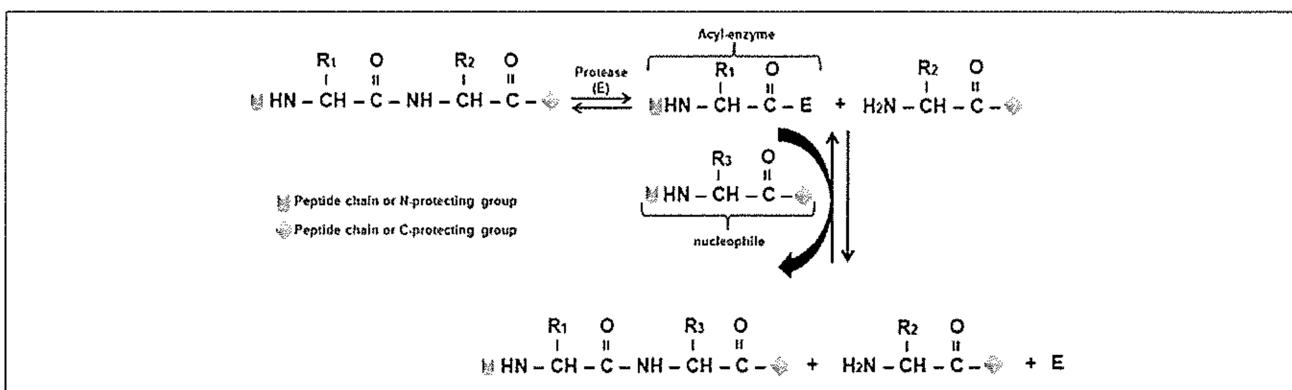

Figure 3–Enzymatic peptides synthesis by transpeptidation mechanism.

a specific reagent, a relatively stronger acid, usually hydrofluoric acid (Borgia and Fields 2000).

The second protocol uses a 9-fluorenylmethyloxycarbonyl (Fmoc) as the N-amino protecting group. This protocol provides a greater degree of chemoselectivity than the Boc protocol, since the Fmoc group is removed under basic conditions (piperidine in N, N-methylpyrrolidone or dimethylformamide), without alteration of the acid-sensitive lateral chains (Andreu and Rivas 2002).

Protection groups of lateral chains are compatible with the Fmoc protection group; these are mainly ether, ester, and urethane derivatives based on t-butanol. Protection groups of lateral chains are removed by the end of the synthesis using TFA (Borgia and Fields 2000).

Enzymatic synthesis

In this method, the peptide bond formation is mediated by an enzyme (protease) in free or immobilized form. The enzymatic method is especially useful in the synthesis of very short peptides (2–5 oligomers) and in the condensation of large peptide fragments (So and others 1998). Proteolytic enzymes such as chymotrypsin, papain, pepsin, subtilisin, termolisin, trypsin, among others, have been used in the presence of organic solvents as catalysts for the synthesis of peptide bonds (Ogino and others 1999).

The enzymatic synthesis of peptides has several advantages over chemical methods, including good stereoselectivity and regioselectivity. However, it has certain shortcomings, such as peptide synthesis being thermodynamically unfavorable in water, as well as the secondary hydrolysis of synthesized peptide chains, which hinders their use in peptide synthesis with long sequences (So and others 1998). Thus, the main practical obstacle to employment of a protease for peptide bond formation is finding suitable conditions to allow bond formation without mediating secondary hydrolysis of the peptide or peptide fragments used as reagents (Bongers and Heimer 1994).

The formation of a peptide bond by enzyme catalysis can occur through several mechanisms, including the reverse hydrolysis reaction of amides and transpeptidation (Machado and others 2004; Boeriu and others 2010).

The mechanism of the reverse hydrolysis reaction is based on the microscopic reversibility principle. This indicates that the peptide bond formation and hydrolysis reaction come from the same intermediate (Figure 2). Thus, the reaction conditions are manipulated to shift the equilibrium towards peptide bond formation.

The transpeptidation mechanism occurs as a result of the break of a peptide bond, with the formation of an active acyl-enzyme intermediate (Figure 3). This intermediate is attacked in the presence of a nucleophile (peptide or amino acid blocked in the α-carboxyl group) and consequently causes the formation of a new peptide bond.

For both mechanisms, the equilibrium should shift to the synthesis reaction direction, requiring the use of protective groups of α-amino and carboxyl substrates, the addition of organic solvents to the media reaction, excess substrates, and the removal of products from the reaction medium (Machado and others 2004).

Synthesis by recombinant DNA technology

This synthesis uses modern methods of cloning and gene expression in microorganisms, allowing the production of a recombinant peptide or several peptides simultaneously. Bacteria are the expression system generally used, with *E. coli* being the most widely used host. Since antimicrobial peptides present a natural destructive activity against the host and relative sensitivity to proteolytic degradation, peptides are often expressed as fusion proteins to

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