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Review

Protein hydrolysis using proteases: An important tool for food biotechnology

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ABSTRACT

This review intended to give a brief idea of the importance of proteases applications. Processes that involve protein hydrolysis steps find wide ranging utilizations, such as cleaning process, proteomic studies, or food biotechnology process. Many positive effects hoped for with food processing can be achieved by protein hydrolysis using specific proteases, changing nutritional, bioactive and functional properties of food proteins, which include improved digestibility, modifications of sensory quality (such as texture or taste), improvement of antioxidant capability or reduction in allergenic compounds. Protease applications in industrial processes are constantly being introduced and can be advantageous compared to chemical processes, by increasing hydrolysis specificity, product preservation and purity, and reducing environmental impact. Differences in specificity between proteases are very important to take in to consideration as a guide for the choice of protease according to the protein source to be hydrolyzed or predicted products. In this present review, some aspects of the processes that involve protein hydrolysis steps are discussed, especially considering the application of specific proteases as a tool on food biotechnology.

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1. Introduction

Processes that involve protein hydrolysis steps find wide ranging applications. Such applications can be found in the detergent or leather industries, in the formulation of samples for amino acid

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analysis or proteomic studies, and in the development of protein hydrolysates designed for nutritional support of special patients [1–7]. For the last two examples, preservation of the properties of the generated products, peptides and amino acids, is especially desirable, thus, the hydrolysis process must be carefully controlled.

Protein hydrolysis, the cleavage of peptide bonds, can be carried out by enzymatic or chemical processes. Chemical processes, including alkaline or acid hydrolysis, tend to be difficult to control, and yield products with modified amino acids. Conventional acid hydrolysis conditions to amino acids determination, using 6 M HCl at 110 °C for more than 24 h, can destroy tryptophan [8]. Alkaline hydrolysis can chemically reduce cystine, arginine, threonine, serine, isoleucine, and/or lysine content, and form unusual amino acid residues such as lysinoalanine or lanthionine [9]. Enzymatic hydrolysis can be performed under mild conditions, and could avoid the extreme environments required by chemical treatments. Usually, enzymatic processes avoid side reactions and do not decrease the nutritional value of the protein source [2]. Additionally, enzymes present substrate specificity which permits the development of protein hydrolysates with better defined chemical and nutritional characteristics [10].

On the other hand, enzymatic processing can present certain difficulties. Total protein hydrolysis by one protease action is still utopic. In addition, relatively small changes in protein structure can cause important damage to enzyme function, and they can present inhibitors, cofactors needs, or suffer autolysis [11–14].

Thus, processes aimed at obtaining hydrolysates with specific and preserved peptides, can consider the protease application as an important tool. But achieving the objectives requires knowing perform more appropriate choice of the protease, and often also to modify this protease for best results in the desired application. In this present review, some aspects of the processes that involve protein hydrolysis steps are discussed, especially considering the application of specific proteases as a tool on food biotechnology.

2. Proteases aspects

Proteases (also termed peptidases, proteolytic enzymes and peptide bond hydrolases) are intimately associated with vital biological pathways. As a result, it is not difficult to imagine their importance, even in the case of the most primitive organisms, and their association with evolutionary processes. The range of variants and specificity reflect these evolutionary modifications. At the same time their similarities permit that common ancestry of distinct species be established. The modifications suffered by proteins can also result in the fact that distinct proteins present similar functions – as proposed for chymotrypsin and subtilysin, which present the same catalytic mechanism for proteolysis by convergent evolution provided by unrelated proteins [8,15–17]. The evolutionary complexity of living organisms provides an enormous range of different proteases with a large repertoire of functions and, subsequently, a wide range of structures and specificities, which arouse interest amongst researchers who seek new applications for proteases [18,19]. The clear importance and complexity of the proteolysis in biochemical functions or pathogenical conditions in all organisms led to the development of *degradomics*, as an experimental field for the identification and characterization of proteases in an organism [19–21].

Advances in the chemical characterization of active sites and structure analysis permit that proteases can be grouped into families by common mechanism or by similar structural features [22]. Despite being relatively simple to classify enzymes, proteases do not always fit clearly into the international system for the classification and nomenclature of enzymes (EC number), which was developed in the 1950s [23]. All enzymes are divided in six classes, and proteases are classified in class 3, as Hydrolases, subclass 3.4., hydrolysis of peptide bonds. So, proteases were divided between 13 sub-classes on the basis of the catalytic reaction.

Nowadays, since the protein structure of the enzymes is better known, other forms of classification can be proposed that consider their chemical structures and thus contain information about their evolutionary families, such as MEROPS databases (peptidase database) [24–26]. Considering, principally, the tertiary structure of the protein and its catalytic sites, proteases can be classified in clans, and clans divided in families. Every clan provides information about the catalytic structure of the proteases. The names take into account the iconic amino acid or metal present in the active site: Aspartic peptidases (A), Cysteine peptidases (C), Metallo peptidases (M), Serine peptidases (S), Mixed catalytic type (P) and Unknown type (U) [24,25,27].

The structure around the active site of the protease determines how the substrate can bind to the sites of the protease. The surface of the protease that is able to accommodate the chain of the substrate is called the *subsite* and it can determine the substrate specificity of a given protease [28]. However, although most enzymes present a large chain/structure, only a few amino acid residues are in fact involved in the active site. Serine proteases, for example, are known for their classical catalytic triad. The geometric relationship between Asp102, His57 and Ser195 of chymotrypsin, the first structure reported for a peptidase, is very well documented [16,27].

Although it is possible to consider that the most important characteristic of proteases is their form of action on substrates, sometimes their specificities are very complex and not clearly defined. However the mode of action can define proteases as exopeptidases or endopeptidases, which are the two main subclasses based on substrate interaction. The peptide substrate runs through the entire length of the active site of an endopeptidase framework and is cleaved in the middle of the molecule. On the other hand, exopeptidades act near the end of polypeptide chains. Furthermore, exopeptidases are termed aminopeptidases if they act at the n-terminus, and carboxypeptidases are those acting on peptide bonds from the c-terminus. Some enzymes present both carboxy- and aminopeptidase forms, such as cathepsins, since their structure can have structural elements that provide negative charge (cathepsin H) to bind the positively charged amino terminus of the substrate, or positive charge (cathepsin X) to bind the negatively charged carboxyl terminus of the substrate [28,29]. Finally, in describing the specificity of endopeptidases, the term oligopeptidase is used to refer to those that act optimally on substrates smaller than proteins.

The specificity of a protease determines the position at which the enzyme will catalyze peptide bond hydrolysis. The enzyme active site has a characteristic arrangement of amino acid residues which define the enzyme–substrate interaction [17]. The knowledge of the specificity of proteases provides information that can lead to a better choice to act on a specific substrate [30,31]. Table 1 presents a compilation of preferential cleavage of some proteases.

These differences in specificity between proteases are very important to take in to consideration as a guide for the choice of protease according to the protein source to be hydrolyzed or predicted products. A same protein chain can produce very different hydrolysates using different proteases. A potato pulp protein, for example, when hydrolysed using four different enzymes [33], endoproteases Alcalase and Novo Pro-D, exopeptidase Corolase, endo and exoproteases mix – Flavourzyme, and their combinations, resulted in distinct hydrolysates. Kamnerdpetch et al. [33] observed that the total hydrolysis degrees were 22, 8, 3 and 2% for Flavourzyme, Alcalase, Novo Pro-D and Corolase, respectively. As can be seen, the determining factor for the degree of hydrolysis was not the fact that the enzymes were endo- or exo-, but the specificity

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