International Journal of Biological Macromolecules 188 (2021) 94–113

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

Immobilization of papain: A review

Veymar G. Tacias-Pascacio [a,b], Roberto Morellon-Sterling [c,d], Daniel Castañeda-Valbuena [b], Ángel Berenguer-Murcia [e], Majid Rasool Kamli [f,g], Olga Tavano [h], Roberto Fernandez-Lafuente [c,i,*]

[a] Facultad de Ciencias de la Nutrición y Alimentos, Universidad de Ciencias y Artes de Chiapas, Lib. Norte Pte. 1150, 29039 Tuxtla Gutiérrez, Chiapas, Mexico
[b] Tecnológico Nacional de México/Instituto Tecnológico de Tuxtla Gutiérrez, Carretera Panamericana Km. 1080, 29050 Tuxtla Gutiérrez, Chiapas, Mexico
[c] Departamento de Biocatálisis. ICP-CSIC./Marie Curie 2, Campus UAM-CSIC Cantoblanco, 28049 Madrid. Spain
[d] Student of Departamento de Biología Molecular, Universidad Autónoma de Madrid, Darwin 2, Campus UAM-CSIC, Cantoblanco, 28049 Madrid. Spain
[e] Departamento de Química Inorgánica e Instituto Universitario de Materiales, Universidad de Alicante, Alicante, Spain
[f] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddad 21589, Saudi Arabia
[g] Center of excellence in Bionanoscience Research, King Abdulaziz University, Jeddad 21589, Saudi Arabia
[h] Faculty of Nutrition, Alfenas Federal Univ., 700 Gabriel Monteiro da Silva St, Alfenas, MG 37130-000, Brazil
[i] Center of Excellence in Bionanoscience Research, External advisory board, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Keywords:
Enzyme immobilization
Enzyme stabilization
Protein hydrolysis

ABSTRACT

Papain is a cysteine protease from papaya, with many applications due to its broad specificity. This paper reviews for first time the immobilization of papain on different supports (organic, inorganic or hybrid supports) presenting some of the features of the utilized immobilization strategies (e.g., epoxide, glutaraldehyde, genipin, glyoxyl for covalent immobilization). Special focus is placed on the preparation of magnetic biocatalysts, which will permit the simple recovery of the biocatalyst even if the medium is a suspension. Problems specific to the immobilization of proteases (e.g., steric problems when hydrolyzing large proteins) are also defined. The benefits of a proper immobilization (enzyme stabilization, widening of the operation window) are discussed, together with some artifacts that may suggest an enzyme stabilization that may be unrelated to enzyme rigidification.

1. Introduction

1.1. Enzymes as industrial biocatalysts

Enzymes are the most efficient catalysts in Nature. They are very active, specific and selective under mild conditions enabling their use under mild conditions (room temperature and atmospheric pressure, saving protection and deprotection steps, focusing on the modification of the target substrate, reducing the amount of side-products, etc. [1–5]. However, their biological origin results in some of their features not being adequate for their industrial implementation: enzymes have moderate stability, may be soluble in water, their extraordinary catalytic properties are limited to the physiological substrate, etc. [6]. Thus, in many instances, enzymes need to be improved before they can be used as industrial catalysts.

There are many different tools at different levels of biocatalyst design to improve enzyme features. In most of them, a great leap has been made in the last couple of decades. Conventional microbiology may provide the researcher with many enzymes, but the development of metagenomics tools has opened the access to all present and past enzyme biodiversity independently from the fact that it may or may not come from a cultivable microorganism [7–9]. Protein designing/engineering has permitted to improve enzyme biocatalytic activity [10] and help in the understanding of the enzyme catalytic mechanism [11,12], substrate specificity [13], and kinetics parameters [14]. Directed evolution permitted to mimic natural evolution in a "fast forward" way, focused on the targeted enzyme properties [15–18]. The new concepts in enzyme chemical modification [19–21] and immobilization [22–25] also permit to further improve upon the enzyme features.

These methodologies to improve enzyme features may be employed in a combined or even a synergetic way (e.g., designing enzymes by genetic tools that can be better improved by chemical modification or immobilization) [26–30]. The combined use of several of these tools permits to explore new concepts in enzymes design, that just a couple of decades ago were no more than a dream. This way, coupling site-directed mutagenesis and enzyme modeling, the researcher may create

* Corresponding author.
E-mail address: rfl@icp.csic.es (R. Fernandez-Lafuente).

https://doi.org/10.1016/j.ijbiomac.2021.08.016
Received 2 July 2021; Received in revised form 22 July 2021; Accepted 3 August 2021
Available online 8 August 2021

an enzyme bearing two active centers (the so-called plurizymes) [31] and later on, by again using enzyme modeling and organic chemistry, to modify only one of them with a specific irreversible inhibitor bearing an organometallic catalyst [32]. This way, a final enzyme bearing two very different active centers may be generated and used in a cascade reaction.

In the manipulation of foods, the use of enzymes has further advantages coupled to traditional chemical routes [33–36]. The use of conventional chemistry or catalysis is not advisable in these instances, as the production of by-products, in many instances with toxic potential, will avoid its consumption. The use of enzymes, thanks to their selectivity, will prevent the production of these toxic by-products. Moreover, enzyme specificity permits to modify just the target compounds, without modifying other desired components of the food. That way, both enzyme features are critical advantages of enzymes in the modification of foods. In this context, proteases are one of the enzymes with a higher relevance [37–47].

1.2. Proteases

Among the different enzymes, proteases, together with carbohydrolases and lipases, are outstanding catalysts for industrial application. Proteases encompass about 60% of the total enzyme market and stand among the most precious commercial enzymes [48]. Proteases are identified as hydrolases, which places them in class 3, and can be more specifically located in subclass 3.4., that is, those that hydrolyze peptide bonds [49]. However, there is a diversity in catalysis mechanisms, which has been used to divide proteases into 13 sub-subclasses. Among the different actions of proteases, the same protein chain can be hydrolyzed at different points depending on the protease in action, that is, on its specificity. In practice, this feature translates into information about which peptide bonds can potentially be broken in the substrate protein chain. Depending on the process to be developed, a greater or lesser specificity, or a more restricted or broader action, may be more or less interesting [39,43]. The specificity of these enzymes refers to how their protein chains, especially the region of their active sites, interact with the substrate. Thanks to the knowledge of this wide range of specificities, it is possible to select the best protease applicable to the specific process [39,43]. These forms of interaction between protease and its substrates are influenced by the amino acid sequence and conformation of its chain. The maintenance of its conformations adequate to the protein-protein interactions guarantee the success of its performance and can even provide an improved performance under a wider range of conditions of the reaction medium of the process in question.

1.3. Papain

Papain was the first protein isolated from papaya and the first cysteine protease that had its structure revealed by means of a three-dimensional X-ray study [50]. Thus, cysteine proteases are identified as papain-like proteases and are a large group of important components. Recently, much attention has turned to one of these proteases, the papain-like protease from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has been shown to play vital roles for the virus virulence, such as replication and capacity for immune evasion [51]. This is because this type of cysteine protease can be found in many living beings, such as viruses, microorganisms, plants or animals. As minutely discussed by Liu et al. (2018) [52], the origin of papain-like proteases probably occurred before the divergence of the main eukaryotic lineages. A striking feature in common is the presence of the active-site containing the catalytic triad Cys-His-Asn. Thanks to this Cysteine in its active site, these proteases are classified within the subclass 3.4.22 (Cysteine endopeptidases). The papaya from *Carica papaya* is classified as EC 3.4.22.2 [53].

Although papain represents a very small percentage of the endopeptidases of *Carica papaya*, only in the range of 5 to 8%, its wide applications placed it in a prominent position in industrial applications. Although its applications in food production processes are very prominent, such as meat tenderization, baking or dairy industry, its applications are wide and varied, including applications in drug production processes and even for direct administration, with their pharmacological actions and production of bioactive peptides [46,54]. With so many interests in this enzyme, its production on a large scale is necessary, and different techniques are proposed. Although the expression of recombinant papain via different microorganisms is a viable way, its direct extraction from papaya fruit tissues is still a way of obtaining it, being possible to extract it from latex, peel and other parts [55].

An interesting feature of this protease is its broad specificity for peptide bonds, allowing a high degree of protein hydrolysis to be achieved. But this enzyme, like other cysteine proteases, has a preference for an amino acid containing a large hydrophobic side chain at position P2 and does not accept Val at P1' [53]. Its structure reveals itself as a single polypeptide chain, folded into two domains of the same size but with conformational differences. This chain is composed of 212 amino acids, with a molecular weight of 23,350 Da, with 7 Cys residues, one of which (Cys 25) composes the active site, together with residues His159 and Asn175 [56].

Although this enzyme has a better performance in media with neutral pH, close to 6.0-7.0, it manages to maintain its activity under a wide range of pH and temperature of the medium [57]. This expands its applications. However, although it maintains its activity, its stability is reduced under more extreme conditions, which can be overcame by enzymatic modification and immobilization techniques. As previously mentioned, processes that increase the stability of protein chains can increase the range of applications of an enzyme, allowing its application to be propitiated in more types of feed media. Although papain is a fantastically applicable protease in its soluble and native form, its immobilized form can have many advantages and even more applications.

2. Enzyme immobilization

2.1. General considerations on enzyme immobilization

Enzyme immobilization was initially developed to solve the problem of enzyme recovery and reuse, as the initial price of enzymes was prohibitive for just a single use in most applications [58–60]. The production of a heterogeneous catalyst permitted the simple recovery of the enzyme after each reaction cycle. In order to reuse the enzyme, it is necessary for the enzyme activity to be maintained after utilization [61–63]. This way, enzyme immobilization and enzyme stability were related concepts from the beginning. To have this good biocatalyst stability, it is possible to use a very stable enzyme (that permits its reuse for many reaction cycles), or to greatly improve the enzyme stability after immobilization, if the initial enzyme stability is not high enough. However, this improvement of the enzyme stability upon immobilization should not be taken for granted [64,65]. Depending on the enzyme and the interaction that the support can establish with it, the immobilization can even lead to enzyme destabilization [65,66]. An appropriate immobilization system to get a stabilized-immobilized enzyme [67] must consider the support (the best ones will be those where there are no more groups able to interact with the enzyme than those introduced by the researcher, like agarose) [68], the reactive groups (that must be abundant, stable, without steric hindrance towards the enzyme support reaction) [69] and the protocol (that must pursue an intense but controlled enzyme support reaction and have some kind of end point) [70], including the used buffers and medium composition [71,72]. Glutaraldehyde [73], glyoxyl [74], epoxides [64,75–77] or vinyl sulfone [78] activated supports are among the best supports for this objective [67]. That way, nowadays an appropriate enzyme immobilization protocol should maintain or even increase the stability of the enzyme, by promoting a significant multipoint covalent or multi-subunit (for multimeric enzymes) immobilization) [24,28,67]. Moreover, enzyme

immobilization has proved to be able to improve enzyme activity, mainly under harsh conditions, widening the range of operational conditions [79–82]. Moreover, immobilization may tune enzyme selectivity or specificity, reduce inhibitions and decrease inactivation by chemicals, or it may be coupled to enzyme purification [22–25]. Enzyme immobilization does not avoid the use of any other strategies to improve enzyme properties. In fact, immobilization may be beneficial when coupled to any other enzyme stabilization technique, such as microbiological, genetic or chemical modification tools [26–30,83–85]. For example, the chemical amination of an enzyme with ethylenediamine may permit to get an enzyme that can be immobilized using several anchoring points at lower pH value and giving a higher intensity of the multipoint covalent attachment, enabling a higher stabilization [83,84]. This way, immobilization is no longer mainly addressed to the enzyme recovery and reuse; it has become a critical point in the development of an enzymatic industrial biocatalyst.

2.2. Protease immobilization

In the case of proteases, their immobilization may have special positive effects, but also some special problems [39,43]. One of the most relevant uses of proteases is in the modification of foods, where other proteins are present in the substrate matrix [39,43]. That means that the enzyme, if used in free form, will be incorporated to the food, and this may not be desired, as some allergic reactions may be produced, even for a minimal percentage of the population, and this prevents partial and controlled modifications. In many instances, the control of the reaction is critical to reach the desired degree of protein hydrolysis, and this is much simpler using immobilized enzymes [38,86–88]. Together with the general advantages of using immobilized enzymes described above, the immobilization of proteases will prevent autolysis (at least if performed in pre-existing or *ex novo* porous biocatalysts), simplifying the protease storage and application [25]. This should improve the operational stability of the proteases, but also makes a direct comparison to the stability of the free and immobilized proteases troublesome, as some artifacts may arise, deriving from the fact that this inactivation cause is prevented by immobilization even if the enzyme rigidity is not improved [39]. The use of immobilized enzymes also offers cleaner results when used to give proteolytic maps, as it is the case of the tryptic maps performed using trypsin used in some instances [89–91].

On the other hand, protease immobilization, when the biocatalyst is going to be used to hydrolyze proteins, presents a specific problem. Due to the large size of the substrate, only properly oriented enzyme molecules may be active by steric hindrances, as the protein molecules will be unable to access those enzyme molecules whose active center is oriented towards the support surface (Fig. 1) [39]. Moreover, a further problem is that no fully properly oriented proteases may give a response to the increase in the enzyme loading of the support quite unexpectedly. Using low enzyme loading, when the immobilization rate is slow and the immobilized enzyme molecules are sufficiently distanced between one another, the active centers of the proteases will be able to hydrolyze proteins (Fig. 2A). Using full enzyme loading, the steric hindrances will promote the actual blocking of the active centers of the proteases for large proteins, which will be active only versus small substrates (Fig. 2B) [80]. Similarly, the results of immobilizing the protease on a support under conditions where a rapid immobilization or a slow one are obtained, should provide a fully different result even using moderate enzyme loadings (Fig. 3). We have not found any references in the literature discussing this. However, using conditions where a low immobilization rate is obtained, the enzyme can diffuse in the particle pores before being immobilized (Fig. 3A). This way, it will be distributed on the support surface and will provide a higher percentage of active molecules than if the enzyme is immobilized so quickly that the enzyme molecules are packed together forming a crown at the beginning of the support pores (Fig. 3B) [92–97]. In some cases, some unexpected effects of the enzyme loading may be found, as is the case of ficin immobilization in the production of milk clotting, where the optimal results were obtained using a fully loaded biocatalyst even though the caseinolytic activity decreased [87].

Another problem is the possible very large size of the substrate proteins. If we are using a protein extract as substrate, it is likely that some of the protein reactants may be really large, and this makes using supports bearing large pore diameters to immobilize proteases necessary, choosing the size not only by the protease size, but considering the size of the larger component of the planned substrate extract (Fig. 4A). This problem may be turned into an advantage if we do not wish to modify some very large proteins (e.g., IgG in milk), as the enzyme will be only able to act versus moderately small protein molecules (Fig. 4B). Again we have not found any bibliography in this regard.

The situation is even more complex if the substrate is a solid, like protein aggregates after oil extraction or using the proteases in textile products. In the first case, the use of chaotropic agents can permit the re-solubilization of the substrate proteins, but this makes having a protease stable enough under these drastic conditions compulsory [79,98] and this may complicate the downstream process. In the case of solids that cannot be re-solubilized, the only way is to use immobilized enzymes that can act versus a solid, that reduces the possibilities towards the immobilization of proteases to the use of nanoparticles [39,99–104] or smart-polymers [105,106] (Fig. 5). Nanoparticles permit enzyme stabilization via multipoint covalent immobilization, but they do not prevent enzyme inactivation by autolysis or interaction with interfaces [107] (Fig. 6). Magnetic nanoparticles may permit the recovery of the biocatalyst even in a suspension product formulation, as it is the case in many foods (also magnetic porous macro-supports may take advantage of the magnetic features) [108]. Smart polymers should have scarce effects on enzyme stability, enabling just the reuse of the enzymes, as long as the final product is a solution and not a suspension [105,106].

That way, papain immobilization is a subject of special relevance to utilize the enzyme in food technology [46]. This is the first review in literature on this subject, as far as we know.

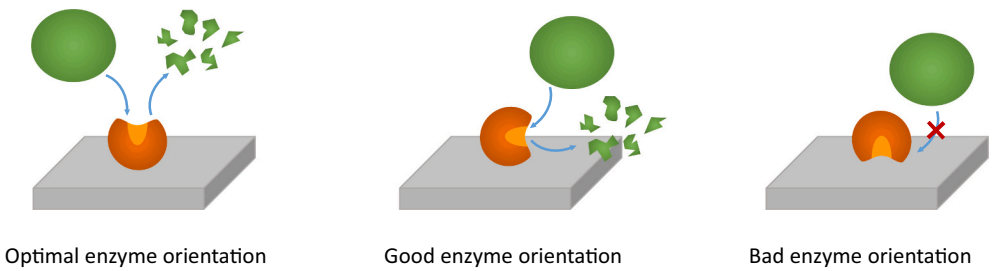

Fig. 1. Effect of the enzyme orientating regarding the support on its activity versus large substrates. If the enzyme active center is oriented towards the support surface, the generation of steric hindrances by the support surface will fully inactivate the immobilized enzyme. Other orientations may result in high levels of activity.

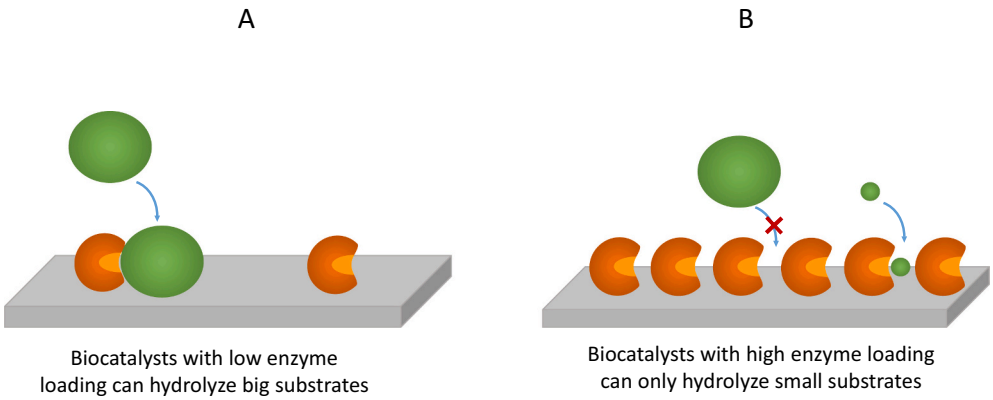

Fig. 2. Effect of protease loading on the activity versus large substrates when the orientation is not the correct one. Using low loadings, even enzyme molecules with active centers not fully oriented towards the medium may be fully active versus large proteins. However, using high loadings, with the enzyme molecules packed together, the generation of steric hindrances by the proximity of other protease molecules can seriously decrease or even supress enzyme activity versus large substrates.

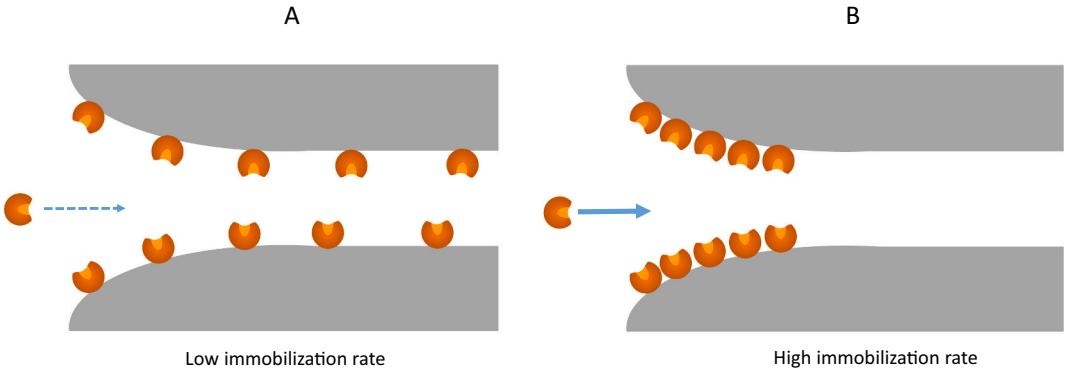

Fig. 3. Effect of the immobilization rate on the enzyme distribution on the pore of a support: A: immobilization rate is much slower than enzyme diffusion rate. This enables a homogenous enzyme distribution along the whole support pores B: immobilization rate is must higher than the enzyme diffusion rate. This will form a crown of enzyme molecules in the outer part of the pores of the support, while the other will be void. This kind of immobilized enzymes will suffer the same problems described in Fig. 2.

3. Immobilization of papain

3.1. Immobilization of papain in organic natural supports

3.1.1. Immobilization of papain in agarose beads

Agarose beads matrix is a support utilized by many researchers due to their good properties: it is commercially available at different and very well defined pore sizes. It is also possible to find it in different particle sizes, it is a highly inert support (in fact, it is used in the size exclusion chromatography where any protein-support interaction will be negative [109,110]), it is very flexible and compatible with mechanical stirring, and there are methods for its activation with very different active groups [68]. It is also transparent, making it possible to study the enzyme fluorescence or UV absorption spectra [68]. That way, it is a very popular support to immobilize enzymes, as it permits full control of the events occurring between enzyme and support: the only groups present are those introduced by the researcher [66]. Thus, this support has been used for immobilizing papain for a long time.

First, we will present the results of immobilization of papain by covalent methods. The first report in that sense that we have found is from 1977. The authors used agarose 4B activated with cyanogen bromide. This reagent was very popular to immobilize enzymes in the past, involving mainly the non-protonated primary amino groups in the immobilization. However, although it is very efficient in immobilizing enzymes under mild conditions [111,112], actual enzyme intense rigidification caused by the enzyme immobilization itself is not expected, as this reagent is used at neutral pH due to the moiety stability problems, and Lys residues are not very reactive under those conditions. This makes getting an intense multipoint covalent immobilization complex, but if properly performed, some enzyme stabilization may be achieved [113]. However, most of the examples of immobilization of papain on agarose that we have found used this activation method. Thus, the protease was immobilized on agarose 4B activated with cyanogen bromide [114]. The loading of the support was 17 mg proteins/g dry agarose. The K_M of the immobilized enzyme was very similar to that of the free enzyme, while the storage stability greatly increased (the activity remained unaltered after 240 days at pH 7.5 and 4 °C). The immobilized enzyme retained its activity unaltered after 4.5 h of incubation in 6 M urea, conditions that decreased the activity of the free enzyme to less than 20%. The authors did not investigate the percentage of improvement due to real enzyme rigidification and due to prevention of autolysis [114,115]. Later, the same activation protocol but using agarose 6B support was utilized to investigate the effect of Cys during enzyme immobilization on the residual activity of the immobilized papain [116]. Cys improved the recovered activity, with a maximum effect when using 200 mM. While the kinetic parameters remained unaltered, the new biocatalyst was more thermostable than the free enzyme and also became more stable at extreme pH values. The optimal temperature for the immobilized enzyme improved from 60 to 80 °C, enlarging the window of enzyme operational conditions also when compared to the enzyme immobilized in absence of Cys [116]. In a further research effort, the authors showed that the enzyme resistance to the inhibition caused by various bivalent metals was increased on the immobilized enzyme, also showing a shift in the optimal pH from 6.5 to 8.0 [117].Papain was also immobilized using glyoxyl groups, that are more adequate to get an intense multipoint immobilization [67,74,118].

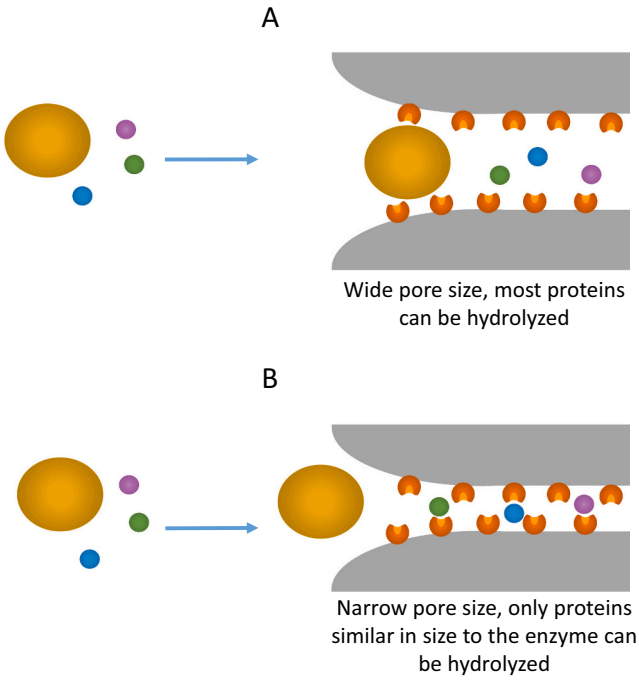

Fig. 4. Effect of the support pore size on the activity of immobilized enzymes versus large and small substrates. If the pore diameter is large, (A), proteins of most sizes can go inside the biocatalysts and the immobilized enzyme will be active versus substrates of all sizes. If the pore of the support is small enough (B), only proteins able to enter the pore will become substrates for the enzyme, and even that may be submitted to high diffusional limitations.

That way, papain was immobilized on 6BCL glyoxyl-agarose and utilized in the hydrolysis of casein and β-lactoglobulin with poor results [119] However, after a preliminary hydrolytic treatment of the substrates employing immobilized trypsin, the immobilized papain yielded the maximum degree of hydrolysis in a shorter time than the free enzyme.

There are not many papers dealing with agarose to immobilize enzymes by physical events [120]. A likely explanation could be that, if the support is not fully coated with the protease, the protein substrate could become adsorbed on the support, making any study complex. The example that we have found is the immobilization of papain on agarose beads activated with immobilized metal chelates. This method is based mainly on the interaction between His (but also amino acids with a lower intensity), and immobilized transition metals. This nowadays is a common support to purify poly-His tagged proteins [121]. Thus, this support was utilized to reversibly immobilize papain [122]. The immobilization permitted to maintain the enzyme activity while improving its thermal stability. The support could be reused after enzyme reuse and utilized in the immobilization of a fresh batch of papain, obtaining a biocatalyst with similar performance [122].

3.1.2. *Immobilization of papain on cellulose beads*

Cellulose is the most abundant biological polymer in Nature, and the beads produced from it have been used in many instances for enzyme immobilization. It has been performed in the form of non-porous nanoparticles [123] or macroporous particles [124,125]. They have been also produced in magnetic formulations, adding ferrite during the bead building [126,127]. These magnetic supports may have some special interest when used in diverse situations, as it has been recently reviewed [108]. And papain has been frequently immobilized on cellulose beads.

One of the support activation methods utilized to immobilize enzymes on cellulose is the direct oxidation of the support with sodium periodate, that produces a di-aldehyde, able to react with primary amino groups of proteins [128–130]. In a first paper, wood chip was oxidized with sodium periodate and used to immobilize papain, with a high retention of enzyme activity [131]. The optimal temperature of the enzyme increased after immobilization by 7 °C. The immobilized enzyme was then used to reduce beer turbidity caused by large peptides. After 28 days of usage, the initial activity was maintained [131].In another research effort, agarose beads were incubated in 1-butyl-3-methyl imidazolium (a ionic liquid) and oxidized with $NaIO_4$, and this support was utilized to immobilize papain, with high activity recovery (stability was not analyzed) [132]. Later, this research group utilized a similarly treated cellulose in different configurations, including powders, membranes and beads [133]. Membranes had the higher papain loading capacity papain loading and immobilized enzyme activity. In an interesting paper, the authors went from cellulose production to the use of the immobilized enzyme on beads formed by this cellulose [134]. First, bacterial cellulose was produced using *Komagataeibacter hansenii*, purified and oxidized with $NaIO_4$ to produce aldehyde groups. This home-made support was used to immobilize papain after surface response methodology optimization (with an optimal immobilization yield over 50% at 45 °C and pH 7.0 and a expressed activity of almost 95%). As the paper did not use higher pH values during immobilization, it is hard to know if these are really the best conditions for papain

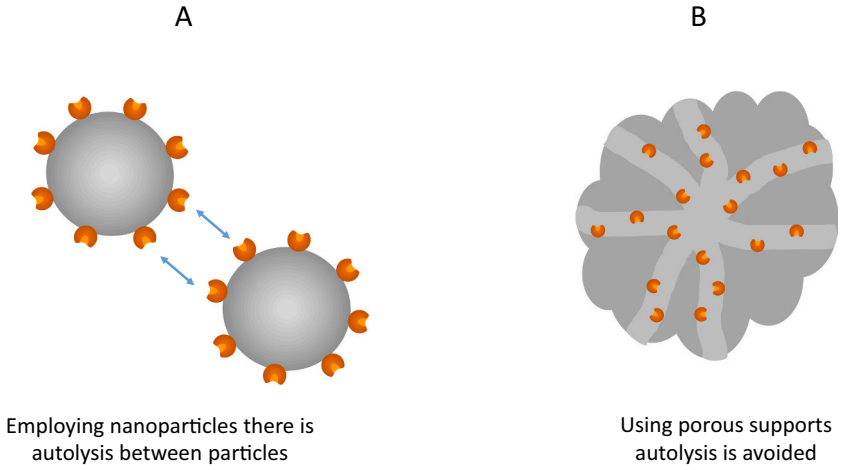

Fig. 5. Prevention of autolysis via immobilization. Enzyme molecules immobilized on non-porous supports can interact with the enzyme molecules located in other particles and produce autolysis, but not with enzyme molecules located in the same particle. (A). When using porous supports, the immobilized enzymes are fully protected from proteolysis from enzyme molecules located in the same or in other particles (B).

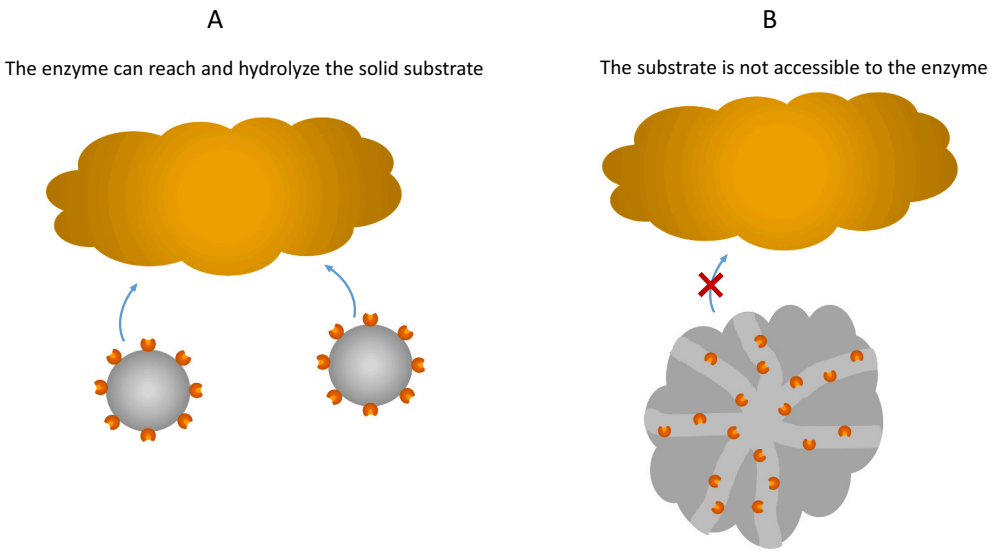

Fig. 6. Hydrolysis of solid substrates by immobilized enzymes. The solid may be hydrolyzed by proteases immobilized on non-porous supports (A), while protease molecules immobilized on porous supports will not have access to the substrate (B).

immobilization on aldehyde activated cellulose, at this pH only terminal amino groups will be reactive (but if the papain suffers autolysis, it may be that each enzyme molecule has more than one of these groups). Immobilization on non-oxidized supports gave a lower immobilization yield, based just on ion exchange. The covalently immobilized enzyme was successfully used in debrided chronic wounds [134].

Many other examples of immobilization of papain in cellulose use glutaraldehyde to covalently immobilize the enzyme to the support. Unfortunately, none of them exploits the versatility of this immobilization strategy. That way the potential of the strategy is under-utilized [73]. The exact mechanism of the covalent enzyme- glutaraldehyde-support linkage is not fully solved [135,136], but the method is very popular. The versatility of glutaraldehyde is based on the heterofunctional character of this support [69], bearing a cationic group (the amino groups in the support), a hydrophobic moiety (the glutaraldehyde cycles) and a chemically reactive group. That way, it is possible to obtain different biocatalysts of the same enzyme using the same support by selecting conditions where one or the other immobilization mechanism is favored as first cause for enzyme immobilization, giving biocatalysts with different stabilities and activities [79,81,137–140]. Moreover, it is possible to use preactivated supports [141] or to treat with glutaraldehyde enzymes that have been previously ionically exchanged on the support [142]. That way, in many instances, glutaraldehyde based immobilization offers very good stabilization results [67] although if the studies are not properly performed, it is not simple to ensure that even a single enzyme-support covalent bond has been formed, the enzyme may be just physically adsorbed on the support. In one interesting paper using glutaraldehyde to immobilize papain, this enzyme was immobilized on microcrystalline cellulose in a 3 step modification. First, the cellulose beads were modified with p-toluenesulfonyl chloride. Then, this group was utilized to attach ethylenediamine to the support, and finally the support was activated using glutaraldehyde [143]. The immobilized enzyme kept high levels of activity and its stability was significantly improved. Another example shows that cellulose beads were incubated in 1-butyl-3-methyl imidazolium chloride, an ionic liquid and modified silane [144]. Then, papain was immobilized following two different protocols: first immobilization by ion exchange and after treatment with glutaraldehyde, or activate the support with glutaraldehyde and then immobilize the enzyme. The best results were obtained using the crosslinking of the previously ionically exchanged enzyme [144]. In a further effort, this research group studied the immobilization of papain in membranes obtained from microcrystalline cellulose Avicel, activated with glutaraldehyde as described above [145]. The enzyme was immobilized on both sides of the membranes and utilized in the modification of wool fiber, but acting only on the external surface of the textile. In another paper, magnetic nanocrystalline cellulose was coated with chitosan and finally activated with glutaraldehyde. The support was used to immobilize papain [146]. This biocatalyst was utilized to produce the dipeptide N-(benzyloxycarbonyl)-alanyl-histidine in deep eutectic solvents [147–151] following a kinetically controlled strategy [152–154]. As this strategy gives a transient maximum yield that depends on the enzyme properties [155–158], results using the immobilized enzyme were higher than those obtained using the free enzyme. After studying the reaction, a dipeptide yield next to 70% was achieved [146]. The biocatalyst could be recovered and reused thanks to its magnetic properties.

3.1.2.1. Immobilization of papain on cotton fabric. A special kind of cellulose is the one derived from cotton fibers. Cotton has been frequently utilized to immobilize enzymes [159–161], and papain has been one of the examples. In a first paper, cotton fiber was oxidized with sodium periodate and used to immobilize papain, with a high retention of enzyme activity [131]. The optimal temperature increased by 7 °C after immobilization. The immobilized enzyme could be used to reduce beer turbidity for 28 days [131]. Researchers used pieces of cotton cloth to incubate them in a sol-gel solution including papain, that way the sol-gel solidified in the cotton textile involving the enzyme [162]. Immobilization increased the pH window of enzyme operation. The biocatalysts could be reused six times maintaining 30% of the initial activity [162]. Later, this biocatalyst was applied to design a cotton cloth bioreactor, used in the hydrolysis of chitosan [163]. The authors did not provide clear evidences that this promiscuous activity is really a reaction catalyzed by papain and not for other components of the extract.

Three papers show the advantages of integrating chemical modification and enzyme immobilization. The papers used a cotton fiber submitted to alkaline treatment and incubation with *p*-toluenesulfonyl chloride, followed by ethylenediamine treatment and activation with glutaraldehyde. In the first paper, papain was modified with pyromellitic acids [164]. The optimal pH was moved from 7.0 to 9.0, with a significant improvement in enzyme stability compared to the immobilized and not modified enzyme. In the second paper, papain was modified by using succinic anhydride and then immobilized on the treated cotton fiber [165]. The optimal temperature increased to 80 °C

and the optimal pH was shifted to pH 9.0. The modified and immobilized enzyme maintained 40% of the activity in the presence of 20 mg/mL of sodium dodecyl sulfate. Stability of the modified and immobilized enzyme improved compared to the unmodified and immobilized one [166]. In the last one, papain was modified using different anhydrides of 1,2,4-benzenetricarboxylic and pyromellitic acids before the modification on the treated cotton fiber. The optimal pH for the immobilized enzyme activity became 9.0 (from an initial value of 6.0 of the free enzyme). The non-modified and immobilized enzyme was less stable at high temperature, alkaline pH values or in the presence of detergents.

3.1.3. Immobilization of papain on chitosan

Chitosan is a polysaccharide derived from chitin, that forms the exoskeleton of arthropods and is also present in some other living beings [167,168]. It is produced by the partial deacetylation of 2-acetyl-glucose amine [169–172]. Chitosan is rich is hydroxyl groups and glucosamine. This makes it a weak anion exchanger, due to the low pK of the amino group. However, it is easy to activate this primary amino group with a variety of activating agents to get a covalent enzyme immobilization, such as glutaraldehyde, epichlorohydrin, divinylsulfone, genipin, etc. [173–180]. The hydroxyl groups make it also possible to use other activating techniques, like oxidation with sodium periodate to form dialdehyde groups. That way, this support provides a wide variety of possibilities to immobilize enzymes [181], and it has been frequently used in papain immobilization. The use of the natural crosslinking reagent genipin [182] to activate the support has special interest, as that way the support will be fully natural and generally recognized as safe (GRAS) by the FAD [178,183–187]. However, we have not found any example of papain immobilization following this strategy. The effect of the spacer arm in the activity versus small and large substrates of covalently immobilized papain in chitosan beads modified with diamines of different lengths was studied, using N-hydroxy succinamide as activation method [188]. It was found that the activity versus N-benzyl-l-arginine ethyl ester was similar independently of the spacer arm, but when casein was used as substrate, higher activity was found using longer spacer arms. Enzyme stability was also improved after immobilization. In this case, the spacer arm length had a negative effect on the final enzyme stability [188].In another paper, the authors immobilized papain on chitosan using glutaraldehyde [189]. The optimal temperature of the immobilized enzymes was increased to 80 °C. The immobilized enzymes retained 95% of their initial activity after 1 h of incubation at 90 °C [189]. In another research effort, papain was immobilized on this support using glutaraldehyde and used to produce chitosan oligomers, a function quite far from the natural function of a protease [190]. The depolymerization efficiency of the papain extract increased after immobilization. However, we cannot rule out that this activity may be associated to other components of papaya latex not detected by the authors of this paper, as the mechanism of this promiscuous activity was not investigated. Another research work shows the immobilization of papain purified using an aqueous two phase system [191–193] just by adding the chitosan particles to the PEG phase, that was the phase where the enzyme was accumulated [194]. The ion exchange of the papain on the chitosan particles gave 90% of immobilization yields and 40% of expressed activity. Another paper shows that papain immobilized on chitosan particles using a glycine buffer via anion exchange had a 90% of expressed activity (only 30% of immobilization yield), and this biocatalyst was successfully utilized as an antibiofilm agent and also as a bactericide versus bacteria in biofilms [195]. The immobilized enzymes were more stable than the free one, by a 6 -7 factor (perhaps just by preventing enzyme autolysis). The enzyme could release from the support, but this fact was utilized to use the material in wound-dressing materials, decreasing the cytotoxicity of free papain [195].

Papain immobilized on chitin and chitosan was utilized to prevent the problem of *Saccharomyces cerevisiae* flocculation during ethanol production [196]. The modification with polyethyleneimine and glutaraldehyde presented a negative effect on enzyme activity, while the best results were found using tripolyphosphate. However, the results were not satisfactory, and the authors finally proposed the use of free enzyme, recovering the enzyme by centrifugation. Papain sensitivity to UV radiation was compared to that of ficin and bromelain in free and immobilized forms [197]. Papain activity decreased at UV intensity of 453 $J{\cdot}m^{-2}$, and its globule size increased using 755 $J{\cdot}m^{-2}$. Immobilization on chitosan matrix of the three proteases leads to the increase in the UV stability, suggesting that the chitosan matrix behaves as photoprotector of the enzymes [197].

3.1.4. Immobilization of papain on alginate

Sodium alginate is utilized in many instances to immobilize cells by the trapping technique [198,199]. To immobilize enzymes, the pores of the sodium alginate beads need to be very small, and for that reason, they are not often utilized to immobilize free enzymes, although some examples may be found [199–202]. However, as this is a sulfhydryl protease that can be affected by metal ions, and alginate can capture them [203–205], protecting the enzyme from the deleterious effects. This immobilization strategy has been used in several cases. Thus, the adsorption of mercury [206], lead [207], and cadmium [208] on papain immobilized on alginate beads has been analyzed, showing how the alginate protects the enzyme from the inactivation promoted by these agents. In another paper, this method of enzyme immobilization was used to produce a wound dressing product [209]. Alginate has some positive effects as wound healing material, and papain produces debridement of necrotic or devitalized tissues. In this case, the controlled papain release from the alginate beads may be an advantage. The development of dressing based on alginate and papain aggregates showed that over 64% of the enzyme was released after 24 h using the Franz cell study [209].

3.2. Immobilization of papain on synthetic organic supports

Synthetic polymeric supports have some advantages compared to the natural polymers in enzyme immobilization [64,210–214]. Using these supports, the chemical structure of the matrix may be designed by the researcher. However, it is difficult that a synthetic polymer may end up with properties as “ideal” as agarose as a matrix for enzyme immobilization [68].

3.2.1. Immobilization of papain on synthetic organic supports beads

This kind of polymeric beads has been frequently utilized to immobilized papain. In 1979, 1.5 mg of papain/g of support were immobilized on nylon grafted with polyacrylamide and activated with glutaraldehyde [215]. Later, papain was immobilized on matrixes formed by radiation induced polymerization of different monomers at low temperatures [216]. Using monofunctional acrylate or methacrylate supports, the activity was higher than that observed when immobilizing the enzyme on supports produced from bifunctional bismethacrylate. Using polyoxyethylene dimethacrylate, the activity of the immobilized papain increased when the number of oxyethylene units was increased. The thermal stability of the immobilized enzyme increased with the hydrophilicity of the monomers and with the monomer concentration [216].

Glutaraldehyde has also been utilized in many instances in this kind of supports. For example, after being anion exchanged in an aminated resin, the immobilized papain was treated with glutaraldehyde [217]. The immobilized enzyme activity increase up to 85 °C, and then slightly decreased at 95 °C. Immobilized enzyme stability was increased using Cys solutions or nitrogen purging during inactivations. The enzyme was used for 30 h in a continuous-flow stirred-tank using both nitrogen purging and 0.01 M cysteine in 2 mM EDTA at 51 °C [217]. In another research effort, papain was ionically exchanged in Dowex MWA-1 (mesh 20–50) and then treated with glutaraldehyde [218]. The presence of 80 mM Cys and 2 mM EDTA maintained more than 95% of the biocatalyst

initial activity for 2 months at 5 °C. Moreover, a macroporous bead formed by N-aminoethyl acrylamide and vinyl alcohol units was utilized to immobilize papain using glutaraldehyde [219]. The papain activity reached a value of 48% - 58%, becoming more stable than the free enzyme. The same research group produced other papain biocatalysts using a support containing primary amino groups prepared using nitrilon fiber and diethylenetriamine, also using the glutaraldehyde chemistry [220]. The recovered activity was around 50%, with a substantial stabilization. In another research, aminated methyl methacrylate-divinylbenzene copolymer beads were utilized to immobilized papain by the glutaraldehyde chemistry [221]. The casein lytic activity of the immobilized enzyme remained over 60% while stability was improved. This immobilization also produced an enzyme less susceptible to inhibition while the optimal temperature reached 70–90 °C [222]. Moreover, the immobilized enzyme could be used in the pH range between 5 and 10, while the free enzyme could be used only at neutral pHs values. The immobilized enzyme maintained almost 100% of the activity after one month at 4 °C [222]. Later, the same research group, using N-alpha-benzoyl-L-arginine amide hydrochloride as substrate studied the intra-particles localization of the immobilized enzyme using X-ray micro-analysis [223]. The released NH_3 was captured by $FeCl_3$, which formed a precipitate. This was utilized to pinpoint active immobilized papain molecules. The experiment revealed that the enzyme is uniformly distributed on the biocatalyst particle [223]. In another research, immobilized papain was used to avoid the bacteria contamination of polyurethane-based ureteral stent, using the glutaraldehyde chemistry [224]. The immobilized enzyme retained 85% of its activity, and was able to reduce the growth of *Staphylococcus aureus* and *Escherichia coli*, reducing also the carbohydrate and protein content in biofilms. This coating also decreased the deposition of magnesium and calcium salts. Moreover, the authors claimed the low cytotoxicity of this coating [224]. In another paper, the enzyme was immobilized on poly(vinyl alcohol) nanofibers prepared by electrospinning, using the glutaraldehyde chemistry [225]. Optimal loading in terms of activity was found when 130 mg/ g of monomer was utilized. The expressed activity was almost 90%. The immobilized biocatalysts maintained its catalytic activity after six reuses [225]. In other publications, papain was immobilized in two epoxy activated supports, Eupergit C and VA Biosynth [226]. Epoxy supports are among the most utilized ones in enzyme immobilization, as they are prepared directly in an activated form, can be stored for long time periods and can react with very different moieties of the enzyme [64,76,227,228]. The enzyme immobilization on these supports, due to the low reactivity of the epoxy groups, follows a two-step mechanism (the enzyme is usually first immobilized to the support by an interaction different from the enzyme-epoxy groups reaction [69,229,230]), except if the enzyme has some Cys residues on the surface [231]). In this first example of papain immobilization using the epoxy chemistry, high levels of activity were maintained, being the stability higher when using VA Biosynth [226]. Papain immobilized on porous poly(glycidyl methacrylate) beads were used in the production of antioxidant peptides by hydrolysis of yeast proteins [232]. The prepared biocatalyst had an enzyme loading of 66.5 mg/g, with an expressed activity of over 60%. 35% of the biocatalyst initial activity was observed after 20 reuses. The enzyme was also immobilized on poly(hydroxyethyl methacrylate-ethylene glycol dimethylacrylate) particles and decorated with fibrous poly(glycidyl methacrylate) to give to the support epoxy groups [233]. Under these conditions, 18.7 mg of papain per g of this support could be immobilized. The V_{max} slightly decreased after immobilization (around a 20%) while K_M increased by over a 50% [233]. Papain was also immobilized on poly(hydroxyethyl methacrylate-*co*-glycidyl methacrylate) cryogel-based, and this biocatalyst was successfully utilized in the fragmentation of immunoglobulins [234]. The biocatalyst presented optimal activities at 70 °C and pH 7.0, while the kinetic constants of the enzyme remained almost unmodified after immobilization.

In all these papers using epoxy supports, the immobilized enzyme was not incubated to increase the enzyme-support multipoint covalent attachment nor was the support blocked as a reaction end point. That way the full prospects for this immobilization protocol have not been fully exploited [228].

Papain has also been covalently immobilized on poly-L-lactic acid polymeric beads using the carbodiimide route, and its anti- *Clostridioides difficile* activity was verified [235].

Physical immobilization of papain on polymeric supports has also been studied. The immobilization of papain in hydrophobic Amberlite XAD-8 permitted to increase the performance of the enzyme in the ethanol esterification of some N-benzyloxycarbonyl (*Z*)-dipeptides [236]. The breakage of the peptide bonds during the esterification was not appreciated. Later, papain was covalently immobilized on low density polyethylene, high density polyethylene, linear low density polyethylene or polycaprolactam beads and used to prevent food contamination [237]. Support immobilized curcumin was utilized as photocrosslinker [237]. The immobilized enzyme became more stable than the free one. The best anti- *Staphylococcus aureus* NCIM 5021 and anti-*Acinetobacter* sp. KC119137.1 activities were obtained using the enzyme immobilized in linear low density polyethylene [237].In another paper, reactive Green 19 dye modified poly(acrylamide-methyl methilacrylate) cryogels were utilized to immobilize papain. Maximum loading was 40.66 mg/g of support g at pH 5.5 and 25 °C [238].

One recurrent subject of study employing these polymeric supports to immobilize papain is the role of the spacer arm. The spacer arm may play several roles. First, the longer the spacer arm, the higher the prospects of getting an intense multipoint covalent immobilization, but also the lower rigidification per each new bond attained [239]. It should be considered that the reactivity of all groups may not be identical when the length increased, e.g., using a diamine to activate the support, the pK of the primary amino groups will be higher when the diamine is larger, and therefore its reactivity will be lower [71]. Moreover, as these groups coat the support surface, they may alter the physical properties of the support and that way tune the possibilities of enzyme-support interactions [66]. The enzyme mobility will be higher when a long spacer arm is used, mainly if one or just some few points of the enzyme react with the support, enabling the action of the immobilized enzymes with large molecules [240].Thus, the importance of the spacer arms in the recovered activity in the covalent papain immobilization was studied using a crosslinked polystyrene or polyacrylamide supports [241]. Results showed that a longer spacer arm enabled a higher immobilization yield and activity in the support. The employment of polyethylene glycol gave the highest activity. The most hydrophilic polyacrylamide support gave a higher activity than the hydrophobic polystyrene one [241]. Later, these biocatalysts were utilized to produce peptides in aqueous-organic solvent mixtures [242]. Among the many studied biocatalysts, papain immobilized on tetraethyleneglycol-crosslinked polystyrene or polystyrene-PEG supports were the most effective catalysts for this reaction [242].

Papain has also been covalently immobilized on poly(λ-methyl L-glutamate)beads, to study the effect of the spacer arms again [243]. Using the azide method to immobilize the enzyme, the activity was good versus the small N-benzyl L-arginine ethyl ester, K_M was increased after immobilization while V_M was decreased and the thermal stability was increased. Immobilized enzyme activity remained unaltered during storage or when used in batch reactions [243]. In another paper, polyethylene and glass surfaces were modified under dichlorosilane-RF-cold-plasma environments, and activated with spacer arms of different lengths [244]. Again, the longer the spacer arm, the longer the activity recovery. In another research, an aminated-polystyrene (0.4–6.0 mmol $NH_2 \cdot g^{-1}$) was utilized to immobilize papain using a flexible spacer arm, aldehyde dextran [245] doubling the activity achieved when the enzyme was directly immobilized in the support [246].

In some instances, several organic materials are utilized to prepare a support with improved features. For example, Roy et al. reported the stabilization of papain (chemically modified using succinic anhydride)

by entrapping it in alginate/starch beads. The immobilized papain retained its activity even after six cycles of hydrolysis, and its storage stability was also increased [247]. In another work, chitosan and dialdehyde starch support were synthesized by coupling flexible dialdehyde starch chain onto the surface of chitosan support, and this was used for papain immobilization. The flexibility of the spacer arm improved the recovery of immobilized enzyme activity over traditional spacer arms [248]. In another interesting study, papain immobilized on fibrous polymer-modified composite beads (poly(methacrylic acid)-grafted chitosan/clay), were used for mercury elimination. The maximum removal capacity of the composite beads has been found to be 4.88 ± 0.21 mg Hg/g when the initial metal concentration and weight of polymer-modified composite beads were 50 mg/L and 0.04 g at pH 7.0, respectively [248]. Papain enzyme was also covalently immobilized onto an interpenetrating network obtained by cryogelation of *N*,*N*′-methylenebisacrylamide cross-linked 2-hydroxyethyl methacrylate and glutaraldehyde cross-linked chitosan. It was found that immobilization enhanced the stability of papain compared to free form, and improved reusability and storage stability [249]. Finally, papain immobilized on choline chloride- lactic acid (ChCl-Lac) deep eutectic solvents-treated chitosan exhibited excellent thermostability as compared to the free enzyme. The results also showed that Deep eutectic solvents could control the active group content, thus achieving the appropriate microporous structure of immobilized enzyme, and better catalytic microenvironment [250].

3.2.2. Immobilization of papain in polymeric membranes

The immobilization of enzymes in membranes may be adequate for some specific applications (e.g., in pharmaceutics, wastewater treatment, biorefinery, biomedicine, food processing) or reactor conformations [251–254]. Thus, there are some examples of immobilization of papain in this kind of supports. In a first example, papain was covalently attached via the glutaraldehyde chemistry to a membrane formed by vinyl alcohol/vinyl butyral copolymer [255]. The biocatalyst was stable under storage conditions, and the stability improved compared to that of the free enzyme in stress thermal inactivation at different pH values. K_M increased after immobilization, although this increase could be diminished when increasing the spacer arm length [255]. Papain has also been immobilized in a fully hydrated polysulfone membrane. Immobilization altered the conformation of the active site of the enzyme, and there are two main enzyme populations [256]. One subpopulation seemed to have a more open active-site cleft than the other subpopulation. This last subpopulation is less sensitive to the pH of the bulk solution the other subpopulation, which had a response to changes in the pH similar to the free enzyme [256]. The addition of guanidine or urea, or the incubation at high temperature, converted the enzymes with the more open active-site cleft on the other subpopulation. This seems to be the active fraction of the immobilized molecules. That way, K_M is higher and V_{max} is lower than those of the free enzyme. This immobilized papain has good storage and operational stabilities, being also more resistant to the presence of guanidine [256].Later, this group immobilized papain on hydroxyethyl cellulose coated polyethersulfone hollow fibers and flat-sheet modified polysulfone membranes [257]. The hollow fiber retained double of the activity of the membrane (25%), and the activity on the membrane biocatalysts decreased when the enzyme loading increased. Immobilization on both supports decreased both K_M and V_{max}. They confirmed the existence of two enzyme subpopulations, one inactive and one active. In a new research effort, this research group immobilized papain on poly(ether)sulfone membrane using the avidin-biotin immobilization strategy [258]. This immobilization strategy is based on the strong affinity between avidin and biotin, and requires the modification of the papain [259–262]. The immobilization via the avidin-biotin strategy gave better activity than the direct immobilization on the support [258]. In fact, K_M decreased and V_{max} increased upon immobilization in this new example. The authors claimed an increased enzyme stability [258]. Other research groups also immobilized papain in polymeric membranes. For example, papain immobilized in a membrane was utilized to analyze the non-linear dynamic performance of a diffusion-reaction, generating external oscillations of pH or substrate [263]. In a last paper on this subject, nylon membranes were acid hydrolyzed and coated with chitosan that was activated with glutaraldehyde, and finally used to immobilize papain [264]. The immobilized enzyme was successfully utilized to purify cystatin from potato juice.

3.2.3. Immobilization of papain using smart polymers

Smart polymers are polymers that change the solubility/insolubility status depending on the reaction conditions (e.g., pH or temperature). If enzymes are attached to these polymeric molecules, the enzyme may be in a soluble status during operation, and by changing the conditions, its precipitation may be achieved and that way the enzyme may be easily recovered and reused [105,265–272]. Although they should have moderate stabilization potential (as they are flexible structures), they may be a good solution to design recyclable biocatalysis to be used versus large substrates, like proteins. This way, there are some examples of immobilization of papain in this kind of polymers. For example, papain was immobilized on poly(methyl methacrylate/N-isopropylacrylamide/methacrylic acid) and poly(styrene/N-isopropylacrylamide/methacrylic acid) latex beads by the carbodiimide method [273]. The immobilized enzyme could be recovered by thermos-flocculation, dispersed by lowering temperature and reused. The biocatalyst prepared in poly(methyl methacrylate/N-isopropylacrylamide/methacrylic acid) was very active versus casein and can be submitted to several cycles of thermal precipitation without decreasing its activity [273]. Papain was later immobilized using the N-hydroxysuccinimide strategy [274–277] in poly (N-isopropylacrylamide) [278]. The optimal pH of the immobilized enzyme was 7.5, optimal temperature was 65 °C. After 24 cycles of thermal flocculation, more than 85% of the enzyme activity was maintained. After storage at 4 °C for two months, the immobilized enzyme maintained more than 70% of the initial activity. Stability was improved at different pH values. The biocatalyst was successfully utilized in the hydrolysis of HCG monoclonal antibody [278].

3.3. Immobilization of papain on inorganic supports

Inorganic materials with pore sizes between 2 and 50 nm are classified as mesoporous materials, and may have very special properties such as uniform vertical pore channels, large pore volume (1.5 cm^3/g), adjustable pore size, thermal, mechanical and chemical stability, high specific surface area (as high as 1500 m^2/g), modulable mesopore inwalls and the possibility of surface modification [279,280]. These properties make them very versatile materials, being attractive for a wide variety of applications such as electronics, separation, energy storage, photocatalytic hydrogen production, solar cells, battery components, environmental rehabilitation, drug delivery, biosensors, clinical treatment and catalysis [279,280]. In the case of biocatalysis, these materials have been widely used in enzyme immobilization, mainly because the properties of the support can be tailored to the biomolecules, making it possible to obtain matrixes with the desired pore structure [281]. In this way, enzymes like lipase from *Candida antarctica* [282] or *Candida rugosa* [283], tyrosinase [284] and many others, have been immobilized by encapsulation, covalent bonding, physical adsorption, and cross-linking in mesoporous materials such as metal oxides, zeolites, carbon structures, hybrid materials, silica and others shaped in different particle morphologies such as spheres, hollow spheres, fibers, rods, etc. [279,281,285]. Papain has been immobilized on activated charcoal by physical adsorption for the removal of mercury. The immobilized papain (papain concentration 40.0 g/L, activated charcoal amount 0.5 g and pH 7) shows a maximum removal reached in the batch study of 99.4%, when initial metal concentration and weight of biocatalyst were 20.0 mg/L and 0.03 g respectively [286]. Papain activated with N-hydroxysuccinimide and N-ethyl-N'-(3-dimethyl

aminopropyl)carbodiimide was immobilized on an amine-modified gold surface and was used to build a Surface Plasmon Resonance Imaging sensor for specific determination of cystatin. The sensor's dynamic response range is between 0 and 0.6 $\mu g.mL^{-1}$, and the detection limit is 0.09 $\mu g.mL^{-1}$. In addition, the sensor produced results in agreement with data reported in the literature with respect to cystatin C determination in blood plasma, urine and saliva [287]. Papain was also immobilized (by hydrofluoric acid etching method) onto Ti_3C_2 MXene nanosheets by physical adsorption and physical adsorption combined with covalent crosslinking with glutaraldehyde. The immobilized papain exhibited enhanced pH and temperature endurances, immobilized papain also showed improved storage stability (39.25% and 65.57% after 20 days of storage at 4 °C) and reusability [288]. The production of enzyme-metal salt nanoflowers is becoming an increasingly popular strategy to immobilize enzymes, usually with very good results in terms of recovered activity and enzyme stability [289–293]. It is based on the biomineralization of the enzyme by the metal salt using some enzyme sites as nucleation point [294]. We have been able to find just one example of the use of this technique for papain, Flower-like papain/$Zn_3(PO_4)_2$ hybrid materials, it was found that the catalytic properties of papain immobilized on hybrid nanoflowers are enhanced compared with that of free papain, and that hybrid nanoflowers exhibited excellent reusability, high thermo stability and long storage life [295].However silicates and inorganic oxide supports are the most frequently used inorganic support to immobilize papain.

3.3.1. *Immobilization of papain in mesoporous silicates*

Mesoporous silicates are excellent candidates for enzyme immobilization as they meet many of the enzyme support requirements such as well-defined pore geometry, narrow pore size distribution, large surface area, thermal and mechanical stability, good dispersion in water, etc., besides the fact that it can also be modified on other materials (for example, graphene and magnetic nanoparticles) [280,296,297]. In addition, the presence of abundant hydroxyl groups on its surface facilitates the binding of enzymes and makes its functionalization through surface modifying agents (mainly trimetoxy-derivatives) feasible [281]. In this sense, the immobilization of papain on mesoporous silicates has also been studied, and has made it possible to take advantage of the excellent characteristics of these materials. The first report that we have found on this regard was from 1978. In this study, papain was immobilized on Spherosil, a porous silica, by two methods: activation with glutaraldehyde and direct bonding to acetal groups, both of them via silane coupling [298]. The second method gave the best results, and the biocatalyst was used on a 1-L packed bed reactor for the continuous processing of beer, finding that the papain reactor was effective for online beer chill proofing [298]. Later, papain was immobilized by aminoorganosilica activated by cyanuric chloride. Immobilized papain reached 40% of enzyme activity retention and, after immobilization, the optimum pH and pH profile of the immobilized papain remained unchangeable [299]. In another publication, papain was crosslinked using glutaraldehyde to a solid dendrimer with silica gel core, and the enzymatic activity of the immobilized papain kept almost unchanged after fifteen cycles of use [300]. In another research effort, it was found that papain immobilized on porous silica beads by cross-linking with glutaraldehyde presented thermal activation in aqueous system at a temperature range from 50 to 90 °C, and the higher the temperature, the more active the immobilized papain. In addition, the durability of the immobilized papain on heating was greatly improved [301]. Sun et al., reported the papain immobilization on aminopropylsilica gel activated with cyanuric chloride. Results showed that the immobilized enzyme had a better pH and thermal stability than the free enzyme, and good operational stability. Additionally, lyophilized immobilized enzyme exhibited much better stability when stored at room temperature for 60 days [302]. In 2004, SiO_2 particles containing amine groups were synthesized by synchronous hydrolysis of tetraethylorthosilicate and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane in Water/Oil microemulsion stabilized in Triton X-100/cyclohexane/ammonium hydroxide system [303]. They immobilized papain on the particles which were treated by glutaraldehyde, and found that, compared with traditional porous silica beads, these particles contain many more amine groups and their amine group content can be easily tuned in the process of synthesis [303]. Later, papain was immobilized on the mesocellular siliceous foams by macromolecular crowding under microwave irradiation. It was observed that the immobilized enzyme exhibited the highest catalytic activity when papain was co-immobilized on mesocellular siliceous foams with bovine serum albumin and the ratio of enzyme to support at 0.4 [304]. In another investigation, siliceous mesocellular foam was employed as carriers in the immobilization of papain. The results showed that pH, thermal, operational, and storage stabilities of the immobilized enzyme were improved due to the large pore microenvironment and the shield of the mesopores of siliceous mesocellular foam [305]. In 2005 Xiao et al. immobilized papain in on the mesoporous molecular sieve MCM-41 under optimal immobilization conditions of the 20 mg/g of concentration of enzyme solution, 0.75% of concentration of glutaraldehyde to activate the support, 2 h, 10–20 °C and pH 7.0, had an activity yield (%) of 55%, and showed a good continually operational stability and its half-life up to 26 days [306]. Later, the same research group carried out the preparation of a nanoporous material from the mesoporous material MCM-41 modified with mesitylene, on which papain was then immobilized using glutaraldehyde chemistry [307]. The synthesized material exhibited a highly ordered arrangement of uniform nanopores with dimensions of around 6.3 nm (under dried conditions, that may be quite different to that under wet conditions), and under the same optimal immobilization conditions, the activity yield (%) of the immobilized papain was around 55% [307]. In another study, papain was immobilized on the mesoporous molecular sieve MCM-48 (with a pore size of 6.2 nm in diameter under dried conditions) using glutaraldehyde as coupling agent. Under optimal immobilization conditions, (20 mg native enzyme/g of the MCM-48 and 0.75% glutaraldehyde, 2 h of immobilization time at 10–20 °C and pH 7.0), immobilized papain was more resistant to extreme pH values and temperatures than the free enzyme, and showed good operational and storage stability, with a half-life up to 2500 min, significantly higher than that of the free enzyme (about 80 min) [308]. In another research effort, Solís et al. used mesoporous silica at 4 °C and pH values between 3.0 and 11.0 to adsorb papain. The best results were observed for papain adsorption at pH 5.0, which led to the enzyme desorption of <5% [309]. On the other hand, a room temperature ionic liquid-decorated mesoporous SBA-15 (RTIL-SBA-15) was synthesized for papain immobilization. It was found that the interaction between the carrier and papain was stronger after ionic liquid [Simim $^{+}$][Cl^{-}] modification of the support, and that the activity of the immobilized papain was improved that way [310]. He et al., reported the immobilization of papain on single-crystal-like, nanoporous silica particle which possesses a high adsorption capacity for the immobilization of papain. They observed that after initial binding to the silica, the papain optimized its conformation to allow more atoms to contact with the surface, and papain global structure was preserved in the course of adsorption [311]. H_2SO_4 treatment plays a key role in the synthesis of carboxyl-modified mesoporous materials, which are efficient carriers for immobilizing enzymes [312]. In this regard, Bian et al., compared the ability of immobilizing papain between SBA-15 and the support after H_2SO_4 treatment. They found that the H_2SO_4 treatment increased the immobilizing ability of SBA-15, and that carboxyl groups made a great contribution to inducing the favorable conformation change of papain, improving the catalytic efficiency, specific activity, and binding affinity of the immobilized enzyme when combined with the substrate [312]. Finally, in another interesting study, papain was immobilized on cage-shape macroporous/mesoporous three-dimensional- ordered material (3DOM) SiO_2 which was prepared using polystyrene colloidal crystal (PS) as template and TEOS as silica source. Results showed that, compared with the free papain, the immobilized papain exhibited higher activity and thermal and pH

stability [313]. Sun et al. immobilized papain on carboxyl-activated silica nanoparticles [314]. They found that the immobilized papain had not only higher activity, but also better pH and thermal stability, reusability and environmental adaptability. However, reusability was complex due to the small size of the particles.

3.3.2. Immobilization of papain on inorganic oxide supports

Inorganic oxide-based materials such as titanium, aluminium, and zirconium oxides are the preferred materials for their use as a support in enzyme immobilization [315]. This is due to their high stability, high mechanical strength, good sorption capacity, high hydrophilicity, and because they are inert under various reaction conditions [281,315]. At this regard, papain was covalently coupled to ZrO_2-coated porous glass by several different methods. It was found that immobilized papain presented a 35 day operational half-life [316]. In another study, two different alumina supports, termed C1 and CPC, were used for the immobilization of papain. Results showed that protein binding was enhanced when the carboxyl groups of the support were activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and the level of papain immobilization was dependent upon the length of the linker used and the mass of protein utilized in the immobilization [317]. Later, the same research group derivatized alumina C1 and CPC using organic phosphate linkers to create free carboxyl groups using a two-step process [318]. Papain binding to these derivatized alumina supports was performed using 1-ethyl-3-(dimethylaminopropyl) carbodiimide (a water soluble carbodiimide). The immobilized protein showed similar kinetic constants when compared to the solution protein, and pH dependence and thermal stability were essentially identical [318]. Finally, in another interesting study, papain and pancreatin immobilized on alumina were used to obtain whey hydrolysates with low phenylalanine content. It was found that the use of papain immobilized on alumina was more advantageous (smaller final Phe content in the peptides) than pancreatin [319].

3.4. Immobilization of papain in hybrid materials

The combination of inorganic-organic or inorganic-inorganic (e.g., silicate and metals) materials may offer on some instances some additional advantages [281,320,321]. The hybrid materials have been used to immobilize a variety of enzymes (oxidoreductases, hydrolases, lyases, etc.) and permit a more accurate adjustment of the mechanical and resistance properties of the biocatalysts to the reactor requirements [281,322,323].In the case of papain, silica spheres with silver nanoparticles deposited on their surface were used in the immobilization of papain. The highest activity recovery rate was obtained using carriers prepared with 0.68% silver nanoparticles. This enabled an increase of 188% in the final activity compared to the use of just silica. Immobilized papain was more thermostable than the free enzyme, and maintained 43% activity after 20 reuses [324]. The same group used a similar support and the glutaraldehyde chemistry, the use of the composite increased by 131% the immobilized activity compared with the carrier without silver nanoparticles [325,326]. Hybrid inorganic/organic gel (with a size of about 0.444 μm) with immobilized papain enzyme was prepared by absorption-flocculation co-immobilization method. After optimization, the maximum enzyme loading efficiency was 91.38%. More than 50% of the initial activity remained after five reuses [327]. Papain was immobilized on chitosan beads prepared by using a cross-linking agent Cu(II) and Zn(II) metal ions. The results showed that immobilized papain had optimum pH 8.0 and optimum temperature 85 °C, very different from free enzyme (pH 6.5 and 55 °C). In addition, in comparison to free enzyme, thermal stability of the immobilized papain was markedly increased, and its residual activity was about 25% after 12 cycles of batch operation [328]. On the other hand, hydrogel composites based on pineapple peel carboxymethyl cellulose, polyvinyl alcohol and mesoporous silica SBA-15 were used to immobilize papain. It showed that in comparison with the free papain, the immobilized papain revealed enhanced pH, thermal and storage stability [329]. Continuing with this topic, Soares et al., immobilized papain on a hybrid bionanomaterial composed of zinc oxide nanoparticles (ZnO NPs) and chitosan for biomedical applications. The prepared bionanomaterial maintained the proteolytic activity of papain and exhibited a nano-triangular structure with a size of 150 nm. In addition, the immobilized papain system decreased the activation of phagocytic cells but did not induce toxicity in an in vitro analysis [330]. Benucci et al., found that papain covalently immobilized on chitosan–clay nanocomposite films using a food-grade activated montmorillonite (Optigel). The biocatalyst was applied for the protein stabilization of two different unfined white wines, and it efficiently reduced both the haze potential and the protein content [331].

3.5. Immobilization of papain in magnetic particles

We will seclude this section of the standard classification, as in this case the main point to classify the supports is its magnetic character (usually using ferrite), that in most cases may be considered hybrid materials (silica/metal, organic/metal) [108,126,332–335].

3.5.1. Immobilization of papain on micro-magnetic porous particles

The ease of handling of a biocatalyst depends on its particle size, as most of the reactors utilize filters to recover the biocatalyst. That way, a large particle size enables an easy handling and recovery of the biocatalyst, but a large particle of the biocatalysts leads to high substrate diffusional limitations [336]. To overcome this drawback, smaller biocatalyst particles may be preferred. However, the recovery for its later reuse becomes more complicated. Faced with this situation, magnetic particles (having the dimension in the micro scale), which can be easily recovered through a magnetic field, emerged as an excellent alternative not only for enzymatic immobilization [108,336,337], but also for the binding of proteins and drugs, and isolation and purification of different biomolecules [338,339]. A magnetic character of the porous microparticle may also present advantages to recover the biocatalysts when used in a suspension (many foods have some solids in suspension), where filtration is no longer feasible [108,340,341]. Moreover, the hyperthermia that can be generated by the ferrite particles may be utilized in this kind of biocatalysts to improve its performance [342–345]. In this context, papain was immobilized by covalent binding on the surface magnetic composite microspheres containing carboxyl groups which was activated by thionylchloride to produce reactive chloride groups which have the capability to react with the free amino groups of enzyme to give amide bonds [346]. The immobilized biocatalysts presented a higher pH, thermal and storage stabilities as well as environmental adaptability and reusability when compared to the soluble one, the being the biocatalyst capture and reuse simpler by its magnetic character [346]. In another research effort, papain was successfully immobilized on magnetic agarose carriers using Cu^{2+} immobilized as a chelate in iminodiacetate (IDA), able to interact with the histidine group of papain surface (His-81). The recovered activity of the immobilized enzyme was 68.4%. The carrier was recovered and reused, and after 5 times, the re-immobilization of papain on the regenerated matrix was 79.71% effective compared to the first cycle and retained maximum enzyme activity [347]. Novel magnetic cellulose nanocrystals (cellulose nanocrystals combined with cationic polyethyleneimine modified Fe_3O_4 nanoparticles) were prepared via electrostatic self-assembly approach and were used as magnetic carriers for efficient immobilization of papain [348]. At an enzyme concentration of 0.4 $mg.mL^{-1}$ and pH of 6.0, the resultant immobilized enzyme exhibited the highest enzymatic activity about 227 $\mu g.min^{-1}.g^{-1}$, and showed enhanced tolerability to n-butyl alcohol, n-hexane and [Cnpy][NTf2], and better pH and thermo stabilities than those of the free papain [348]. In another example, papain was immobilized on a new type of magnetic metal chelating carrier prepared using chitosan as raw material, nano Fe_3O_4 as magnetic material, SiO_2 as porogen, iminodiacetic acid (IDA) as a chelating

ligand, and binding with transition metal ion (Cu_2^+) [349]. The immobilized papain presented enhanced enzyme activity, good operational stability and reusability [349]. In another interesting study, papain was immobilized via cross-linking method on magnetic porous polymer microspheres which were prepared using sodium alginate, magnetic Fe_3O_4 particles and polyethylene glycol as a pore-forming agent [350]. Results showed that the immobilized papain exhibited shifted the optimal pH to more alkaline values, higher thermal stability and activity in casein hydrolysis than free papain. In addition, the immobilized papain retained nearly 72% of residual activity after seven reuses in casein hydrolysis reaction [350].

3.5.2. Immobilization of papain on magnetic nanoparticles

The use of nanoparticles, usually non-porous ones, is a good alternative to immobilize proteases that are going to act versus very large or even solid substrates [39]. In these instances, the loading capacity of the support is determined by the diameter of the particles, the smallest diameter, the higher specific area and, that way, the higher loading capacity [107]. However, very small nanoparticles cannot be easily handled. This drawback has been solved using magnetic nanoparticles, with superparamagnetic properties (that is, they do not generate a magnetic field, but can respond to one, e.g., in the presence of a magnet) [351,352]. This way, they can be recovered by using a potent enough magnet such as an electromagnet for industrial purposes. Magnetic nanoparticles commonly consist of magnetic elements such as cobalt, iron and nickel and their chemical compounds; however, in the food area, the most frequently used magnetic nanoparticles are iron oxides, mainly the superparamagnetic nanoparticles of Fe_3O_4, since they do not have toxicity, have good biocompatibility and do not retain residual magnetism after elimination of the external magnetic field [353]. However, the use of this kind of support has some limitations [107,108]:

- All enzymes are on the surface (that is also the advantage), that is, the enzyme is subject to all inactivation causes related to interaction with external interfaces, may suffer proteolysis,

- Small particles will have moderate geometrical congruence with the enzyme, this will decrease the possibilities of stabilizing the enzyme due to multipoint covalent immobilization.
- The loading may be similar to appropriate porous supports in the best case (e.g., agarose 10B can immobilize over 100 mg of enzyme/wet gram of support).
- Prices tend to be much higher than those of commercial macroparticles supports.
- In many instances, during handling or enzyme immobilization, the nanoparticles aggregate, losing the advantages of using nanoparticles.

The advantages are that the enzymes (if properly oriented) [30] may attack any substrate (even a solid) [39,107]. Moreover, substrate or pH gradients are prevented (although in some instances this may not be an advantage). Only if the advantages overcome the drawbacks, this will be recommended.

Papain has been immobilized on these materials in some instances. For instance, magnetic poly(HEMA-GMA) nanoparticles were synthesized by using emulsion polymerization technique, functionalized with, Cibacron Blue F3GA (a dye with high affinity for some proteins) and then evaluated in the papain immobilization [354]. Maximum papain adsorption onto the nanoparticles was found to be 764.0 mg/g polymer in pH 7.0 HEPES buffer. Immobilized papain was much more stable than the free form, and among casein, BSA, IgG and cytochrome C as substrates, the highest catalytic efficiency was achieved with IgG [354]. In another publication, antibacterial non-toxic Ag/CuFe2O4 magnetic nanoparticles were solvothermally synthesized and used as an efficient magnetic precursor for papain immobilization. All prepared samples exhibited stronger antibacterial properties against *Staphyloccocus aureus* than versus *Escherichia coli*. Moreover, the antibacterial activity of the enzyme increased by papain immobilization [355]. In another research effort, papain was immobilized by covalent bonding onto biocompatible Fe_3O_4/SF nanoparticles under optimal immobilization conditions (pH 6.0, 60 min of hydrolysis time and an enzyme/support ratio of 10.0 mg/g) [356]. In comparison with free papain, the immobilized papain exhibits a higher effective activity, broader working pH and temperature, and retained 70% and 85% of initial activity after eight consecutive operations and storage of 28 days, respectively [356]. Later, covalent immobilization of papain through glutaraldehyde treatment on cysteine functionalized iron oxide nanoparticle coated glass beads was investigated. Immobilized papain showed thermal and pH stabilization, high activity, the possibility of reuse for 5 times retaining of 81% of its initial activity, and the possibility of storage for 6 months without loss of activity [357]. In addition, in another research effort, it was found that papain immobilized on gold nanoparticles largely preserved its activity and enhanced the stability, allowing the reuse of the linked enzyme many times without any significant loss of its catalytic performance [358].In another publication the use of magnetic gold nanocomposites for papain immobilization was reported. The loading amount of papain on these nanocomposites was 54 mg/g of support. The Michaelis-Menten kinetic constant and maximum reaction velocity for immobilized papain were 0.308×10^5 $g.mL^{-1}$ and 5.4 $g.mL^{-1}.s^{-1}$ respectively, while the activity recovery of the immobilized papain reached to 47 (±5) % compared to native papain [359]. Other example shows the preparation of immobilized papain prepared by magnetic Fe_3O_4/P (GMA-EDGMA-St) composite carrier had an average particle size of 196 nm. Compared with the free enzyme, the magnetically immobilized papain indicated higher acid-base tolerance and thermal stability, pH tolerance increased from 7.0 to 8.0, and temperature tolerance increased from 60 °C to 65 °C [360].

4. Future developments

The use of some supports and activation methods (e.g., genipin) that have been approved for use in human and products consumed by humans will expand the use of immobilized papain biocatalyst in the next future. However, immobilization nowadays should not just be justified by the possibility of enzyme reuse (in food manipulation, to prevent the food contamination by the protease or to improve the control of the reaction may be additional justification of the use of immobilized papain). That way, immobilization should compensate the expenses of the immobilization process, which means that immobilization should be accompanied by significant improvements in enzyme features, e.g., stability via multipoint covalent immobilization (that permit the use of the enzyme under much more drastic conditions), tailoring enzyme specificity (using steric or diffusion limitation to selectively hydrolyze target proteins while not hydrolyzing other ones), etc. Immobilization of proteases in general and of papain in particular should pursue a complete control of the enzyme orientation to permit the full activity of the immobilized enzyme molecules even versus very large substrates (e.g., coupling site-directed mutagenesis and tailor-made supports). Magnetic nanoparticles seem a good option as supports to immobilize papain when it is going to be used in the hydrolysis of a substrate in the form of a suspension (because the proteins are aggregated, or the medium contains other solids), but it is not required in other uses (e.g., peptide synthesis). It seems obvious that papain immobilization has the same shortcomings and expectations than the immobilization of any enzyme. The use of an immobilized biocatalyst may be justified only when the gains are higher than the costs, and to be able to compensate this, papain immobilization should be performed using optimized protocols to take the maximum profit of immobilization in improving enzyme features.

CRediT authorship contribution statement

Veymar G. Tacias-Pascacio, Roberto Morellon-Sterling, Daniel

Castañeda-Valbuena, performed the initial literature search, all authors contributed to the writing and final editing of the paper, Roberto Fernandez-Lafuente designed the paper and supervised the writing.

Acknowledgements

We gratefully recognize the support from the Ministerio de Ciencia e Innovación from Spanish Government (project number CTQ2017-86170-R) and CSIC for the project AEP045. The FPU fellowship (Ministerio de Educacion) for Mr. Morellon–Sterling is gratefully recognized. Dr. Tacias-Pascacio thanks the financial support from "Programa para el Desarrollo Profesional Docente" (PRODEP) from Mexican Government. ABM would like to thank Ministerio de Ciencia Innovación y Universidades and FEDER (Project RTI2018-095291-B-I00) and the Generalitat Valenciana (PROMETEOII/2018/076) for financial support.

References

[1] A. Schmid, J.S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, Industrial biocatalysis today and tomorrow, Nature 409 (2001) 258–268, https://doi.org/10.1038/35051736.
[2] D.J. Pollard, J.M. Woodley, Biocatalysis for pharmaceutical intermediates: the future is now, Trends Biotechnol. 25 (2007) 66–73, https://doi.org/10.1016/j.tibtech.2006.12.005.
[3] J.-M. Choi, S.-S. Han, H.-S. Kim, Industrial applications of enzyme biocatalysis: current status and future aspects, Biotechnol. Adv. 33 (2015) 1443–1454, https://doi.org/10.1016/j.biotechadv.2015.02.014.
[4] R.A. Sheldon, J.M. Woodley, Role of biocatalysis in sustainable chemistry, Chem. Rev. 118 (2018) 801–838, https://doi.org/10.1021/acs.chemrev.7b00203.
[5] M.T. Reetz, Biocatalysis in organic chemistry and biotechnology: past, present, and future, J. Am. Chem. Soc. 135 (2013) 12480–12496, https://doi.org/10.1021/ja405051f.
[6] H.E. Schoemaker, D.L. Mink, M.G. WubboLts, Dispelling the myths - biocatalysis in industrial synthesis, Science (80-.) 299 (2003) 1694–1697, https://doi.org/10.1126/science.1079237.
[7] J.M. Vieites, M.-E. Guazzaroni, A. Beloqui, P.N. Golyshin, M. Ferrer, Metagenomics approaches in systems microbiology, FEMS Microbiol. Rev. 33 (2009) 236–255, https://doi.org/10.1111/j.1574-6976.2008.00152.x.
[8] P.D. Schloss, J. Handelsman, Biotechnological prospects from metagenomics, Curr. Opin. Biotechnol. 14 (2003) 303–310, https://doi.org/10.1016/S0958-1669(03)00067-3.
[9] M. Ferrer, A. Beloqui, K. Timmis, P. Golyshin, Metagenomics for mining new genetic resources of microbial communities, J. Mol. Microbiol. Biotechnol. 16 (2009) 109–123, https://doi.org/10.1159/000142898.
[10] T.-S. Kim, S.K.S. Patel, C. Selvaraj, W.-S. Jung, C.-H. Pan, Y.C. Kang, J.-K. Lee, A highly efficient sorbitol dehydrogenase from gluconobacter oxydans G624 and improvement of its stability through immobilization, Sci. Rep. 6 (2016) 33438, https://doi.org/10.1038/srep33438.
[11] J.-S. Kim, S.K.S. Patel, M.K. Tiwari, C. Lai, A. Kumar, Y.S. Kim, V.C. Kalia, J.-K. Lee, Phe-140 determines the catalytic efficiency of arylacetonitrilase from Alcaligenes faecalis, Int. J. Mol. Sci. 21 (2020) 7859, https://doi.org/10.3390/ijms21217859.
[12] C. Selvaraj, G. Krishnasamy, S.S. Jagtap, S.K.S. Patel, S.S. Dhiman, T.-S. Kim, S. K. Singh, J.-K. Lee, Structural insights into the binding mode of d-sorbitol with sorbitol dehydrogenase using QM-polarized ligand docking and molecular dynamics simulations, Biochem. Eng. J. 114 (2016) 244–256, https://doi.org/10.1016/j.bej.2016.07.008.
[13] H. Gao, J. Li, D. Sivakumar, T.S. Kim, S.K.S. Patel, V.C. Kalia, I.W. Kim, Y. W. Zhang, J.K. Lee, NADH oxidase from lactobacillus reuteri: a versatile enzyme for oxidized cofactor regeneration, Int. J. Biol. Macromol. 123 (2019) 629–636, https://doi.org/10.1016/j.ijbiomac.2018.11.096.
[14] P. Ramachandran, S.S. Jagtap, S.K.S. Patel, J. Li, Y. Chan Kang, J.K. Lee, Role of the non-conserved amino acid asparagine 285 in the glycone-binding pocket of Neosartorya fischeri ß-glucosidase, RSC Adv. 6 (2016) 48137–48144, https://doi.org/10.1039/c5ra28017f.
[15] U.T. Bornscheuer, M. Pohl, Improved biocatalysts by directed evolution and rational protein design, Curr. Opin. Chem. Biol. 5 (2001) 137–143, https://doi.org/10.1016/S1367-5931(00)00182-4.
[16] H. Renata, Z.J. Wang, F.H. Arnold, Expanding the enzyme universe: accessing non-natural reactions by mechanism-guided directed evolution, Angew. Chemie Int. Ed. 54 (2015) 3351–3367, https://doi.org/10.1002/anie.201409470.
[17] P.A. Romero, F.H. Arnold, Exploring protein fitness landscapes by directed evolution, Nat. Rev. Mol. Cell Biol. 10 (2009) 866–876, https://doi.org/10.1038/nrm2805.
[18] V.G.H. Eijsink, S. GÅseidnes, T.V. Borchert, B.Van Den Burg, Directed evolution of enzyme stability, Biomol. Eng. 22 (2005) 21–30, https://doi.org/10.1016/j.bioeng.2004.12.003.
[19] J.M. Chalker, G.J.L. Bernardes, Y.A. Lin, B.G. Davis, Chemical modification of proteins at cysteine: opportunities in chemistry and biology, Chem. Asian J. 4 (2009) 630–640, https://doi.org/10.1002/asia.200800427.
[20] C.D. Spicer, B.G. Davis, Selective chemical protein modification, Nat. Commun. 5 (2014) 4740, https://doi.org/10.1038/ncomms5740.
[21] O. Boutureira, G.J.L. Bernardes, Advances in chemical protein modification, Chem. Rev. 115 (2015) 2174–2195, https://doi.org/10.1021/cr500399p.
[22] R.C. Rodrigues, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R. Fernández-Lafuente, Modifying enzyme activity and selectivity by immobilization, Chem. Soc. Rev. 42 (2013) 6290–6307, https://doi.org/10.1039/c2cs35231a.
[23] O. Barbosa, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R.C. Rodrigues, R. Fernandez-Lafuente, Strategies for the one-step immobilization-purification of enzymes as industrial biocatalysts, Biotechnol. Adv. 33 (2015) 435–456, https://doi.org/10.1016/j.biotechadv.2015.03.006.
[24] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Improvement of enzyme activity, stability and selectivity via immobilization techniques, Enzym. Microb. Technol. 40 (2007) 1451–1463, https://doi.org/10.1016/j.enzmictec.2007.01.018.
[25] C. Garcia-Galan, Á. Berenguer-Murcia, R. Fernandez-Lafuente, R.C. Rodrigues, Potential of different enzyme immobilization strategies to improve enzyme performance, Adv. Synth. Catal. 353 (2011) 2885–2904, https://doi.org/10.1002/adsc.201100534.
[26] R.C. Rodrigues, Á. Berenguer-Murcia, R. Fernandez-Lafuente, Coupling chemical modification and immobilization to improve the catalytic performance of enzymes, Adv. Synth. Catal. 353 (2011) 2216–2238, https://doi.org/10.1002/adsc.201100163.
[27] R.C. Rodrigues, O. Barbosa, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R. Fernandez-Lafuente, Amination of enzymes to improve biocatalyst performance: coupling genetic modification and physicochemical tools, RSC Adv. 4 (2014) 38350–38374, https://doi.org/10.1039/c4ra04625k.
[28] R. Fernandez-Lafuente, Stabilization of multimeric enzymes: strategies to prevent subunit dissociation, Enzym. Microb. Technol. 45 (2009) 405–418, https://doi.org/10.1016/j.enzmictec.2009.08.009.
[29] N. Rueda, J.C.S. dos Santos, C. Ortiz, R. Torres, O. Barbosa, R.C. Rodrigues, Á. Berenguer-Murcia, R. Fernandez-Lafuente, Chemical modification in the design of immobilized enzyme biocatalysts: drawbacks and opportunities, Chem. Rec. 16 (2016) 1436–1455, https://doi.org/10.1002/tcr.201600007.
[30] K. Hernandez, R. Fernandez-Lafuente, Control of protein immobilization: coupling immobilization and site-directed mutagenesis to improve biocatalyst or biosensor performance, Enzym. Microb. Technol. 48 (2011) 107–122, https://doi.org/10.1016/j.enzmictec.2010.10.003.
[31] G. Santiago, M. Martínez-Martínez, S. Alonso, R. Bargiela, C. Coscolín, P. N. Golyshin, V. Guallar, M. Ferrer, Rational engineering of multiple active sites in an ester hydrolase, Biochemistry 57 (2018) 2245–2255, https://doi.org/10.1021/acs.biochem.8b00274.
[32] S. Alonso, G. Santiago, I. Cea-Rama, L. Fernandez-Lopez, C. Coscolín, J. Modregger, A.K. Ressmann, M. Martínez-Martínez, H. Marrero, R. Bargiela, M. Pita, J.L. Gonzalez-Alfonso, M.L. Briand, D. Rojo, C. Barbas, F.J. Plou, P. N. Golyshin, P. Shahgaldian, J. Sanz-Aparicio, V. Guallar, M. Ferrer, Genetically engineered proteins with two active sites for enhanced biocatalysis and synergistic chemo- and biocatalysis, Nat. Catal. 3 (2020) 319–328, https://doi.org/10.1038/s41929-019-0394-4.
[33] N.A. Dierick, Biotechnology aids to improve feed and feed digestion: enzymes and fermentation, Arch. Tierernaehr. 39 (1989) 241–261, https://doi.org/10.1080/17450398909429530.
[34] Y. Zhang, S. He, B.K. Simpson, Enzymes in food bioprocessing — novel food enzymes, applications, and related techniques, Curr. Opin. Food Sci. 19 (2018) 30–35, https://doi.org/10.1016/j.cofs.2017.12.007.
[35] S. Raveendran, B. Parameswaran, S.B. Ummalyma, A. Abraham, A.K. Mathew, A. Madhavan, S. Rebello, A. Pandey, Applications of microbial enzymes in food industry, Food Technol. Biotechnol. 56 (2018) 16–30, https://doi.org/10.17113/ftb.56.01.18.5491.
[36] J. Synowiecki, B. Grzybowska, A. Zdzieblo, Sources, properties and suitability of new thermostable enzymes in food processing, Crit. Rev. Food Sci. Nutr. 46 (2006) 197–205, https://doi.org/10.1080/10408690590957296.
[37] J.G. dos Santos Aguilar, H.H. Sato, Microbial proteases: production and application in obtaining protein hydrolysates, Food Res. Int. 103 (2018) 253–262, https://doi.org/10.1016/j.foodres.2017.10.044.
[38] V.G. Tacias-Pascacio, R. Morellon-Sterling, E.-H. Siar, O. Tavano, Á. Berenguer-Murcia, R. Fernandez-Lafuente, Use of alcalase in the production of bioactive peptides: a review, Int. J. Biol. Macromol. 165 (2020) 2143–2196, https://doi.org/10.1016/j.ijbiomac.2020.10.060.
[39] O.L. Tavano, A. Berenguer-Murcia, F. Secundo, R. Fernandez-Lafuente, Biotechnological applications of proteases in food technology, Compr. Rev. Food Sci. Food Saf. 17 (2018) 412–436, https://doi.org/10.1111/1541-4337.12326.
[40] K.M. Sharma, R. Kumar, S. Panwar, A. Kumar, Microbial alkaline proteases: optimization of production parameters and their properties, J. Genet. Eng. Biotechnol. 15 (2017) 115–126, https://doi.org/10.1016/j.jgeb.2017.02.001.
[41] A. Razzaq, S. Shamsi, A. Ali, Q. Ali, M. Sajjad, A. Malik, M. Ashraf, Microbial proteases applications, Front. Bioeng. Biotechnol. 7 (2019) 1–20, https://doi.org/10.3389/fbioe.2019.00110.
[42] M.A. Mazorra-Manzano, J.C. Ramírez-Suarez, R.Y. Yada, Plant proteases for bioactive peptides release: a review, Crit. Rev. Food Sci. Nutr. 58 (2018) 2147–2163, https://doi.org/10.1080/10408398.2017.1308312.
[43] O.L. Tavano, Protein hydrolysis using proteases: an important tool for food biotechnology, J. Mol. Catal. B Enzym. 90 (2013) 1–11, https://doi.org/10.1016/j.molcatb.2013.01.011.
[44] R. Morellon-Sterling, H. El-Siar, O.L. Tavano, Á. Berenguer-Murcia, R. Fernández-Lafuente, Ficin: a protease extract with relevance in biotechnology and

biocatalysis, Int. J. Biol. Macromol. 162 (2020) 394–404, https://doi.org/10.1016/j.ijbiomac.2020.06.144.
[45] F.J. Contesini, R.R. de Melo, H.H. Sato, An overview of bacillus proteases: from production to application, Crit. Rev. Biotechnol. 38 (2018) 321–334, https://doi.org/10.1080/07388551.2017.1354354.
[46] J. Fernández-Lucas, D. Castañeda, D. Hormigo, New trends for a classical enzyme: papain, a biotechnological success story in the food industry, Trends Food Sci. Technol. 68 (2017) 91–101, https://doi.org/10.1016/j.tifs.2017.08.017.
[47] M. Sharma, Y. Gat, S. Arya, V. Kumar, A. Panghal, A. Kumar, A review on microbial alkaline protease: an essential tool for various industrial approaches, Ind. Biotechnol. 15 (2019) 69–78, https://doi.org/10.1089/ind.2018.0032.
[48] M. Naveed, F. Nadeem, T. Mehmood, M. Bilal, Z. Anwar, F. Amjad, Protease—a versatile and ecofriendly biocatalyst with multi-industrial applications: an updated review, Catal. Lett. 151 (2021) 307–323, https://doi.org/10.1007/s10562-020-03316-7.
[49] J.K. McDonald, An overview of protease specificity and catalytic mechanisms: aspects related to nomenclature and classification, Histochem. J. 17 (1985) 773–785, https://doi.org/10.1007/BF01003313.
[50] J. Drenth, J.N. Jansonius, R. Koekoek, H.M. Swen, B.G. Wolthers, Structure of papain, Nature 218 (1968) 929–932, https://doi.org/10.1038/218929a0.
[51] X. Gao, B. Qin, P. Chen, K. Zhu, P. Hou, J.A. Wojdyla, M. Wang, S. Cui, Crystal structure of SARS-CoV-2 papain-like protease, Acta Pharm. Sin. B 11 (2021) 237–245, https://doi.org/10.1016/j.apsb.2020.08.014.
[52] J. Liu, A. Sharma, M.J. Niewiara, R. Singh, R. Ming, Q. Yu, Papain-like cysteine proteases in Carica papaya: lineage-specific gene duplication and expansion, BMC Genomics 19 (2018) 26, https://doi.org/10.1186/s12864-017-4394-y.
[53] BRENDA, (n.d.). http://www.brenda-enzymes.org/ (accessed June 30, 2021).
[54] V.G. Tacias-Pascacio, D. Castañeda-Valbuena, R. Morellon-Sterling, O. Tavano, Á. Berenguer-Murcia, G. Vela-Gutiérrez, I.A. Rather, R. Fernandez-Lafuente, Bioactive peptides from fisheries residues: a review of use of papain in proteolysis reactions, Int. J. Biol. Macromol. 184 (2021) 415–428, https://doi.org/10.1016/j.ijbiomac.2021.06.076.
[55] J. Jain, Review on isolation and purification of papain enzyme from papaya fruit, Int. J. Eng. Appl. Sci. Technol. 5 (2020) 193–197, https://doi.org/10.33564/IJEAST.2020.v05i06.028.
[56] I.G. Kamphuis, K.H. Kalk, M.B.A. Swarte, J. Drenth, Structure of papain refined at 1.65 Å resolution, J. Mol. Biol. 179 (1984) 233–256, https://doi.org/10.1016/0022-2836(84)90467-4.
[57] E. Amri, F. Mamboya, Papain, a plant enzyme of biological importance: a review, Am. J. Biochem. Biotechnol. 8 (2012) 99–104, https://doi.org/10.3844/ajbbsp.2012.99.104.
[58] K. Mosbach, Immobilized enzymes, Trends Biochem. Sci. 5 (1980) 1–3, https://doi.org/10.1016/S0968-0004(80)80065-X.
[59] A. Kilara, K.M. Shahani, T.P. Shukla, The use of immobilized enzymes in the food industry: a review, CRC Crit. Rev. Food Sci. Nutr. 12 (1979) 161–198, https://doi.org/10.1080/10408397909527276.
[60] A.M. Klibanov, Enzyme stabilization by immobilization, Anal. Biochem. 93 (1979) 1–25, https://doi.org/10.1016/S0003-2697(79)80110-4.
[61] R.A. Sheldon, S. van Pelt, Enzyme immobilisation in biocatalysis: why, what and how, Chem. Soc. Rev. 42 (2013) 6223–6235, https://doi.org/10.1039/C3CS60075K.
[62] R. Di Cosimo, J. Mc Auliffe, A.J. Poulose, G. Bohlmann, Industrial use of immobilized enzymes, Chem. Soc. Rev. 42 (2013) 6437–6474, https://doi.org/10.1039/c3cs35506c.
[63] A. Liese, L. Hilterhaus, Evaluation of immobilized enzymes for industrial applications, Chem. Soc. Rev. 42 (2013) 6236–6249, https://doi.org/10.1039/c3cs35511j.
[64] C. Mateo, O. Abian, G. Fernández-Lorente, J. Pedroche, R. Fernández-Lafuente, J. M. Guisan, A. Tam, M. Daminati, Epoxy sepabeads: a novel epoxy support for stabilization of industrial enzymes via very intense multipoint covalent attachment, Biotechnol. Prog. 18 (2002) 629–634, https://doi.org/10.1021/bp010171n.
[65] D. Carballares, R. Morellon-Sterling, X. Xu, F. Hollmann, R. Fernandez-Lafuente, Immobilization of the peroxygenase from Agrocybe aegerita. The effect of the immobilization pH on the features of an ionically exchanged dimeric peroxygenase, Catalysts 11 (2021) 560, https://doi.org/10.3390/catal11050560.
[66] J.C.S. Dos Santos, O. Barbosa, C. Ortiz, A. Berenguer-Murcia, R.C. Rodrigues, R. Fernandez-Lafuente, Importance of the support properties for immobilization or purification of enzymes, ChemCatChem 7 (2015) 2413–2432, https://doi.org/10.1002/cctc.201500310.
[67] R.C. Rodrigues, Á. Berenguer-Murcia, D. Carballares, R. Morellon-Sterling, R. Fernandez-Lafuente, Stabilization of enzymes via immobilization, Biotechnol. Adv. (2021). Submitted.
[68] P. Zucca, R. Fernandez-Lafuente, E. Sanjust, Agarose and its derivatives as supports for enzyme immobilization, Molecules 21 (2016) 1577, https://doi.org/10.3390/molecules21111577.
[69] O. Barbosa, R. Torres, C. Ortiz, Á. Berenguer-Murcia, R.C. Rodrigues, R. Fernandez-Lafuente, Heterofunctional supports in enzyme immobilization: from traditional immobilization protocols to opportunities in tuning enzyme properties, Biomacromolecules 14 (2013) 2433–2462, https://doi.org/10.1021/bm400762h.
[70] J. Pedroche, M. del Mar Yust, C. Mateo, R. Fernández-Lafuente, J. Girón-Calle, M. Alaiz, J. Vioque, J.M. Guisán, F. Millán, Effect of the support and experimental conditions in the intensity of the multipoint covalent attachment of proteins on glyoxyl-agarose supports: correlation between enzyme-support linkages and thermal stability, Enzym. Microb. Technol. 40 (2007) 1160–1166, https://doi.org/10.1016/j.enzmictec.2006.08.023.
[71] R. Morellon-Sterling, E.-H. Siar, S.A. Braham, D. de Andrades, J. Pedroche, M. Del C. Millán, R. Fernandez-Lafuente, R. Fernandez-Lafuente, Effect of amine length in the interference of the multipoint covalent immobilization of enzymes on glyoxyl agarose beads, J. Biotechnol. 329 (2021) 128–142, https://doi.org/10.1016/j.jbiotec.2021.02.005.
[72] S.A. Braham, R. Morellon-Sterling, D. de Andrades, R.C. Rodrigues, E.-H. Siar, A. Aksas, J. Pedroche, M. Del C. Millán, R. Fernandez-Lafuente, Effect of tris buffer in the intensity of the multipoint covalent immobilization of enzymes in glyoxyl-agarose beads, Appl. Biochem. Biotechnol. (2021), https://doi.org/10.1007/s12010-021-03570-4 in press.
[73] O. Barbosa, C. Ortiz, Á. Berenguer-Murcia, R. Torres, R.C. Rodrigues, R. Fernandez-Lafuente, Glutaraldehyde in bio-catalysts design: a useful crosslinker and a versatile tool in enzyme immobilization, RSC Adv. 4 (2014) 1583–1600, https://doi.org/10.1039/c3ra45991h.
[74] C. Mateo, J.M. Palomo, M. Fuentes, L. Betancor, V. Grazu, F. López-Gallego, B.C. C. Pessela, A. Hidalgo, G. Fernández-Lorente, R. Fernández-Lafuente, J. M. Guisán, Glyoxyl agarose: a fully inert and hydrophilic support for immobilization and high stabilization of proteins, Enzym. Microb. Technol. 39 (2006) 274–280, https://doi.org/10.1016/j.enzmictec.2005.10.014.
[75] L. Hilterhaus, B. Minow, J. Müller, M. Berheide, H. Quitmann, M. Katzer, O. Thum, G. Antranikian, A.P. Zeng, A. Liese, Practical application of different enzymes immobilized on sepabeads, Bioprocess Biosyst. Eng. 31 (2008) 163–171, https://doi.org/10.1007/s00449-008-0199-3.
[76] E. Katchalski-Katzir, D.M. Kraemer, Eupergit® C, a carrier for immobilization of enzymes of industrial potential, J. Mol. Catal. B Enzym. 10 (2000) 157–176, https://doi.org/10.1016/S1381-1177(00)00124-7.
[77] C. Mateo, V. Grazú, B.C.C. Pessela, T. Montes, J.M. Palomo, R. Torres, F. López-Gallego, R. Fernández-Lafuente, J.M. Guisán, Advances in the design of new epoxy supports for enzyme immobilization–stabilization, Biochem. Soc. Trans. 35 (2007) 1593–1601, https://doi.org/10.1042/BST0351593.
[78] J.C.S.Dos Santos, N. Rueda, O. Barbosa, J.F. Fernández-Sánchez, A.L. Medina-Castillo, T. Ramón-Márquez, M.C. Arias-Martos, M.C. Millán-Linares, J. Pedroche, M.D.M. Yust, L.R.B. Gonçalves, R. Fernandez-Lafuente, Characterization of supports activated with divinyl sulfone as a tool to immobilize and stabilize enzymes via multipoint covalent attachment. Application to chymotrypsin, RSC Adv. 5 (2015) 20639–20649, https://doi.org/10.1039/c4ra16926c.
[79] E.-H. Siar, S. Arana-Peña, O. Barbosa, M. Zidoune, R. Fernandez-Lafuente, Immobilization/stabilization of ficin extract on glutaraldehyde-activated agarose beads. Variables that control the final stability and activity in protein hydrolyses, Catalysts 8 (2018) 149, https://doi.org/10.3390/catal8040149.
[80] E.-H. Siar, H. Zaak, J.F. Kornecki, M.N. Zidoune, O. Barbosa, R. Fernandez-Lafuente, Stabilization of ficin extract by immobilization on glyoxyl agarose. Preliminary characterization of the biocatalyst performance in hydrolysis of proteins, Process Biochem. 58 (2017) 98–104, https://doi.org/10.1016/j.procbio.2017.04.009.
[81] L. Dal Magro, J.F. Kornecki, M.P. Klein, R.C. Rodrigues, R. Fernandez-Lafuente, Pectin lyase immobilization using the glutaraldehyde chemistry increases the enzyme operation range, Enzym. Microb. Technol. 132 (2020), 109397, https://doi.org/10.1016/j.enzmictec.2019.109397.
[82] L. Dal Magro, J.F. Kornecki, M.P. Klein, R.C. Rodrigues, R. Fernandez-Lafuente, Optimized immobilization of polygalacturonase from aspergillus Niger following different protocols: improved stability and activity under drastic conditions, Int. J. Biol. Macromol. 138 (2019) 234–243, https://doi.org/10.1016/j.ijbiomac.2019.07.092.
[83] G. Fernandez-Lorente, C.A. Godoy, A.A. Mendes, F. Lopez-Gallego, V. Grazu, B. De las Rivas, J.M. Palomo, J. Hermoso, R. Fernandez-Lafuente, J.M. Guisan, Solid-phase chemical amination of a lipase from Bacillus thermocatenulatus to improve its stabilization via covalent immobilization on highly activated glyoxyl-agarose, Biomacromolecules 9 (2008) 2553–2561, https://doi.org/10.1021/bm800609g.
[84] R.C. Rodrigues, C.A. Godoy, G. Volpato, M.A.Z. Ayub, R. Fernandez-Lafuente, J. M. Guisan, Immobilization-stabilization of the lipase from thermomyces lanuginosus: critical role of chemical amination, Process Biochem. 44 (2009) 963–968, https://doi.org/10.1016/j.procbio.2009.04.015.
[85] C.I. Giunta, I. Cea-Rama, S. Alonso, M.L. Briand, R. Bargiela, C. Coscolín, P.F. X. Corvini, M. Ferrer, J. Sanz-Aparicio, P. Shahgaldian, Tuning the properties of natural promiscuous enzymes by engineering their nano-environment, ACS Nano 14 (2020) 17652–17664, https://doi.org/10.1021/acsnano.0c08716.
[86] L. Cai, L. Xiao, C. Liu, T. Ying, Functional properties and bioactivities of pine nut (Pinus gerardiana) protein isolates and its enzymatic hydrolysates, Food Bioprocess Technol. 6 (2013) 2109–2117, https://doi.org/10.1007/s11947-012-0885-7.
[87] E.-H. Siar, R. Morellon-Sterling, M.N. Zidoune, R. Fernandez-Lafuente, Use of glyoxyl-agarose immobilized ficin extract in milk coagulation: unexpected importance of the ficin loading on the biocatalysts, Int. J. Biol. Macromol. 144 (2020) 419–426, https://doi.org/10.1016/j.ijbiomac.2019.12.140.
[88] F.A. Payne, C.L. Hicks, P.S. Shen, Predicting optimal cutting time of coagulating milk using diffuse reflectance, J. Dairy Sci. 76 (1993) 48–61, https://doi.org/10.3168/jds.S0022-0302(93)77322-1.
[89] M. Luan, J. Wu, Trypsin immobilized in sol-gel for high-throughput protein peptide mapping, Chin. J. Anal. Chem. 34 (2006) 1707–1710.
[90] S. Iyer, J. Olivares, Trypsin digestion of proteins on intact immobilized pH gradient strips for surface matrix-assisted laser desorption/ionization time-of-

flight mass spectrometric analysis, Rapid Commun. Mass Spectrom. 17 (2003) 2323–2326, https://doi.org/10.1002/rcm.1199.
[91] J. Gobom, E. Nordhoff, R. Ekman, P. Roepstorff, Rapid micro-scale proteolysis of proteins for MALDI-MS peptide mapping using immobilized trypsin, Int. J. Mass Spectrom. Ion Process. 169–170 (1997) 153–163, https://doi.org/10.1016/S0168-1176(97)00216-4.
[92] J. Rocha-Martín, B. de Las Rivas, R. Muñoz, J.M. Guisán, F. López-Gallego, Rational co-immobilization of bi-enzyme cascades on porous supports and their applications in bio-redox reactions with in situ recycling of soluble cofactors, ChemCatChem 4 (2012) 1279–1288, https://doi.org/10.1002/cctc.201200146.
[93] L. Trobo-Maseda, A.H. Orrego, J.M. Guisan, J. Rocha-Martin, Coimmobilization and colocalization of a glycosyltransferase and a sucrose synthase greatly improves the recycling of UDP-glucose: glycosylation of resveratrol 3-O-ß-D-glucoside, Int. J. Biol. Macromol. 157 (2020) 510–521, https://doi.org/10.1016/j.ijbiomac.2020.04.120.
[94] J.M. Bolivar, A. Hidalgo, L. Sánchez-Ruiloba, J. Berenguer, J.M. Guisán, F. López-Gallego, Modulation of the distribution of small proteins within porous matrixes by smart-control of the immobilization rate, J. Biotechnol. 155 (2011) 412–420, https://doi.org/10.1016/j.jbiotec.2011.07.039.
[95] K.E. Dennis, D.S. Clark, J.E. Bailey, Y.K. Cho, Y.H. Park, Immobilization of enzymes in porous supports: effects of support-enzyme solution contacting, Biotechnol. Bioeng. 26 (1984) 892–900, https://doi.org/10.1002/bit.260260812.
[96] D.D. Do, D.S. Clark, J.E. Bailey, Modeling enzyme immobilization in porous solid supports, Biotechnol. Bioeng. 24 (1982) 1527–1546, https://doi.org/10.1002/bit.260240707.
[97] D.D. Do, M.M. Hossain, A new method to determine active enzyme distribution, effective diffusivity, rate constant for main reaction and rate constant for deactivation, Biotechnol. Bioeng. 29 (1987) 545–551, https://doi.org/10.1002/bit.260290502.
[98] N. Ranjbari, M. Razzaghi, R. Fernandez-Lafuente, F. Shojaei, M. Satari, A. Homaei, Improved features of a highly stable protease from Penaeus vannamei by immobilization on glutaraldehyde activated graphene oxide nanosheets, Int. J. Biol. Macromol. 130 (2019) 564–572, https://doi.org/10.1016/j.ijbiomac.2019.02.163.
[99] S.A. Ansari, Q. Husain, Potential applications of enzymes immobilized on/in nano materials: a review, Biotechnol. Adv. 30 (2012) 512–523, https://doi.org/10.1016/j.biotechadv.2011.09.005.
[100] H. Vaghari, H. Jafarizadeh-Malmiri, M. Mohammadlou, A. Berenjian, N. Anarjan, N. Jafari, S. Nasiri, Application of magnetic nanoparticles in smart enzyme immobilization, Biotechnol. Lett. 38 (2016) 223–233, https://doi.org/10.1007/s10529-015-1977-z.
[101] N. Feng, H. Zhang, Y. Li, Y. Liu, L. Xu, Y. Wang, X. Fei, J. Tian, A novel catalytic material for hydrolyzing cow's milk allergenic proteins: papain-Cu3(PO4) 2·3H2O-magnetic nanoflowers, Food Chem. 311 (2020), 125911, https://doi.org/10.1016/j.foodchem.2019.125911.
[102] X. Zhu, Y. Li, G. Yang, M. Lv, L. Zhang, Covalent immobilization of alkaline proteinase on amino-functionalized magnetic nanoparticles and application in soy protein hydrolysis, Biotechnol. Prog. 35 (2019), e2756, https://doi.org/10.1002/btpr.2756.
[103] B. Gao, L. He, D. Wei, L. Zhang, Identification and magnetic immobilization of a pyrophilous aspartic protease from Antarctic psychrophilic fungus, J. Food Biochem. 42 (2018), e12691, https://doi.org/10.1111/jfbc.12691.
[104] R.D.S. Azevedo, I.P.G. Amaral, A.C.M. Ferreira, T.S. Espósito, R.S. Bezerra, Use of fish trypsin immobilized onto magnetic-chitosan composite as a new tool to detect antinutrients in aquafeeds, Food Chem. 257 (2018) 302–309, https://doi.org/10.1016/j.foodchem.2018.03.034.
[105] G. Cirillo, F.P. Nicoletta, M. Curcio, U.G. Spizzirri, N. Picci, F. Iemma, Enzyme immobilization on smart polymers: catalysis on demand, React. Funct. Polym. 83 (2014) 62–69, https://doi.org/10.1016/j.reactfunctpolym.2014.07.010.
[106] M. Sardar, R. Agarwal, A. Kumar, M.N. Gupta, Noncovalent immobilization of enzymes on an enteric polymer eudragit S-100, Enzym. Microb. Technol. 20 (1997) 361–367, https://doi.org/10.1016/S0141-0229(96)00152-4.
[107] E.P. Cipolatti, A. Valério, R.O. Henriques, D.E. Moritz, J.L. Ninow, D.M.G. Freire, E.A. Manoel, R. Fernandez-Lafuente, D. De Oliveira, Nanomaterials for biocatalyst immobilization-state of the art and future trends, RSC Adv. 6 (2016) 104675–104692, https://doi.org/10.1039/c6ra22047a.
[108] J. Del Arco, A.R. Alcántara, R. Fernández-Lafuente, J. Fernández-Lucas, Magnetic micro-macro biocatalysts applied to industrial bioprocesses, Bioresour. Technol. 322 (2021), 124547, https://doi.org/10.1016/j.biortech.2020.124547.
[109] P. Hong, S. Koza, E.S.P. Bouvier, A review size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates, J. Liq. Chromatogr. Relat. Technol. 35 (2012) 2923–2950, https://doi.org/10.1080/10826076.2012.743724.
[110] H.G. Barth, B.E. Boyes, C. Jackson, Size exclusion chromatography and related separation techniques, Anal. Chem. 70 (1998) 251–278, https://doi.org/10.1021/a1980015t.
[111] J. Schnapp, Y. Shalitin, Immobilization of enzymes by covalent binding to amine supports via cyanogen bromide activation, Biochem. Biophys. Res. Commun. 70 (1976) 8–14, https://doi.org/10.1016/0006-291X(76)91101-3.
[112] M.R. Smith, C. Ratledge, S. Crook, Properties of cyanogen bromide-activated, agarose-immobilized catechol 1,2-dioxygenase from freeze-dried extracts of nocardia sp. NCIB 10503, Enzym. Microb. Technol. 12 (1990) 945–949, https://doi.org/10.1016/0141-0229(90)90114-6.
[113] A. Ballesteros, J.M. Guisan, J. Serrano, Influence of the activation degree of the support on the properties of agarose-Nuclease, in: Enzym. Eng. Proc. Int. Enzym. Eng. Conf, Springer US, Boston, MA, 1982, pp. 223–224, https://doi.org/10.1007/978-1-4615-9290-7_47.
[114] A. Kilara, K.M. Shahani, F.W. Wagner, Preparation and properties of immobilized papain and lipase, Biotechnol. Bioeng. 19 (1977) 1703–1714, https://doi.org/10.1002/bit.260191109.
[115] A. Kilara, K.M. Shahani, F.W. Wagner, The kinetic properties of lactase, papain and lipase immobilized on a single support, J. Food Biochem. 1 (1978) 261–273, https://doi.org/10.1111/j.1745-4514.1978.tb00185.x.
[116] A.A. Homaei, R.H. Sajedi, R. Sariri, S. Seyfzadeh, R. Stevanato, Cysteine enhances activity and stability of immobilized papain, Amino Acids 38 (2010) 937–942, https://doi.org/10.1007/s00726-009-0302-3.
[117] A. Homaei, Enhanced activity and stability of papain immobilized on CNBr-activated sepharose, Int. J. Biol. Macromol. 75 (2015) 373–377, https://doi.org/10.1016/j.ijbiomac.2015.01.055.
[118] C. Mateo, O. Abian, M. Bernedo, E. Cuenca, M. Fuentes, G. Fernandez-Lorente, J. M. Palomo, V. Grazu, B.C.C. Pessela, C. Giacomini, G. Irazoqui, A. Villarino, K. Ovsejevi, F. Batista-Viera, R. Fernandez-Lafuente, J.M. Guisán, Some special features of glyoxyl supports to immobilize proteins, Enzym. Microb. Technol. 37 (2005) 456–462, https://doi.org/10.1016/j.enzmictec.2005.03.020.
[119] T.B. Pessato, O.L. Tavano, Hydrolysis of casein and ß-lactoglobulin by immobilized papain after pre-treatment with immobilized trypsin, Acta Aliment. 44 (2015) 570–577, https://doi.org/10.1556/066.2015.44.0029.
[120] T. Jesionowski, J. Zdarta, B. Krajewska, Enzyme immobilization by adsorption: a review, Adsorption 20 (2014) 801–821, https://doi.org/10.1007/s10450-014-9623-y.
[121] J. Porath, B. Olin, Immobilized metal affinity adsorption and immobilized metal affinity chromatography of biomaterials. Serum protein affinities for gel-immobilized iron and nickel ions, Biochemistry 22 (1983) 1621–1630, https://doi.org/10.1021/bi00276a015.
[122] S. Afaq, J. Iqbal, Immobilization and stabilization of papain on chelating sepharose: a metal chelate regenerable carrier, Electron. J. Biotechnol. 4 (2001) 32–36, https://doi.org/10.2225/vol4-issue3-fulltext-1.
[123] E. Lam, K.B. Male, J.H. Chong, A.C.W. Leung, J.H.T. Luong, Applications of functionalized and nanoparticle-modified nanocrystalline cellulose, Trends Biotechnol. 30 (2012) 283–290, https://doi.org/10.1016/j.tibtech.2012.02.001.
[124] M.L. Cacicedo, M.C. Castro, I. Servetas, L. Bosnea, K. Boura, P. Tsafrakidou, A. Dima, A. Terpou, A. Koutinas, G.R. Castro, Progress in bacterial cellulose matrices for biotechnological applications, Bioresour. Technol. 213 (2016) 172–180, https://doi.org/10.1016/j.biortech.2016.02.071.
[125] M.E. Culica, A.-L. Chibac-Scutaru, T. Mohan, S. Coseri, Cellulose-based biogenic supports, remarkably friendly biomaterials for proteins and biomolecules, Biosens. Bioelectron. 182 (2021), 113170, https://doi.org/10.1016/j.bios.2021.113170.
[126] A. Gennari, A.J. Führ, G. Volpato, C.F. Volken de Souza, Magnetic cellulose: versatile support for enzyme immobilization - a review, Carbohydr. Polym. 246 (2020), 116646, https://doi.org/10.1016/j.carbpol.2020.116646.
[127] M.A.C.M. Haniffa, K. Munawar, C.Y. Chee, S. Pramanik, A. Halilu, H.A. Illias, M. Rizwan, R. Senthilnithy, K.R.R. Mahanama, A. Tripathy, M.F. Azman, Cellulose supported magnetic nanohybrids: synthesis, physicomagnetic properties and biomedical applications-a review, Carbohydr. Polym. 267 (2021), 118136, https://doi.org/10.1016/j.carbpol.2021.118136.
[128] X.-J. Huang, P.-C. Chen, F. Huang, Y. Ou, M.-R. Chen, Z.-K. Xu, Immobilization of Candida rugosa lipase on electrospun cellulose nanofiber membrane, J. Mol. Catal. B Enzym. 70 (2011) 95–100, https://doi.org/10.1016/j.molcatb.2011.02.010.
[129] S. Varavinit, N. Chaokasem, S. Shobsngob, Covalent immobilization of a glucoamylase to bagasse dialdehyde cellulose, World J. Microbiol. Biotechnol. 17 (2001) 721–725, https://doi.org/10.1023/A:1012984802624.
[130] M. Singh, A.R. Ray, P.V.K. Verma, S.K. Guha, Potential biosoluble carriers: biocompatibility and biodegradability of oxidized cellulose, Biomater. Med. Devices Artif. Organs 7 (1979) 495–512, https://doi.org/10.3109/10731197909118964.
[131] F. Jin, K. Toda, Preparation of immobilized papain covalently bound on natural cellulose for treatment of beer, Biotechnol. Lett. 10 (1988) 221–223, https://doi.org/10.1007/BF01134834.
[132] Y.M. Yuan, Q.J. Li, S.K. Yao, W. Zhang, J.F. Zhang, Immobilization of papain on regenerated cellulose from ionic liquids, Appl. Mech. Mater. 448–453 (2013) 1651–1655, https://doi.org/10.4028/www.scientific.net/AMM.448-453.1651.
[133] Y.M. Yuan, Q.J. Li, S.K. Yao, J.X. Gong, J.F. Zhang, Different patterns of ionic liquids-regenerated cellulose carriers for papain immobilization, Adv. Mater. Res. 864–867 (2013) 319–323, https://doi.org/10.4028/www.scientific.net/AMR.864-867.319.
[134] N.F. Vasconcelos, A.P. Cunha, N.M.P.S. Ricardo, R.S. Freire, L. De A.P. Vieira, A.I. S. Brígida, M. De F. Borges, M. De F. Rosa, R.S. Vieira, F.K. Andrade, Papain immobilization on heterofunctional membrane bacterial cellulose as a potential strategy for the debridement of skin wounds, Int. J. Biol. Macromol. 165 (2020) 3065–3077, https://doi.org/10.1016/j.ijbiomac.2020.10.200.
[135] I. Migneault, C. Dartiguenave, M.J. Bertrand, K.C. Waldron, Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking, Biotechniques. 37 (2004) 790–802, https://doi.org/10.2144/04375RV01.
[136] Y. Wine, N. Cohen-Hadar, A. Freeman, F. Frolow, Elucidation of the mechanism and end products of glutaraldehyde crosslinking reaction by X-ray structure analysis, Biotechnol. Bioeng. 98 (2007) 711–718, https://doi.org/10.1002/bit.21459.

[137] D. de Andrades, N.G. Graebin, M.K. Kadowaki, M.A.Z. Ayub, R. Fernandez-Lafuente, R.C. Rodrigues, Immobilization and stabilization of different ß-glucosidases using the glutaraldehyde chemistry: optimal protocol depends on the enzyme, Int. J. Biol. Macromol. 129 (2019) 672–678, https://doi.org/10.1016/j.ijbiomac.2019.02.057.
[138] P.G. Vazquez-Ortega, M.T. Alcaraz-Fructuoso, J.A. Rojas-Contreras, J. López-Miranda, R. Fernandez-Lafuente, Stabilization of dimeric ß-glucosidase from aspergillus Niger via glutaraldehyde immobilization under different conditions, Enzym. Microb. Technol. 110 (2018) 38–45, https://doi.org/10.1016/j.enzmictec.2017.12.007.
[139] H. Zaak, S. Peirce, T. de Albuquerque, M. Sassi, R. Fernandez-Lafuente, Exploiting the versatility of aminated supports activated with glutaraldehyde to immobilize ß-galactosidase from aspergillus oryzae, Catalysts. 7 (2017) 250, https://doi.org/10.3390/catal7090250.
[140] O. Barbosa, R. Torres, C. Ortiz, R. Fernandez-Lafuente, Versatility of glutaraldehyde to immobilize lipases: effect of the immobilization protocol on the properties of lipase B from Candida antarctica, Process Biochem. 47 (2012) 1220–1227, https://doi.org/10.1016/j.procbio.2012.04.019.
[141] L. Betancor, F. López-Gallego, A. Hidalgo, N. Alonso-Morales, G.D.-O.C. Mateo, R. Fernández-Lafuente, J.M. Guisán, Different mechanisms of protein immobilization on glutaraldehyde activated supports: effect of support activation and immobilization conditions, Enzym. Microb. Technol. 39 (2006) 877–882, https://doi.org/10.1016/j.enzmictec.2006.01.014.
[142] F. López-Gallego, L. Betancor, C. Mateo, A. Hidalgo, N. Alonso-Morales, G. Dellamora-Ortiz, J.M. Guisán, R. Fernández-Lafuente, Enzyme stabilization by glutaraldehyde crosslinking of adsorbed proteins on aminated supports, J. Biotechnol. 119 (2005) 70–75, https://doi.org/10.1016/j.jbiotec.2005.05.021.
[143] G. Guo, L. Ma, W. Xie, G. Chen, L. Gu, Activation of microcrystalline cellulose by two-step method and its use in immobilization of papain, Zhongshan Daxue Xuebao Acta Sci. Nat. Univ. Sunyatseni 40 (1980) 45–47.
[144] S.K. Yao, Q.J. Li, W. Zhang, J.X. Gong, J.F. Zhang, Ionic liquid-regenerated cellulose beads as solid support matrices for papain immobilization, Adv. Mater. Res. 535–537 (2012) 2349–2352, https://doi.org/10.4028/www.scientific.net/AMR.535-537.2349.
[145] Q. Li, Z. Zhao, Y. Yuan, J. Gong, J. Zhang, Immobilization of papain with ionic liquids-regenerated cellulose membrane for wool fabric modification, Gaodeng Xuexiao Huaxue Xuebao 36 (2015) 2590–2597, https://doi.org/10.7503/cjcu20150498.
[146] J. Xiong, S.-L. Cao, M.-H. Zong, W.-Y. Lou, X. Wu, Biosynthesis of alanyl-histidine dipeptide catalyzed by papain immobilized on magnetic nanocrystalline cellulose in deep eutectic solvents, Appl. Biochem. Biotechnol. 192 (2020) 573–584, https://doi.org/10.1007/s12010-020-03345-3.
[147] Q. Zhang, K. De Oliveira Vigier, S. Royer, F. Jérôme, Deep eutectic solvents: syntheses, properties and applications, Chem. Soc. Rev. 41 (2012) 7108, https://doi.org/10.1039/c2cs35178a.
[148] A.P. Abbott, D. Boothby, G. Capper, D.L. Davies, R.K. Rasheed, Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, J. Am. Chem. Soc. 126 (2004) 9142–9147, https://doi.org/10.1021/ja048266j.
[149] Y. Dai, J. van Spronsen, G.-J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as new potential media for green technology, Anal. Chim. Acta 766 (2013) 61–68, https://doi.org/10.1016/j.aca.2012.12.019.
[150] D. Lindberg, M. de la Fuente Revenga, M. Widersten, Deep eutectic solvents (DESs) are viable cosolvents for enzyme-catalyzed epoxide hydrolysis, J. Biotechnol. 147 (2010) 169–171, https://doi.org/10.1016/j.jbiotec.2010.04.011.
[151] H. Zhao, C. Zhang, T.D. Crittle, Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil, J. Mol. Catal. B Enzym. 85–86 (2013) 243–247, https://doi.org/10.1016/j.molcatb.2012.09.003.
[152] V. Kasche, Mechanism and yields in enzyme catalysed equilibrium and kinetically controlled synthesis of ß-lactam antibiotics, peptides and other condensation products, Enzym. Microb. Technol. 8 (1986) 4–16, https://doi.org/10.1016/0141-0229(86)90003-7.
[153] V. Schellenberger, H.-D. Jakubke, Protease-catalyzed kinetically controlled peptide synthesis, Angew. Chem. Int. Ed. Engl. 30 (1991) 1437–1449, https://doi.org/10.1002/anie.199114371.
[154] V. Kasche, U. Haufler, L. Riechmann, [26] Equilibrium and kinetically controlled synthesis with enzymes: semisynthesis of penicillins and peptides, in: Methods Enzymol, 1987, pp. 280–292, https://doi.org/10.1016/S0076-6879(87)36028-8.
[155] O. Barbosa, C. Ortiz, R. Torres, R. Fernandez-Lafuente, Effect of the immobilization protocol on the properties of lipase B from Candida antarctica in organic media: enantiospecifc production of atenolol acetate, J. Mol. Catal. B Enzym. 71 (2011) 124–132, https://doi.org/10.1016/j.molcatb.2011.04.008.
[156] R. Fernández-Lafuente, O. Hernández-Justiz, C. Mateo, M. Terreni, G. Fernández-Lorente, M.A. Moreno, J. Alonso, J.L. García-López, J.M. Guisan, Biotransformations catalyzed by multimeric enzymes: stabilization of tetrameric ampicillin acylase permits the optimization of ampicillin synthesis under dissociation conditions, Biomacromolecules 2 (2001) 95–104, https://doi.org/10.1021/bm000072i.
[157] O. Hernández-Justiz, M. Terreni, G. Pagani, J. Garcia, J. Guisán, R. Fernández-Lafuente, Evaluation of different enzymes as catalysts for the production of ß-lactam antibiotics following a kinetically controlled strategy, Enzym. Microb. Technol. 25 (1999) 336–343, https://doi.org/10.1016/S0141-0229(99)00050-2.
[158] I. Estruch, A.R. Tagliani, J.M. Guisán, R. Fernández-Lafuente, A.R. Alcántara, L. Toma, M. Terreni, Immobilization of the acylase from Escherichia coli on glyoxyl-agarose gives efficient catalyst for the synthesis of cephalosporins, Enzym. Microb. Technol. 42 (2008) 121–129, https://doi.org/10.1016/j.enzmictec.2007.08.013.
[159] N. Albayrak, S.-T. Yang, Production of galacto-oligosaccharides from lactose by aspergillus oryzae ß-galactosidase immobilized on cotton cloth, Biotechnol. Bioeng. 77 (2002) 8–19, https://doi.org/10.1002/bit.1195.
[160] N. Albayrak, S.-T. Yang, Immobilization of aspergillus oryzae ß-galactosidase on tosylated cotton cloth, Enzym. Microb. Technol. 31 (2002) 371–383, https://doi.org/10.1016/S0141-0229(02)00115-1.
[161] T. Nikolic, M. Kostic, J. Praskalo, B. Pejic, Z. Petronijevic, P. Skundric, Sodium periodate oxidized cotton yarn as carrier for immobilization of trypsin, Carbohydr. Polym. 82 (2010) 976–981, https://doi.org/10.1016/j.carbpol.2010.06.028.
[162] F.-Y. Li, Y.-J. Xing, X. Ding, Immobilization of papain on cotton fabric by sol–gel method, Enzym. Microb. Technol. 40 (2007) 1692–1697, https://doi.org/10.1016/j.enzmictec.2006.09.007.
[163] F.-Y. Li, Z.-L. Zhong, Y.-J. Xing, X. Ding, A cotton cloth bioreactor with immobilized papain, in: Proc. Fiber Soc. 2009 Spring Conf, 2009, pp. 789–791.
[164] S. Li, H. Nie, H. Zhang, Y. Xue, L. Zhu, Cotton fabric as a carrier to immobilize chemically modified papain, in: ICAFPM 2009 - Proc. 2009 Int. Conf. Adv. Fibers Polym. Mater., Shanghai, 2009, pp. 883–886.
[165] Y. Xue, H. Nie, S. Li, L. Zhu, Study on the immobilization of the chemically modified papain and its properties, Chem. Bull. 72 (2009) 277–280. https://www.scopus.com/record/display.uri?eid=2-s2.0-67249105929&origin=inward&txGid=c4e7ef4f5b85bf233f7501d4969d5991.
[166] Y. Xue, H. Nie, L. Zhu, S. Li, H. Zhang, Immobilization of modified papain with anhydride groups on activated cotton fabric, Appl. Biochem. Biotechnol. 160 (2010) 109–121, https://doi.org/10.1007/s12010-009-8588-x.
[167] M. Rinaudo, Chitin and chitosan: properties and applications, Prog. Polym. Sci. 31 (2006) 603–632, https://doi.org/10.1016/j.progpolymsci.2006.06.001.
[168] M.N. Ravi Kumar, A review of chitin and chitosan applications, React. Funct. Polym. 46 (2000) 1–27, https://doi.org/10.1016/S1381-5148(00)00038-9.
[169] A. Hirai, H. Odani, A. Nakajima, Determination of degree of deacetylation of chitosan by 1H NMR spectroscopy, Polym. Bull. 26 (1991) 87–94, https://doi.org/10.1007/BF00299352.
[170] S. Mima, M. Miya, R. Iwamoto, S. Yoshikawa, Highly deacetylated chitosan and its properties, J. Appl. Polym. Sci. 28 (1983) 1909–1917, https://doi.org/10.1002/app.1983.070280607.
[171] R.F. Weska, J.M. Moura, L.M. Batista, J. Rizzi, L.A.A. Pinto, Optimization of deacetylation in the production of chitosan from shrimp wastes: use of response surface methodology, J. Food Eng. 80 (2007) 749–753, https://doi.org/10.1016/j.jfoodeng.2006.02.006.
[172] K.H. Sjoholm, M. Cooney, S.D. Minteer, Effects of degree of deacetylation on enzyme immobilization in hydrophobically modified chitosan, Carbohydr. Polym. 77 (2009) 420–424, https://doi.org/10.1016/j.carbpol.2009.02.006.
[173] L. Zang, J. Qiu, X. Wu, W. Zhang, E. Sakai, Y. Wei, Preparation of magnetic chitosan nanoparticles as support for cellulase immobilization, Ind. Eng. Chem. Res. 53 (2014) 3448–3454, https://doi.org/10.1021/ie404072s.
[174] G. Bayramoglu, M. Yilmaz, M. Yakup Arica, Preparation and characterization of epoxy-functionalized magnetic chitosan beads: laccase immobilized for degradation of reactive dyes, Bioprocess Biosyst. Eng. 33 (2010) 439–448, https://doi.org/10.1007/s00449-009-0345-6.
[175] W.S. Adriano, D.B. Mendonça, D.S. Rodrigues, E.J. Mammarella, R.L.C. Giordano, Improving the properties of chitosan as support for the covalent multipoint immobilization of chymotrypsin, Biomacromolecules 9 (2008) 2170–2179, https://doi.org/10.1021/bm8002754.
[176] B.B. Pinheiro, N.S. Rios, E. Rodríguez Aguado, R. Fernandez-Lafuente, T. M. Freire, P.B.A. Fechine, J.C.S. dos Santos, L.R.B. Gonçalves, Chitosan activated with divinyl sulfone: a new heterofunctional support for enzyme immobilization. application in the immobilization of lipase B from Candida antarctica, Int. J. Biol. Macromol. 130 (2019) 798–809, https://doi.org/10.1016/j.ijbiomac.2019.02.145.
[177] J.A. Silva, G.P. Macedo, D.S. Rodrigues, R.L.C. Giordano, L.R.B. Gonçalves, Immobilization of Candida antarctica lipase B by covalent attachment on chitosan-based hydrogels using different support activation strategies, Biochem. Eng. J. 60 (2012) 16–24, https://doi.org/10.1016/j.bej.2011.09.011.
[178] M.P. Klein, C.R. Hackenhaar, A.S.G. Lorenzoni, R.C. Rodrigues, T.M.H. Costa, J. L. Ninow, P.F. Hertz, Chitosan crosslinked with genipin as support matrix for application in food process: support characterization and ß-d-galactosidase immobilization, Carbohydr. Polym. 137 (2016) 184–190, https://doi.org/10.1016/j.carbpol.2015.10.069.
[179] R.-S. Juang, F.-C. Wu, R.-L. Tseng, Use of chemically modified chitosan beads for sorption and enzyme immobilization, Adv. Environ. Res. 6 (2002) 171–177, https://doi.org/10.1016/S1093-0191(00)00078-2.
[180] F. Zheng, B.-K. Cui, X.-J. Wu, G. Meng, H.-X. Liu, J. Si, Immobilization of laccase onto chitosan beads to enhance its capability to degrade synthetic dyes, Int. Biodeterior. Biodegrad. 110 (2016) 69–78, https://doi.org/10.1016/j.ibiod.2016.03.004.
[181] R.A.A. Muzzarelli, Immobilization of enzymes on chitin and chitosan, Enzym. Microb. Technol. 2 (1980) 177–184, https://doi.org/10.1016/0141-0229(80)90044-7.
[182] V.G. Tacias-Pascacio, E. García-Parra, G. Vela-Gutiérrez, J.J. Virgen-Ortiz, Á. Berenguer-Murcia, A.R. Alcántara, R. Fernandez-Lafuente, Genipin as an emergent tool in the design of biocatalysts: mechanism of reaction and applications, Catalysts. 9 (2019) 1035, https://doi.org/10.3390/catal9121035.
[183] H.-F. Ma, G. Meng, B.-K. Cui, J. Si, Y.-C. Dai, Chitosan crosslinked with genipin as supporting matrix for biodegradation of synthetic dyes: laccase immobilization

and characterization, Chem. Eng. Res. Des. 132 (2018) 664–676, https://doi.org/10.1016/j.cherd.2018.02.008.
[184] S. Chiou, T. Hung, R. Giridhar, W. Wu, Immobilization of lipase to chitosan beads using a natural cross-linker, Prep. Biochem. Biotechnol. 37 (2007) 265–275, https://doi.org/10.1080/10826060701386752.
[185] P.E. Kidibule, J. Costa, A. Atrei, F.J. Plou, M. Fernandez-Lobato, R. Pogni, Production and characterization of chitooligosaccharides by the fungal chitinase Chit42 immobilized on magnetic nanoparticles and chitosan beads: selectivity, specificity and improved operational utility, RSC Adv. 11 (2021) 5529–5536, https://doi.org/10.1039/D0RA10409D.
[186] P.C. Lima, I. Gazoni, A.M.G. de Carvalho, D. Bresolin, D. Cavalheiro, D. de Oliveira, E. Rigo, ß-galactosidase from kluyveromyces lactis in genipin-activated chitosan: an investigation on immobilization, stability, and application in diluted UHT milk, Food Chem. 349 (2021), 129050, https://doi.org/10.1016/j.foodchem.2021.129050.
[187] C. Wang, P.-X. Chen, Q. Xiao, Q.-M. Yang, H.-F. Weng, Y.-H. Zhang, A.-F. Xiao, Chitosan activated with genipin: a nontoxic natural carrier for tannase immobilization and its application in enhancing biological activities of tea extract, Mar. Drugs. 19 (2021) 166, https://doi.org/10.3390/md19030166.
[188] K. Itoyama, H. Tanibe, T. Hayashi, Y. Ikada, Spacer effects on enzymatic activity of papain immobilized onto porous chitosan beads, Biomaterials 15 (1994) 107–112, https://doi.org/10.1016/0142-9612(94)90258-5.
[189] F. He, R. Zhuo, L. Liu, M. Xu, Studies on the preparation and characterization of immobilized papain, Acta Polym. Sin. 640 (2000).
[190] H. Lin, H. Wang, C. Xue, M. Ye, Preparation of chitosan oligomers by immobilized papain, Enzym. Microb. Technol. 31 (2002) 588–592, https://doi.org/10.1016/S0141-0229(02)00138-2.
[191] M. Rito-Palomares, Practical application of aqueous two-phase partition to process development for the recovery of biological products, J. Chromatogr. B 807 (2004) 3–11, https://doi.org/10.1016/j.jchromb.2004.01.008.
[192] J.A. Asenjo, B.A. Andrews, Aqueous two-phase systems for protein separation: a perspective, J. Chromatogr. A 1218 (2011) 8826–8835, https://doi.org/10.1016/j.chroma.2011.06.051.
[193] J.A. Asenjo, B.A. Andrews, Aqueous two-phase systems for protein separation: phase separation and applications, J. Chromatogr. A 1238 (2012) 1–10, https://doi.org/10.1016/j.chroma.2012.03.049.
[194] M. Li, E. Su, P. You, X. Gong, M. Sun, D. Xu, D. Wei, Purification and in situ immobilization of papain with aqueous two-phase system, PLoS One. 5 (2010), e15168, https://doi.org/10.1371/journal.pone.0015168.
[195] D.R. Baidamshina, V.A. Koroleva, S.S. Olshannikova, E.Y. Trizna, M.I. Bogachev, V.G. Artyukhov, M.G. Holyavka, A.R. Kayumov, Biochemical properties and anti-biofilm activity of chitosan-immobilized papain, Mar. Drugs. 19 (2021) 197, https://doi.org/10.3390/md19040197.
[196] D.F. Silva, H. Rosa, A.F.A. Carvalho, P. Oliva-Neto, Immobilization of papain on chitin and chitosan and recycling of soluble enzyme for deflocculation of Saccharomyces cerevisiae from bioethanol distilleries, Enzyme Res. 2015 (2015) 1–10, https://doi.org/10.1155/2015/573721.
[197] M. Holyavka, S. Pankova, V. Koroleva, Y. Vyshkvorkina, A. Lukin, M. Kondratyev, V. Artyukhov, Influence of UV radiation on molecular structure and catalytic activity of free and immobilized bromelain, ficin and papain, J. Photochem. Photobiol. B Biol. 201 (2019), 111681, https://doi.org/10.1016/j.jphotobiol.2019.111681.
[198] O. Smidsrod, G. Skjak-Braek, Alginate as immobilization matrix for cells, Trends Biotechnol. 8 (1990) 71–78, https://doi.org/10.1016/0167-7799(90)90139-O.
[199] M. Kierstan, C. Bucke, The immobilization of microbial cells, subcellular organelles, and enzymes in calcium alginate gels, Biotechnol. Bioeng. 19 (1977) 387–397, https://doi.org/10.1002/bit.260190309.
[200] J. Klein, J. Stock, K.D. Vorlop, Pore size and properties of spherical ca-alginate biocatalysts, Eur. J. Appl. Microbiol. Biotechnol. 18 (1983) 86–91, https://doi.org/10.1007/BF00500829.
[201] S. Hosseini, M. Varidi, Optimization of microbial rennet encapsulation in alginate – chitosan nanoparticles, Food Chem. 352 (2021), 129325, https://doi.org/10.1016/j.foodchem.2021.129325.
[202] N. Popovic, D. Pržulj, M. Mladenovic, O. Prodanovic, S. Ece, K. Ilic Đurdic, R. Ostafe, R. Fischer, R. Prodanovic, Immobilization of yeast cell walls with surface displayed laccase from Streptomyces cyaneus within dopamine-alginate beads for dye decolorization, Int. J. Biol. Macromol. 181 (2021) 1072–1080, https://doi.org/10.1016/j.ijbiomac.2021.04.115.
[203] S.K. Papageorgiou, F.K. Katsaros, E.P. Kouvelos, J.W. Nolan, H. Le Deit, N. K. Kanellopoulos, Heavy metal sorption by calcium alginate beads from Laminaria digitata, J. Hazard. Mater. 137 (2006) 1765–1772, https://doi.org/10.1016/j.jhazmat.2006.05.017.
[204] Y. Li, F. Liu, B. Xia, Q. Du, P. Zhang, D. Wang, Z. Wang, Y. Xia, Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites, J. Hazard. Mater. 177 (2010) 876–880, https://doi.org/10.1016/j.jhazmat.2009.12.114.
[205] T. Gotoh, K. Matsushima, K.-I. Kikuchi, Preparation of alginate–chitosan hybrid gel beads and adsorption of divalent metal ions, Chemosphere 55 (2004) 135–140, https://doi.org/10.1016/j.chemosphere.2003.11.016.
[206] A. Bhattacharyya, S. Dutta, P. De, P. Ray, S. Basu, Removal of mercury (II) from aqueous solution using papain immobilized on alginate bead: optimization of immobilization condition and modeling of removal study, Bioresour. Technol. 101 (2010) 9421–9428, https://doi.org/10.1016/j.biortech.2010.06.126.
[207] A. Bhattacharyya, S. Chatterjee, S. Dutta, P. De, S. Basu, Removal and recovery of lead (II) from simulated solution using alginate immobilized papain (AIP), Environ. Eng. Manag. J. 13 (2014) 835–846, https://doi.org/10.30638/eemj.2014.088.
[208] S. Chatterjee, S. Dutta, S. Basu, Removal and recovery of cadmium (II) using immobilized papain, in: Recycl. Reuse Mater. Their Prod, 2013, pp. 125–142. https://www.scopus.com/record/display.uri?eid=2-s2.0-85054691577&origin=inward&txGid=1f5a7c04e4717f9b2e2a4193f19e51cb.
[209] R.N.F.Moreira Filho, N.F. Vasconcelos, F.K. Andrade, M. De F. Rosa, R.S. Vieira, Papain immobilized on alginate membrane for wound dressing application, Colloids Surf. B: Biointerfaces 194 (2020), 111222, https://doi.org/10.1016/j.colsurfb.2020.111222.
[210] F. Contesini, J. de Alencar Figueira, H. Kawaguti, P. de Barros Fernandes, P. de Oliveira Carvalho, M. da Graça Nascimento, H. Sato, Potential applications of carbohydrases immobilization in the food industry, Int. J. Mol. Sci. 14 (2013) 1335–1369, https://doi.org/10.3390/ijms14011335.
[211] J.M. Palomo, G. Muñoz, G. Fernández-Lorente, C. Mateo, M. Fuentes, J.M. Guisan, R. Fernández-Lafuente, Modulation of mucor miehei lipase properties via directed immobilization on different hetero-functional epoxy resins, J. Mol. Catal. B Enzym. 21 (2003) 201–210, https://doi.org/10.1016/S1381-1177(02)00224-2.
[212] R. Torres, C. Mateo, G. Fernandez-Lorente, C. Ortiz, M. Fuentes, J.M. Palomo, J. M. Guisan, R. Fernandez-Lafuente, A novel heterofunctional epoxy-amino sepabeads for a new enzyme immobilization protocol: immobilization-stabilization of ß-galactosidase from aspergillus oryzae, Biotechnol. Prog. 19 (2003) 1056–1060, https://doi.org/10.1021/bp025771g.
[213] J.X. Wong, K. Ogura, S. Chen, B.H.A. Rehm, Bioengineered polyhydroxyalkanoates as immobilized enzyme scaffolds for industrial applications, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00156.
[214] S. Aggarwal, A. Chakravarty, S. Ikram, A comprehensive review on incredible renewable carriers as promising platforms for enzyme immobilization & thereof strategies, Int. J. Biol. Macromol. 167 (2021) 962–986, https://doi.org/10.1016/j.ijbiomac.2020.11.052.
[215] C.G. Beddows, H. Gil, J.T. Guthrie, The use of graft copolymers as enzyme supports, Polym. Bull. 3 (1980) 645–653, https://doi.org/10.1007/BF00285301.
[216] M. Kumakura, I. Kaetsu, Properties of immobilized papain by radiation polymerization, Polym. J. 16 (1984) 113–117, https://doi.org/10.1295/polymj.16.113.
[217] R.Y. Chiou, L.R. Beuchat, Characteristics and application of immobilized papain in a continuous-flow reactor, Biotechnol. Appl. Biochem. 8 (1986) 529–536. http://www.ncbi.nlm.nih.gov/pubmed/3814357.
[218] R.Y.-Y. Chiou, L.R. Beuchat, Immobilization of papain on an anion exchange resin by physical adsorption followed by cross linking with glutaraldehyde, J. Food Biochem. 11 (1987) 163–176, https://doi.org/10.1111/j.1745-4514.1987.tb00120.x.
[219] Y.-F. Li, J.-R. Li, L.-D. Fu, Y.-Z. Li, Study on the immobilization of papain with a macroporous bead carrier of copolymer containing monomer units of N-aminoethyl acrylamide and vinyl alcohol, Chin. J. Polym. Sci. 18 (2000) 25–31.
[220] Y.F. Li, F.Y. Jia, J.R. Li, G. Liu, Y.Z. Li, Papain immobilization on a nitrilon fibre carrier containing primary amine groups, Biotechnol. Appl. Biochem. 33 (2001) 29, https://doi.org/10.1042/BA19990015.
[221] L. Ding, Z. Yao, T. Li, M. Qiu, Q. Yue, J. Chai, Synthesis of macroporous polymer carrier and immobilization of papain, Turk. J. Chem. 12 (2003) 491–495.
[222] L. Ding, Z. Yao, T. Li, Q. Yue, J. Chai, Study on papain immobilization on a macroporous polymer carrier, Turk. J. Chem. 27 (2003) 627–637.
[223] L. Ding, Z. Yao, T. Li, M. Qiu, X-ray microanalysis of the activity of immobilized papain, Guang Pu Xue Yu Guang Pu Fen Xi 23 (2003) 400–402. http://www.ncbi.nlm.nih.gov/pubmed/12961907.
[224] C. Maria Manohar, M. Doble, Papain immobilized polyurethane as an ureteral stent material, J Biomed Mater Res B Appl Biomater 104 (2016) 723–731, https://doi.org/10.1002/jbm.b.33627.
[225] I.E. Moreno-Cortez, J. Romero-García, V. González-González, D.I. García-Gutierrez, M.A. Garza-Navarro, R. Cruz-Silva, Encapsulation and immobilization of papain in electrospun nanofibrous membranes of PVA cross-linked with glutaraldehyde vapor, Mater. Sci. Eng. C. 52 (2015) 306–314, https://doi.org/10.1016/j.msec.2015.03.049.
[226] H. Eckstein, H.J. Renner, H. Brun, Immobilization of papain on epoxy-polymers for papain-catalyzed peptide synthesis, Biomed. Biochim. Acta. 50 (1991), S114-7. http://www.ncbi.nlm.nih.gov/pubmed/1820030.
[227] T. Boller, C. Meier, S. Menzler, Eupergit oxirane acrylic beads: how to make enzymes fit for biocatalysis, Org. Process. Res. Dev. 6 (2002) 509–519, https://doi.org/10.1021/op015506w.
[228] C. Mateo, O. Abian, R. Fernandez-Lafuente, J.M. Guisan, Increase in conformational stability of enzymes immobilized on epoxy-activated supports by favoring additional multipoint covalent attachment, Enzym. Microb. Technol. 26 (2000) 509–515, https://doi.org/10.1016/S0141-0229(99)00188-X.
[229] C. Mateo, G. Fernández-Lorente, O. Abian, R. Fernández-Lafuente, J.M. Guisán, Multifunctional epoxy supports: a new tool to improve the covalent immobilization of proteins. The promotion of physical adsorptions of proteins on the supports before their covalent linkage, Biomacromolecules 1 (2000) 739–745, https://doi.org/10.1021/bm000071q.
[230] C. Mateo, R. Torres, G. Fernández-Lorente, C. Ortiz, M. Fuentes, A. Hidalgo, F. López-Gallego, O. Abian, J.M. Palomo, L. Betancor, J.M. Guisan, R. Fernández-Lafuente, Epoxy-amino groups: a new tool for improved immobilization of proteins by the epoxy method, Biomacromolecules 4 (2003) 772–777, https://doi.org/10.1021/bm0257661.
[231] V. Grazu, F. López-Gallego, J.M. Guisán, Tailor-made design of penicillin G acylase surface enables its site-directed immobilization and stabilization onto

commercial mono-functional epoxy supports, Process Biochem. 47 (2012) 2538–2541, https://doi.org/10.1016/j.procbio.2012.07.010.
[232] W.J. Diao, S.P. Zhang, P. Wang, W.Q. Zhou, G.H. Ma, Z.G. Su, Y.F. Zhang, Enzymatic hydrolysis of yeast proteins to antioxidant peptides by papain immobilized on gigaporous microspheres, Bioprod. Biosyst. Eng. 41 (2013), https://doi.org/10.3969/j.issn.1005-9954.2013.11.001 (1-4+13).
[233] G. Bayramoglu, B. Filiz Senkal, M. Yilmaz, M. Yakup Arica, Immobilization and stabilization of papain on poly(hydroxyethyl methacrylate–ethylenglycol dimethacrylate) beads grafted with epoxy functional polymer chains via surface-initiated-atom transfer radical polymerization (SI-ATRP), Bioresour. Technol. 102 (2011) 9833–9837, https://doi.org/10.1016/j.biortech.2011.08.042.
[234] C. Armutcu, M.E. Çorman, E. Bayram, L. Uzun, Purification of fab and fc using papain immobilized cryogel bioreactor separator system, J. Chromatogr. B 1158 (2020), 122396, https://doi.org/10.1016/j.jchromb.2020.122396.
[235] M. Teske, T. Kießlich, N. Grabow, S. Illner, J. Fischer, H. Bahl, Immobilizing hydrolytic active papain on biodegradable PLLA for biofilm inhibition in cardiovascular applications, Curr. Dir. Biomed. Eng. 6 (2020) 172–175, https://doi.org/10.1515/cdbme-2020-3044.
[236] K. Kawashiro, H. Ishizaki, S. Sugiyama, H. Hayashi, Esterification of N-benzyloxycarbonyldipeptides in ethanol-water with immobilized papain, Biotechnol. Bioeng. 42 (1993) 309–314, https://doi.org/10.1002/bit.260420307.
[237] C.M. Manohar, V. Prabhawathi, P.M. Sivakumar, M. Doble, Design of a papain immobilized antimicrobial food package with curcumin as a crosslinker, PLoS One. 10 (2015), e0121665, https://doi.org/10.1371/journal.pone.0121665.
[238] M. Uygun, R. Yavaser, D.A. Uygun, Reversible papain immobilization onto poly (AAm–MMA)-based cryogels, Bull. Mater. Sci. 39 (2016) 1039–1046, https://doi.org/10.1007/s12034-016-1235-6.
[239] S. Arana-Peña, D. Carballares, R. Morellon-Sterlling, Á. Berenguer-Murcia, A. R. Alcántara, R.C. Rodrigues, R. Fernandez-Lafuente, Enzyme co-immobilization: always the biocatalyst designers' choice…or not? Biotechnol. Adv. (2021) https://doi.org/10.1016/j.biotechadv.2020.107584.
[240] G. Penzol, P. Armisén, R. Fernández-Lafuente, L. Rodés, J.M. Guisán, Use of dextrans as long and hydrophilic spacer arms to improve the performance of immobilized proteins acting on macromolecules, Biotechnol. Bioeng. 60 (1998) 518–523, https://doi.org/10.1002/(SICI)1097-0290(19981120)60:4<518::AID-BIT14>3.0.CO;2-D.
[241] V.G. Jayakumari, V.N.R. Pillai, Immobilization of papain on crosslinked polymer supports: role of the macromolecular matrix on enzymic activity, J. Appl. Polym. Sci. 42 (1991) 583–590, https://doi.org/10.1002/app.1991.070420303.
[242] V.G. Jayakumari, V.N.R. Pillai, Peptide synthesis catalysed by papain immobilised on polymer supports: effect of the macromolecular structure and reaction conditions on synthesis, Proc. Indian Acad. Sci. Chem. Sci. 103 (1991) 133–148, https://doi.org/10.1007/BF02843563.
[243] T. Hayashi, C. Hirayama, M. Iwatsuki, Papain immobilization onto porous poly (?-methyl L-glutamate) beads, J. Appl. Polym. Sci. 44 (1992) 143–150, https://doi.org/10.1002/app.1992.070440115.
[244] R. Ganapathy, S. Manolache, M. Sarmadi, F. Denes, Immobilization of papain on cold-plasma functionalized polyethylene and glass surfaces, J. Biomater. Sci. Polym. Ed. 12 (2001) 1027–1049, https://doi.org/10.1163/156856201753252543.
[245] V.G. Tacias-Pascacio, C. Ortiz, N. Rueda, Á. Berenguer-Murcia, N. Acosta, I. Aranaz, C. Civera, R. Fernandez-Lafuente, A.R. Alcántara, Dextran aldehyde in biocatalysis: more than a mere immobilization system, Catalysts 9 (2019) 622, https://doi.org/10.3390/catal9070622.
[246] B. Shen, R. Wei, X. Liu, J. Zhu, P. Ouyang, Flexible immobilization of papain on amino polystyrene support, Huagong Xuebao J. Chem. Ind. Eng. 56 (2005) 1070–1075.
[247] J.J. Roy, S. Sumi, K. Sangeetha, T.E. Abraham, Chemical modification and immobilization of papain, J. Chem. Technol. Biotechnol. 80 (2005) 184–188, https://doi.org/10.1002/jctb.1177.
[248] A.Ü. Metin, E. Alver, Fibrous polymer-grafted chitosan/clay composite beads as a carrier for immobilization of papain and its usability for mercury elimination, Bioprocess Biosyst. Eng. 39 (2016) 1137–1149, https://doi.org/10.1007/s00449-016-1590-0.
[249] R. Yavaser, A.A. Karagözler, Covalent immobilization of papain onto poly (hydroxyethyl methacrylate)-chitosan cryogels for apple juice clarification, Food Sci. Technol. Int. 26 (2020) 629–641, https://doi.org/10.1177/1082013220919307.
[250] K.-P. Lin, G.-J. Feng, F.-L. Pu, X.-D. Hou, S.-L. Cao, Enhancing the thermostability of papain by immobilizing on deep eutectic solvents-treated chitosan with optimal microporous structure and catalytic microenvironment, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.576266.
[251] A.P. Echavarría, C. Torras, J. Pagán, A. Ibarz, Fruit juice processing and membrane technology application, Food Eng. Rev. 3 (2011) 136–158, https://doi.org/10.1007/s12393-011-9042-8.
[252] V.C. Gekas, Artificial membranes as carriers for the immobilization of biocatalysts, Enzym. Microb. Technol. 8 (1986) 450–460, https://doi.org/10.1016/0141-0229(86)90046-3.
[253] S. Gupta, A. Bhattacharya, C.N. Murthy, Tune to immobilize lipases on polymer membranes: techniques, factors and prospects, Biocatal. Agric. Biotechnol. 2 (2013) 171–190, https://doi.org/10.1016/j.bcab.2013.04.006.
[254] Y. Cen, Y. Liu, Y. Xue, Y. Zheng, Immobilization of enzymes in/on membranes and their applications, Adv. Synth. Catal. 361 (2019) 5500–5515, https://doi.org/10.1002/adsc.201900439.
[255] P. Zhuang, D. Allan Butterfield, Structural and enzymatic characterizations of papain immobilized onto vinyl alcohol/vinyl butyral copolymer membrane, J. Membr. Sci. 66 (1992) 247–257, https://doi.org/10.1016/0376-7388(92)87015-P.
[256] D. Allan Butterfield, J. Lee, S. Ganapathi, D. Bhattacharyya, Biofunctional membranes part IV. Active-site structure and stability of an immobilized enzyme, papain, on modified polysulfone membranes studied by electron paramagnetic resonance and kinetics, J. Membr. Sci. 91 (1994) 47–64, https://doi.org/10.1016/0376-7388(94)00044-1.
[257] S. Ganapathi, D.A. Butterfield, D. Bhattacharyya, Flat-sheet and hollow fiber membrane bioreactors: a study of the kinetics and active site conformational changes of immobilized papain including sorption studies of reaction constituents, J. Chem. Technol. Biotechnol. 64 (1995) 157–164, https://doi.org/10.1002/jctb.280640208.
[258] A. Bhardwaj, J. Lee, K. Glauner, S. Ganapathi, D. Bhattacharyya, D.A. Butterfield, Biofunctional membranes: an EPR study of active site structure and stability of papain non-covalently immobilized on the surface of modified poly(ether) sulfone membranes through the avidin-biotin linkage, J. Membr. Sci. 119 (1996) 241–252, https://doi.org/10.1016/0376-7388(96)00124-X.
[259] V. Hynninen, L. Vuori, M. Hannula, K. Tapio, K. Lahtonen, T. Isoniemi, E. Lehtonen, M. Hirsimäki, J.J. Toppari, M. Valden, V.P. Hytönen, Improved antifouling properties and selective biofunctionalization of stainless steel by employing heterobifunctional silane-polyethylene glycol overlayers and avidin-biotin technology, Sci. Rep. 6 (2016) 29324, https://doi.org/10.1038/srep29324.
[260] M. Roth, A. Jeltsch, Biotin-avidin microplate assay for the quantitative analysis of enzymatic methylation of DNA by DNA methyltransferases, Biol. Chem. 381 (2000) 269–272, https://doi.org/10.1515/BC.2000.035.
[261] N. Dontha, W.B. Nowall, W.G. Kuhr, Generation of biotin/avidin/enzyme nanostructures with maskless photolithography, Anal. Chem. 69 (1997) 2619–2625, https://doi.org/10.1021/ac9702094.
[262] S. Luo, D.R. Walt, Avidin-biotin coupling as a general method for preparing enzyme-based fiber-optic sensors, Anal. Chem. 61 (1989) 1069–1072, https://doi.org/10.1021/ac00185a005.
[263] M. Nakaiwa, T. Ohmori, Dynamic behavior of an immobilized papain membrane system under forced oscillations, ACH - Models Chem. 135 (1998) 329–342.
[264] H.-L. Nie, S.-N. Su, H.-T. Zhang, Y. Xue, S.-B. Li, Y.-T. Zhou, T.-X. Chen, L.-M. Zhu, C. Branford-White, Papain immobilization on chitosan-coated nylon membrane: preparation and its application in cystatin separation, in: 2009 3rd Int. Conf. Bioinforma. Biomed. Eng., IEEE, 2009, pp. 1–4, https://doi.org/10.1109/ICBBE.2009.5163165.
[265] H.M. Abdel-Mageed, R.A. Radwan, N.Z. AbuelEzz, H.A. Nasser, A.A. El Shamy, R. M. Abdelnaby, N.A. EL Gohary, Bioconjugation as a smart immobilization approach for a-amylase enzyme using stimuli-responsive eudragit-L100 polymer: a robust biocatalyst for applications in pharmaceutical industry, Artif. Cells Nanomedicine Biotechnol. 47 (2019) 2361–2368, https://doi.org/10.1080/21691401.2019.1626414.
[266] A. Herberg, X. Yu, D. Kuckling, Responsive polymers as smart carriers in tunable catalytic processes, in: Smart Polym. Catal. Tunable Catal, Elsevier, 2019, pp. 115–147, https://doi.org/10.1016/B978-0-12-811840-5.00006-X.
[267] G. Levin, S. Gómez, A. Glodowsky, O. Cascone, M.J. Hernáiz, Two-step enzymatic strategy for the synthesis of a smart phenolic polymer and further immobilization of a ß-galactosidase able to catalyze transglycosydation reaction, Int. J. Biol. Macromol. 117 (2018) 264–270, https://doi.org/10.1016/j.ijbiomac.2018.05.177.
[268] T. Palai, P.K. Bhattacharya, Bioconjugates from smart polymers and ß-galactosidase: synthesis, characterization and application in producing galacto-oligosaccharides from lactose, J. Indian Chem. Soc. 95 (2018) 917–924.
[269] L.-T. Li, Y.-P. Zhu, Z.-Q. Jiang, Z.-L. Ai, X.-T. Li, Immobilization of hyperthermophilic xylanase from thermotoga maritima on the smart polymer eudragit S-100 and its application in pulp pre-bleaching, Chung-Kuo Tsao Chih China Pulp Pap. 24 (2005) 1–5.
[270] T. Shimoboji, E. Larenas, T. Fowler, A.S. Hoffman, P.S. Stayton, Temperature-induced switching of enzyme activity with smart polymer-enzyme conjugates, Bioconjug. Chem. 14 (2003) 517–525, https://doi.org/10.1021/bc025615v.
[271] I. Roy, A. Gupta, S.K. Khare, V.S. Bisaria, M.N. Gupta, Immobilization of xylan-degrading enzymes from melanocarpus albomyces IIS 68 on the smart polymer eudragit L-100, Appl. Microbiol. Biotechnol. 61 (2003) 309–313, https://doi.org/10.1007/s00253-002-1213-3.
[272] I. Galaev, "Smart" polymers and what they could do in biotechnology and medicine, Trends Biotechnol. 17 (1999) 335–340, https://doi.org/10.1016/S0167-7799(99)01345-1.
[273] A. Kondo, K. Imura, K. Nakama, K. Higashitani, Preparation of immobilized papain using thermosensitive latex particles, J. Ferment. Bioeng. 78 (1994) 241–245, https://doi.org/10.1016/0922-338X(94)90297-6.
[274] J.-P. Chen, S.-H. Chiu, A poly(N-isopropylacrylamide-co-N-acryloxysuccinimide-co-2-hydroxyethyl methacrylate) composite hydrogel membrane for urease immobilization to enhance urea hydrolysis rate by temperature swing, Enzym. Microb. Technol. 26 (2000) 359–367, https://doi.org/10.1016/S0141-0229(99)00181-7.
[275] J.F. Cabrita, L.M. Abrantes, A.S. Viana, N-hydroxysuccinimide-terminated self-assembled monolayers on gold for biomolecules immobilisation, Electrochim. Acta 50 (2005) 2117–2124, https://doi.org/10.1016/j.electacta.2004.09.019.
[276] M. Yan, S.X. Cai, M.N. Wybourne, J.F.W. Keana, N-hydroxysuccinimide ester functionalized perfluorophenyl azides as novel photoactive heterobifunctional crosslinking reagents. the covalent immobilization of biomolecules to polymer surfaces, Bioconjug. Chem. 5 (1994) 151–157, https://doi.org/10.1021/bc00026a007.

[277] D. Ataman Sadik, H. Eksi-Kocak, G. Ertas, I.H. Boyaci, M. Mutlu, Mixed-monolayer of N-hydroxysuccinimide-terminated cross-linker and short alkanethiol to improve the efficiency of biomolecule binding for biosensing, Surf. Interface Anal. 50 (2018) 866–878, https://doi.org/10.1002/sia.6489.
[278] J.-F. Mei, T.-T. Huang, J.-S. Chen, Y. Yi, K.-Y. Zhu, G.-Q. Ying, Properties of immobilized papain on thermally sensitive carrier and its application for HCG antibody digestion, Chin. J. Pharm. Biotechnol. 50 (2013) 210–214.
[279] M. Moritz, M. Geszke-Moritz, Mesoporous materials as multifunctional tools in biosciences: principles and applications, Mater. Sci. Eng. C. 49 (2015) 114–151, https://doi.org/10.1016/j.msec.2014.12.079.
[280] J. Yao, N. Sun, C. Deng, Recent advances in mesoporous materials for sample preparation in proteomics research, TrAC Trends Anal. Chem. 99 (2018) 88–100, https://doi.org/10.1016/j.trac.2017.11.016.
[281] J. Zdarta, A. Meyer, T. Jesionowski, M. Pinelo, A general overview of support materials for enzyme immobilization: characteristics, properties, practical utility, Catalysts. 8 (2018) 92, https://doi.org/10.3390/catal8020092.
[282] C. Cai, Y. Gao, Y. Liu, N. Zhong, N. Liu, Immobilization of Candida antarctica lipase B onto SBA-15 and their application in glycerolysis for diacylglycerols synthesis, Food Chem. 212 (2016) 205–212, https://doi.org/10.1016/j.foodchem.2016.05.167.
[283] Y. Chen, Y. Xu, X.M. Wu, Efficient improving the activity and enantioselectivity of Candida rugosa lipase for the resolution of naproxen by enzyme immobilization on MCM-41 mesoporous molecular sieve, Int. J. Bioautomation. 19 (2015) 325–334.
[284] P.A. Mangrulkar, R. Yadav, J.S. Meshram, N.K. Labhsetwar, S.S. Rayalu, Tyrosinase-immobilized MCM-41 for the detection of phenol, Water Air Soil Pollut. 223 (2012) 819–825, https://doi.org/10.1007/s11270-011-0904-8.
[285] X. Yang, P. Qiu, J. Yang, Y. Fan, L. Wang, W. Jiang, X. Cheng, Y. Deng, W. Luo, Mesoporous materials–based electrochemical biosensors from enzymatic to nonenzymatic, Small 17 (2021) 1904022, https://doi.org/10.1002/smll.201904022.
[286] S. Dutta, A. Bhattacharyya, P. De, P. Ray, S. Basu, Removal of mercury from its aqueous solution using charcoal-immobilized papain (CIP), J. Hazard. Mater. 172 (2009) 888–896, https://doi.org/10.1016/j.jhazmat.2009.07.085.
[287] E. Gorodkiewicz, J. Luszczyn, Surface plasmon resonance imaging (SPRI) sensor for cystatin determination based on immobilized papain, Protein Pept. Lett. 18 (2011) 23–29, https://doi.org/10.2174/092986611794328663.
[288] C. Li, X. Feng, L. Sun, L. Zhou, J. Sun, Z. Wang, D. Liao, P. Lan, X. Lan, Non-covalent and covalent immobilization of papain onto Ti3C2 MXene nanosheets, Enzym. Microb. Technol. 148 (2021), 109817, https://doi.org/10.1016/j.enzmictec.2021.109817.
[289] M. Zhang, Y. Zhang, C. Yang, C. Ma, J. Tang, Enzyme-inorganic hybrid nanoflowers: classification, synthesis, functionalization and potential applications, Chem. Eng. J. 415 (2021), 129075, https://doi.org/10.1016/j.cej.2021.129075.
[290] Y. Li, H. Wu, Z. Su, Enzyme-based hybrid nanoflowers with high performances for biocatalytic, biomedical, and environmental applications, Coord. Chem. Rev. 416 (2020), 213342, https://doi.org/10.1016/j.ccr.2020.213342.
[291] M. Bilal, M. Asgher, S.Z.H. Shah, H.M.N. Iqbal, Engineering enzyme-coupled hybrid nanoflowers: the quest for optimum performance to meet biocatalytic challenges and opportunities, Int. J. Biol. Macromol. 135 (2019) 677–690, https://doi.org/10.1016/j.ijbiomac.2019.05.206.
[292] T.D. Tran, M. Il Kim, Organic-inorganic hybrid nanoflowers as potent materials for biosensing and biocatalytic applications, BioChip J. 12 (2018) 268–279, https://doi.org/10.1007/s13206-018-2409-7.
[293] C. Altinkaynak, S. Tavlasoglu, N. Özdemir, I. Ocsoy, A new generation approach in enzyme immobilization: organic-inorganic hybrid nanoflowers with enhanced catalytic activity and stability, Enzym. Microb. Technol. 93–94 (2016) 105–112, https://doi.org/10.1016/j.enzmictec.2016.06.011.
[294] S. Escobar, S. Velasco-Lozano, C.-H. Lu, Y.-F. Lin, M. Mesa, C. Bernal, F. López-Gallego, Understanding the functional properties of bio-inorganic nanoflowers as biocatalysts by deciphering the metal-binding sites of enzymes, J. Mater. Chem. B 5 (2017) 4478–4486, https://doi.org/10.1039/C6TB03295H.
[295] B. Zhang, P. Li, H. Zhang, L. Fan, H. Wang, X. Li, L. Tian, N. Ali, Z. Ali, Q. Zhang, Papain/Zn3(PO4)2 hybrid nanoflower: preparation, characterization and its enhanced catalytic activity as an immobilized enzyme, RSC Adv. 6 (2016) 46702–46710, https://doi.org/10.1039/C6RA05308D.
[296] A. Costantini, V. Califano, Lipase immobilization in mesoporous silica nanoparticles for biofuel production, Catalysts. 11 (2021) 629, https://doi.org/10.3390/catal11050629.
[297] M. Hartmann, X. Kostrov, Immobilization of enzymes on porous silicas – benefits and challenges, Chem. Soc. Rev. 42 (2013) 6277, https://doi.org/10.1039/c3cs60021a.
[298] P. Monsan, B. Duteurtre, M. Moll, G. Durand, Use of papain immobilized on spherosil for beer chillproofing, J. Food Sci. 43 (1978) 424–427, https://doi.org/10.1111/j.1365-2621.1978.tb02320.x.
[299] S.A. Ogii, V.V. Ianishpol'skii, V.A. Tertykh, Properties of papain, immobilized on organosilica, Ukr. Biokhim. Zh. 62 (1990) 48–52. http://www.ncbi.nlm.nih.gov/pubmed/1965071.
[300] L.J. Liu, Preparation of dendrimers from macromolecular cores and papain immobilization, Chin. Chem. Lett. 9 (1998) 263–264.
[301] R. Zhuo, H. Feng, L. Liu, M. Xu, Thermal activation of immobilized papain, Chin. J. Polym. Sci. 16 (1998) 142–146. English Ed.
[302] S. Sun, G. Yang, H. Liu, H. Sun, C. Liu, A new method to immobilize enzyme and its application to the papain, Chem. J. Internet. 4 (2002) 14–22.
[303] F.L. Cheng, L.L. Chen, W. Wang, L.L. Liu, L. Deng, Immobilization and stabilization of papain on SiO2 particles containing amine groups, Sheng Wu Gong Cheng Xue Bao 20 (2004) 287–290.
[304] M.-Q. Liu, A.-M. Wang, H. Wang, C. Zhou, Z.-Q. Du, S.-M. Zhu, M. Yang, J. Zhang, S.-B. Shen, Immobilization of papain by macromolecular crowding in mesopores under microwave irradiation, Guocheng Gongcheng Xuebao Chin. J. Process Eng. 9 (2009) 157–160.
[305] Z.-C. Zou, Q. Wei, W. Na, H. Sun, Z.-R. Nie, Immobilization of papain on siliceous mesocellular foam, Wuji Cailiao Xuebao J. Inorg. Mater. 24 (2009) 702–706.
[306] N. Xiao, B.-C. Zhao, Y.-H. Wang, R.-Y. Ma, Immobilization of papain on mesoporous molecular sieve MCM-41, Beijing Huagong Daxue Xuebao (Ziran Kexueban) J. Beijing Univ. Chem. Technol. 32 (2005) 41–43. Natural Sci. Ed.
[307] B.-C. Zhao, N. Xiao, R.-Y. Ma, B. Shi, Preparation of nanoporous material MCM-41 as well as immobilization of papain on it, Beijing Huagong Daxue Xuebao (Ziran Kexueban) J. Beijing Univ. Chem. Technol. 33 (2006) 8–11. Natural Sci. Ed.
[308] B. Zhao, B. Shi, R. Ma, Immobilization of papain on the mesoporous molecular sieve MCM-48, Eng. Life Sci. 5 (2005) 436–441, https://doi.org/10.1002/elsc.200520094.
[309] S. Solís, J. Paniagua, J.C. Martínez, M. Asomoza, Immobilization of papain on mesoporous silica: pH effect, J. Sol-Gel Sci. Technol. 37 (2006) 125–127, https://doi.org/10.1007/s10971-006-6431-1.
[310] W. Bian, B. Yan, N. Shi, F. Qiu, L.-L. Lou, B. Qi, S. Liu, Room temperature ionic liquid (RTIL)-decorated mesoporous silica SBA-15 for papain immobilization: RTIL increased the amount and activity of immobilized enzyme, Mater. Sci. Eng. C. 32 (2012) 364–368, https://doi.org/10.1016/j.msec.2011.11.006.
[311] J. He, M. Wu, X. Feng, X. Shao, W. Cai, Immobilization of papain on nanoporous silica, RSC Adv. 4 (2014) 13304–13312, https://doi.org/10.1039/C3RA47346E.
[312] W. Bian, L.-L. Lou, B. Yan, C. Zhang, S. Wu, S. Liu, Immobilization of papain by carboxyl-modified SBA-15: rechecking the carboxyl after excluding the contribution of H2SO4 treatment, Microporous Mesoporous Mater. 143 (2011) 341–347, https://doi.org/10.1016/j.micromeso.2011.03.017.
[313] J.Z. Tian, T. Jing, X.M. Huang, Y.J. Zheng, Q.G. Deng, Z. Li, Cage-shape macroporous/mesoporous 3DOM SiO2 immobilized papain, Adv. Mater. Res. 236–238 (2011) 954–957, https://doi.org/10.4028/www.scientific.net/AMR.236-238.954.
[314] L. Sun, H. Liang, Q. Yuan, T. Wang, H. Zhang, Study on a carboxyl-activated carrier and its properties for papain immobilization, J. Chem. Technol. Biotechnol. 87 (2012) 1083–1088, https://doi.org/10.1002/jctb.3714.
[315] P. Zucca, E. Sanjust, Inorganic materials as supports for covalent enzyme immobilization: methods and mechanisms, Molecules 19 (2014) 14139–14194, https://doi.org/10.3390/molecules190914139.
[316] H.H. Weetall, R.D. Mason, Studies on immobilized papain, Biotechnol. Bioeng. 15 (1973) 455–466, https://doi.org/10.1002/bit.260150303.
[317] D. Hyndman, T.G. Flynn, G. Lever, R. Burrell, Protein immobilization to alumina supports: I. Characterization of alumina-organophosphate ligand interactions and use in the attachment of papain, Biotechnol. Bioeng. 40 (1992) 1319–1327, https://doi.org/10.1002/bit.260401105.
[318] D. Hyndman, R. Burrell, G. Lever, T.G. Flynn, Protein immobilization to alumina supports: II. Papain immobilization to alumina via organophosphate linkers, Biotechnol. Bioeng. 40 (1992) 1328–1336, https://doi.org/10.1002/bit.260401106.
[319] V.D.M. Silva, L.M. De Marco, W.O. Afonso, D.C.F. Lopes, J. Januario, M.J. B. Aguiar, A.L.P. Starling, M.P.C. Silvestre, Preparation of low-phenylalanine whey hydrolysates, using papain and pancreatin immobilized on activated carbon and alumina, Am. J. Food Technol. 2 (2007) 327–341, https://doi.org/10.3923/ajft.2007.327.341.
[320] T.C. Coutinho, J.O.D. Malafatti, E.C. Paris, P.W. Tardioli, C.S. Farinas, Hydroxyapatite-CoFe2O4 magnetic nanoparticle composites for industrial enzyme immobilization, use, and recovery, ACS Appl. Nano Mater. 3 (2020) 12334–12345, https://doi.org/10.1021/acsanm.0c02811.
[321] W.-C. Huang, W. Wang, C. Xue, X. Mao, Effective enzyme immobilization onto a magnetic chitin nanofiber composite, ACS Sustain. Chem. Eng. 6 (2018) 8118–8124, https://doi.org/10.1021/acssuschemeng.8b01150.
[322] S. Seino, K. Watanabe, T. Shikakura, M. Abe, T. Nakagawa, Y. Koga, T. A. Yamamoto, Enzyme immobilization on gold/Fe-oxide composite nanoparticles using a methionine tag, Colloids Surf. A Physicochem. Eng. Asp. 459 (2014) 298–301, https://doi.org/10.1016/j.colsurfa.2014.06.049.
[323] E. Krisch, D. Balogh-Weiser, J. Klimko, B. Gyarmati, K. Laszlo, L. Poppe, A. Szilagyi, Composite beads of silica gel, alginate and poly(aspartic acid) for the immobilization of a lipase enzyme, Express Polym Lett 13 (2019) 512–523, https://doi.org/10.3144/expresspolymlett.2019.43.
[324] A.-M. Wang, J. Zong, H. Wang, S.-B. Shen, Application of silver nanoparticles in immobilization of papain, Guocheng Gongcheng Xuebao Chin. J. Process Eng. 7 (2007) 1018–1021.
[325] A.-M. Wang, J. Zong, H. Wang, S.-B. Shen, Study of papain immobilized on silver/silica composite, Huaxue Gongcheng Chem. Eng. 36 (2008) (60–62 74).
[326] A. Wang, H. Wang, C. Zhou, Z. Du, S. Zhu, S. Shen, Ag-induced efficient immobilization of papain on silica spheres, Chin. J. Chem. Eng. 16 (2008) 612–619, https://doi.org/10.1016/S1004-9541(08)60129-9.
[327] M. Wu, Q. He, Y. Zuo, F. Wang, Y. Sun, Studies on immobilization of papain based on inorganic-organic hybrid gel of micro-nano-scale, Acta Chim. Sin. 69 (2011) 1475–1482.
[328] S.E. Cahyaningrum, S.J. Narsito, R. Santoso, Agustini, preparation and properties of papain immobilized onto metal ions cross-linked chitosan beads, Res. J. Pharm. Biol. Chem. Sci. 4 (2013) 120–126.

[329] H. Dai, S. Ou, Z. Liu, H. Huang, Pineapple peel carboxymethyl cellulose/polyvinyl alcohol/mesoporous silica SBA-15 hydrogel composites for papain immobilization, Carbohydr. Polym. 169 (2017) 504–514, https://doi.org/10.1016/j.carbpol.2017.04.057.
[330] A.M.B.F. Soares, L.M.O. Gonçalves, R.D.S. Ferreira, J.M. de Souza, R. Fangueiro, M.M.M. Alves, F.A.A. Carvalho, A.N. Mendes, W. Cantanhêde, Immobilization of papain enzyme on a hybrid support containing zinc oxide nanoparticles and chitosan for clinical applications, Carbohydr. Polym. 243 (2020), 116498, https://doi.org/10.1016/j.carbpol.2020.116498.
[331] I. Benucci, C. Lombardelli, I. Cacciotti, M. Esti, Papain covalently immobilized on chitosan–clay nanocomposite films: application in synthetic and real white wine, Nanomaterials. 10 (2020) 1622, https://doi.org/10.3390/nano10091622.
[332] K. Chinnaiah, K. Gurushankar, K. Kannan, A. Asadi, S. Dinesh, C. Thangamani, Magnetic nanoparticles for immobilization of enzyme and their applications - a review, Int. J. Pharm. Res. 12 (2020) 2689–2699, https://doi.org/10.31838/ijpr/2020.SP2.300.
[333] S.S. Nadar, V.K. Rathod, Magnetic-metal organic framework (magnetic-MOF): a novel platform for enzyme immobilization and nanozyme applications, Int. J. Biol. Macromol. 120 (2018) 2293–2302, https://doi.org/10.1016/j.ijbiomac.2018.08.126.
[334] M. Bilal, Y. Zhao, T. Rasheed, H.M.N. Iqbal, Magnetic nanoparticles as versatile carriers for enzymes immobilization: a review, Int. J. Biol. Macromol. 120 (2018) 2530–2544, https://doi.org/10.1016/j.ijbiomac.2018.09.025.
[335] Z. Al-Qodah, M. Al-Shannag, M. Al-Busoul, I. Penchev, W. Orfali, Immobilized enzymes bioreactors utilizing a magnetic field: a review, Biochem. Eng. J. 121 (2017) 94–106, https://doi.org/10.1016/j.bej.2017.02.003.
[336] M. Shinkai, H. Honda, T. Kobayashi, Preparation of fine magnetic particles and application for enzyme immobilization, Biocatalysis 5 (1991) 61–69, https://doi.org/10.3109/10242429109014855.
[337] L. Zeng, K. Luo, Y. Gong, Preparation and characterization of dendritic composite magnetic particles as a novel enzyme immobilization carrier, J. Mol. Catal. B Enzym. 38 (2006) 24–30, https://doi.org/10.1016/j.molcatb.2005.10.007.
[338] M. Koneracká, P. Kopcansky, M. Timko, C.N. Ramchand, Z.M. Saiyed, T. Michael, A.De Sequeira, Immobilization of enzymes on magnetic particles, in: J.M. Guisan (Ed.), Immobil. Enzym. Cells, 2 nd, Humana Press, Totowa, New Jersey, 2006, pp. 217–228.
[339] M. Koneracká, P. Kopcanský, M. Antalik, M. Timko, C.N. Ramchand, D. Lobo, R. V. Mehta, R.V. Upadhyay, Immobilization of proteins and enzymes to fine magnetic particles, J. Magn. Magn. Mater. 201 (1999) 427–430, https://doi.org/10.1016/S0304-8853(99)00005-0.
[340] L. Dal Magro, V.C.C. Silveira, E.W. de Menezes, E.V. Benvenutti, S. Nicolodi, P. F. Hertz, M.P. Klein, R.C. Rodrigues, Magnetic biocatalysts of pectinase and cellulase: synthesis and characterization of two preparations for application in grape juice clarification, Int. J. Biol. Macromol. 115 (2018) 35–44, https://doi.org/10.1016/j.ijbiomac.2018.04.028.
[341] H. Shahrestani, A. Taheri-Kafrani, A. Soozanipour, O. Tavakoli, Enzymatic clarification of fruit juices using xylanase immobilized on 1,3,5-triazine-functionalized silica-encapsulated magnetic nanoparticles, Biochem. Eng. J. 109 (2016) 51–58, https://doi.org/10.1016/j.bej.2015.12.013.
[342] S.Y. Lam, R.C.Y. Yeung, T.-H. Yu, K.-H. Sze, K.-B. Wong, A rigidifying salt-bridge favors the activity of thermophilic enzyme at high temperatures at the expense of low-temperature activity, PLoS Biol. 9 (2011), e1001027, https://doi.org/10.1371/journal.pbio.1001027.
[343] I. Armenia, M.V. Grazú Bonavia, L. De Matteis, P. Ivanchenko, G. Martra, R. Gornati, J.M. de la Fuente, G. Bernardini, Enzyme activation by alternating magnetic field: importance of the bioconjugation methodology, J. Colloid Interface Sci. 537 (2019) 615–628, https://doi.org/10.1016/j.jcis.2018.11.058.
[344] Y.I. Andreeva, A.S. Drozdov, D. Avnir, V.V. Vinogradov, Enzymatic nanocomposites with radio frequency field-modulated activity, ACS Biomater. Sci. Eng. 4 (2018) 3962–3967, https://doi.org/10.1021/acsbiomaterials.8b00838.
[345] B.P. Krishnan, L.O. Prieto-López, S. Hoefgen, L. Xue, S. Wang, V. Valiante, J. Cui, Thermomagneto-responsive smart biocatalysts for malonyl-coenzyme a synthesis, ACS Appl. Mater. Interfaces 12 (2020) 20982–20990, https://doi.org/10.1021/acsami.0c04344.
[346] H. Lei, W. Wang, L.-L. Chen, X.-C. Li, B. Yi, L. Deng, The preparation and catalytically active characterization of papain immobilized on magnetic composite microspheres, Enzym. Microb. Technol. 35 (2004) 15–21, https://doi.org/10.1016/j.enzmictec.2004.03.007.
[347] L.-L. Liu, L.-X. Zeng, T. Liu, D. Le, Oriented immobilization of papain on metal chelating carriers, Sheng Wu Gong Cheng Xue Bao 21 (2005) 789–793. http://www.ncbi.nlm.nih.gov/pubmed/16285522.
[348] F. Zhang, R. Wang, C. Zhen, B. Li, Magnetic cellulose nanocrystals: synthesis by electrostatic self-assembly approach and efficient use for immobilization of papain, J. Mol. Catal. B Enzym. 134 (2016) 164–171, https://doi.org/10.1016/j.molcatb.2016.11.017.
[349] Y.-J. Gu, M.-L. Zhu, Y.-L. Li, C.-H. Xiong, Research of a new metal chelating carrier preparation and papain immobilization, Int. J. Biol. Macromol. 112 (2018) 1175–1182, https://doi.org/10.1016/j.ijbiomac.2018.02.079.
[350] H. Wang, D. Shao, Study on immobilization of papain with magnetic porous alginate microspheres, IOP Conf. Ser. Mater. Sci. Eng. 397 (2018), 012031, https://doi.org/10.1088/1757-899X/397/1/012031.
[351] S. Santra, R.P. Bagwe, D. Dutta, J.T. Stanley, G.A. Walter, W. Tan, B.M. Moudgil, R.A. Mericle, Synthesis and characterization of fluorescent, radio-opaque, and paramagnetic silica nanoparticles for multimodal bioimaging applications, Adv. Mater. 17 (2005) 2165–2169, https://doi.org/10.1002/adma.200500018.
[352] N. Noginova, F. Chen, T. Weaver, E.P. Giannelis, A.B. Bourlinos, V.A. Atsarkin, Magnetic resonance in nanoparticles: between ferro- and paramagnetism, J. Phys. Condens. Matter. 19 (2007), 246208, https://doi.org/10.1088/0953-8984/19/24/246208.
[353] M. Cao, Z. Li, J. Wang, W. Ge, T. Yue, R. Li, V.L. Colvin, W.W. Yu, Food related applications of magnetic iron oxide nanoparticles: enzyme immobilization, protein purification, and food analysis, Trends Food Sci. Technol. 27 (2012) 47–56, https://doi.org/10.1016/j.tifs.2012.04.003.
[354] P. Alpay, D.A. Uygun, Usage of immobilized papain for enzymatic hydrolysis of proteins, J. Mol. Catal. B Enzym. 111 (2015) 56–63, https://doi.org/10.1016/j.molcatb.2014.11.001.
[355] K. Atacan, M. Özacar, M. Özacar, Investigation of antibacterial properties of novel papain immobilized on tannic acid modified Ag/CuFe2O4 magnetic nanoparticles, Int. J. Biol. Macromol. 109 (2018) 720–731, https://doi.org/10.1016/j.ijbiomac.2017.12.066.
[356] W. Sheng, Y. Xi, L. Zhang, T. Ye, X. Zhao, Enhanced activity and stability of papain by covalent immobilization on porous magnetic nanoparticles, Int. J. Biol. Macromol. 114 (2018) 143–148, https://doi.org/10.1016/j.ijbiomac.2018.03.088.
[357] S. Chauhan, L.S.B. Upadhyay, The quest for reusability: the facile and stable immobilization of papain on cysteine functionalized iron oxide nanoparticles activated glass surface, Indian J. Biochem. Biophys. 58 (2021) 141–147.
[358] A. Homaei, H. Barkheh, R. Sariri, R. Stevanato, Immobilized papain on gold nanorods as heterogeneous biocatalysts, Amino Acids 46 (2014) 1649–1657, https://doi.org/10.1007/s00726-014-1724-0.
[359] B. Sahoo, S.K. Sahu, D. Bhattacharya, D. Dhara, P. Pramanik, A novel approach for efficient immobilization and stabilization of papain on magnetic gold nanocomposites, Colloids Surf. B: Biointerfaces 101 (2013) 280–289, https://doi.org/10.1016/j.colsurfb.2012.07.003.
[360] D. Yu, K. Chen, J. Liu, Z. Pan, L. Jiang, L. Wang, W. Elfalleh, Application of magnetic immobilized papain on passivated rice bran lipase, Int. J. Biol. Macromol. 157 (2020) 51–59, https://doi.org/10.1016/j.ijbiomac.2020.04.132.