Trends in Food Science & Technology 68 (2017) 91–101

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

Review

New trends for a classical enzyme: Papain, a biotechnological success story in the food industry

Jesús Fernández-Lucas [a, b, *], Daniel Castañeda [b], Daniel Hormigo [a, **]

[a] Applied Biotechnology Group, Department of Pharmacy and Biotechnology, School of Biomedical Sciences, Universidad Europea de Madrid, Urbanización El Bosque, Calle Tajo s/n, 28670 Villaviciosa de Odón, Madrid, Spain
[b] Grupo de Investigación en Desarrollo Agroindustrial Sostenible, Department of Agroindustrial Engineering, School of Environmental Sciences, Universidad de la Costa, Cra. 55 #58-66, Barranquilla, Colombia

ARTICLE INFO

Article history:
Received 4 April 2017
Received in revised form
19 August 2017
Accepted 23 August 2017
Available online 24 August 2017

Keywords:
Cysteine proteases
Papain
Enzyme biotechnology
Biocatalysis
Immobilization
Industrial processes

ABSTRACT

Background: In recent years, proteases have arisen as standard biocatalysts in many industrial processes in different fields, such as pharmaceutical, medicine, detergent manufacturing and food science. Among them, papain is undoubtedly one of the most frequently studied and widely used proteases in the food industry around the world. However, the latest advances in recombinant papain expression systems, genetically engineered biocatalysts, new purification and isolation strategies, and enzymatic immobilization will enhance the development of new applications of papain, as well as improve and optimize classical applications.

Scope and approach: This review addresses not only the latest advances in classic applications, such as meat tenderization and protein hydrolysates, but also the most innovative applications in different industries such as food, animal feed, bioactive peptides production, water treatment, baking and brewing, among many others.

In addition, papain is a perfect example of a successful industrial enzyme that covers all the steps of the biocatalytic cycle that are necessary for the industrial implementation of any biocatalyst. This cycle includes the production and extraction of the enzyme concerned (from natural or recombinant sources), functional and structural characterization, genetic improvement, immobilization and, finally, industrial application. This review describes the complete biocatalytic cycle of papain.

Key findings and conclusions: Papain is clearly a case of industrial and commercial success over the last 40 years. The key to this success has been continual biotechnological and process engineering innovation, which has opened up a new range of possibilities for this exciting biocatalyst. However, further efforts are needed in protein engineering and characterization of new mutants to reach the full potential of this enzyme.

1. Introduction

Within the global industrial enzymes market, the food industry enzymes sector is the major segment and is expected to grow from nearly $1.5 billion in 2016 to $1.9 billion in 2021 (BCC Research Biotechnology Report, 2011. Enzymes in industrial applications: Global markets).

Proteases (also called peptidases or proteinases) are enzymes that hydrolyse the peptidic linkages in protein into shorter fragments (peptides) and eventually into their components, amino acids. Proteases are important enzymes in living organisms, being involved in many different biological processes.

Protease enzymes are used in a large variety of applications, mainly in the detergent and pharmaceutical industries, followed by the food industry. Since proteases represent more than 60% of the enzyme market share, with an expected CAGR (compound annual growth rate) of 5.3% from 2014 to 2019, they are the most important type of commercialized enzymes in the world. Leading producers worldwide include Novo Industries, DSM, DuPont Industries, BASF, Genencor International, and Roche (Feijoo-Siota & Villa, 2011;

* Corresponding author. Applied Biotechnology Group, Department of Pharmacy and Biotechnology, School of Biomedical Sciences, Universidad Europea de Madrid, Urbanización El Bosque, Calle Tajo s/n, 28670 Villaviciosa de Odón, Madrid, Spain.
** Corresponding author.
E-mail addresses: jesus.fernandez2@universidadeuropea.es (J. Fernández-Lucas), daniel.hormigo@universidadeuropea.es (D. Hormigo).

http://dx.doi.org/10.1016/j.tifs.2017.08.017
0924-2244/

Kumar, Singh, Sangwan, & Gill, 2014).

According to the nature of the active site, proteases can be divided into seven mechanistic classes: serine proteases, S (EC 3.4.21); cysteine proteases, C (EC 3.4.22); aspartic and glutamic proteases, D or E (EC 3.4.23); metalloproteases, M (EC 3.4.24); threonine proteases, T (EC 3.4.25); and asparagine peptide lyases, N (EC 4.3) (Rawlings, Barrett, & Bateman, 2011). According to the MEROPS database, cysteine proteases are divided into ten clans, and most plant cysteine proteases belong to the C1 family, also known as the papain family (papain-like proteases) Rawlings, Barrett, & Bateman, 2010). Papain-like proteases are found in most known organisms, such as viruses, protozoa, plants, invertebrates, and vertebrates. Most of the C1 family members are endopeptidases, but some of them display a wide variety of activities, including aminopeptidases, dipeptidyl peptidases and enzymes with both exo- and endo-peptidase activities (Feijoo-Siota & Villa, 2011; Novinec & Lenarčič, 2013; Rawlings et al., 2010).

Papain (EC 3.4.22.2), also called Papaya proteinase I (PPI), is a 23.4 kDa, 212 residue cysteine endopeptidase belonging to subfamily C1A of papain-like proteases (Rawlings et al., 2010). The commercial importance of papain is mainly due to its strong proteolytic activity against a broad range of protein substrates, and because it is active across a broad range of operational conditions. These interesting characteristics have allowed papain to lead the proteases market, outselling other plant derived proteases, such as bromelain (*Ananas comosus*) and ficin (*Ficus carica*), as well as fungal source proteases (Market Research Future, 2017. Global Meat Tenderizing Agents Market Research Report- Forecast to 2023). Major producers of papain include India, Sri Lanka, Democratic Republic of Congo, Zaire, Tanzania, Uganda, Mexico, Brazil and Argentina. As regards the global papain market, the main importers are concentrated in Europe and USA, with a market size of about 150–200 and 300–400 tons per year, respectively. Furthermore, the Japanese market is relatively small (less than 50 tons per year). Some of the main limiting factors of the global and sustainable supply of papain are the high climatic dependence of papaya crops, as well as the economical and political issues of some producing countries (IDEA, 2000. Commercialisation Bulletin 13 Papain Report). This fact makes the search for alternative sources of papain a priority for producing companies. Fortunately, the latest advances in recombinant papain expression may provide a solution to this problem.

This review presents papain as an industrial success paradigm in Biocatalysis, where all the steps necessary for the industrial implementation of any enzyme have been effectively addressed (Fig. 1). The review provides a detailed description of each step, including the different strategies of isolation and purification from natural and recombinant sources, operational conditions, genetic modifications to improve its functional characteristics, enzyme immobilization, and industrial applications. It also describes the most recent trends in meat tenderization, dairy industry, production of protein hydrolysates and bioactive peptides, food allergens removal, brewing and baking industry, animal feed and water treatment, among other fields. Finally, we look at some industrial uses of papain not strictly focused on the food industry, but which demonstrate the enormous versatility of this exciting enzyme, such as certain biomedical approaches, antimicrobial food packaging, caries removal agent, or tooth-whitening additive in toothpastes.

2. Production and purification strategies

2.1. Plant derived papain

Papain can be obtained from the latex of the papaya plant (*Carica papaya*), which is a natural source of other endopeptidases, such as chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30) and glycyl endopeptidase (EC 3.4.22.25). In fact, papain is a minor constituent (5–8%) among the papaya endopeptidases (Feijoo-Siota & Villa, 2011).

Purification of papain from papaya latex has traditionally been carried out using precipitation methods, reaching high yields of up to 53 g of crude enzyme per kg of latex. Although these methods have routinely been used in industry, they can only achieve up to 39% purity of papain (Nitsawang, Hatti-Kaul, & Kanasawud, 2006). An alternative and more efficient purification strategy involves the use of various chromatographic techniques (ion exchange or affinity). In these types of techniques the initial pre-processing of the latex is essential before samples can be applied on a chromatography column. This processing consists in a sun- or spray-drying process after latex extraction from plant (Feijoo-Siota & Villa, 2011).

Current techniques usually include aqueous two-phase systems (ATPS). These systems may be composed of two polymers in aqueous solution, or one polymer and salt in aqueous solution. ATPS techniques have shown great potential for downstream processing of papain and other proteases, since they allow clarification, concentration and purification of the target product in a one-pot process (Nitsawang et al., 2006). In this respect, it is worth mentioning that polymer–salt–water systems have aroused greater interest in the industry, since they are cheaper and show less viscosity than polymer–polymer–water systems. In this regard, polyethylene glycol–phosphate based systems are the most employed (Rocha & Nerli, 2013). Recent studies have demonstrated that the use of alginate as macro-ligand in PEG based systems improves papain purification. In a recent study, Rocha et al. (2016) described a PEG-citrate buffer system able to purify papain from latex with a 20% of recovery. They observed that by adding alginate (0.1% w/w) and $CaCl_2$ they could recover up to 72% of papain and recycle PEG for purification. This strategy is very promising, since it is low cost, easy to scale up, accurate and environmentally friendly (Rocha et al., 2016).

In 2014, He et al. reported an efficient method of large scale papain purification from unclarified papaya juice feedstock in batch adsorption system. In this study, the authors employed a reversed phase expanded bed adsorption chromatography (RP-EBAC) using a FastlineTM-10-EBAC column packed with AmberliteTM-XAD-7HP. This technique allowed the authors to purify papain in a single operation with a purification factor of 7.04 and a purity of 75% (He, bin Tuan Chik, & Chong, 2014).

After the purification process, crude papain usually has to be treated with reducing agents in order to protect the free cysteine thiol groups from oxidation, preserving its protease activity. When needed, free thiol groups of papain can be regenerated by the addition of low molecular mass thiols, such as cysteine or dithiothreitol.

2.2. Recombinant papain

The high world demand of papain for industrial uses makes necessary the search for alternative sources from traditional extraction from the latex of *C. papaya*. Despite the high recovery of papain from *Carica papaya* plant material (up to 53 g of crude enzyme per kg of wet latex), this natural source presents several drawbacks. First, papain only represents about 5–8% of total cysteine proteases in the latex, which entails an expensive and ponderous isolation strategy (Nitsawang et al., 2006). Furthermore, isolated papain is highly susceptible to oxidation, so it must immediately be conserved at low temperatures and direct air contact must be avoided. Another major disadvantage is the dependence of papaya crops on external factors, such as climate, soil, pests, etc., which avoids a continuous supply of papain.