



Screening of plant peptidases for the synthesis of arginine-based surfactants

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ABSTRACT

Partially purified preparations with proteolytic activity, obtained from South American native plants, were used as biocatalysts in condensation reactions of *N*-protected arginine alkyl ester derivatives with decylamine and dodecylamine in low-water content systems. The final products are cationic surfactants with potential application as emulsifiers and preservatives. Most of the proteolytic extracts were obtained from latex of species belonging to the *Asclepiadaceae* family (araujain from *Araujia hortorum*, asclepain c from *Asclepias curassavica* and funastrain from *Funastrum clausum*). Hieronymain was obtained from unripe fruits of *Bromelia hieronymi* (*Bromeliaceae*). Plant proteases from commercial sources (papain and bromelain) were also tested as catalysts in the same reactions. Araujain and funastrain furnished good reaction conversions (60–84%, with a ratio synthesis/hydrolysis of 2–5) similar to those obtained with commercial papain. Moreover, araujain was the biocatalyst which rendered the best conversions (60%) for the synthesis of the two novel Bz-Arg-NH-dodecylamide (Bz-Arg-NHC₁₂) and Bz-Arg-NH-decylamide (Bz-Arg-NHC₁₀) derivatives. Moderate to poor conversions (10–50%, showing a ratio synthesis/hydrolysis of 0.5–1) were achieved with asclepain c, hieronymain and bromelain. The screening presented in this work revealed that, although these are structurally similar, their behavior for the synthesis of this kind of products differ among them.

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1. Introduction

The use of partially purified enzymes as catalysts in multistep synthetic organic chemistry has become widespread [1–3]. In particular, the realization that certain hydrolases are stable and active in non-aqueous media has promoted a lot of work on the asymmetric synthesis of esters and amides [4–8]. An increasing number of lipases, esterases and peptidases are readily available for these reactions. Moreover, the possibility to alter their natural substrate specificity, enantioselectivity, regioselectivity and chemoselectivity by means of medium and protein engineering, broaden their applications [9].

Peptidases are among the best characterized hydrolases because they are easy to handle, do not need expensive cofactors and exist in a great variety of selectivities [10]. These advantages make them good catalysts for hydrolysis, amidation and esterification reactions [4–8,10]. Most of industrial peptidases are from microbial sources due to their simple large-scale production. Furthermore, they can be engineered to create specialized enzymes, which may suit par-

ticular applications. Animal and plant peptidases are also largely used in industry. Papain from the latex of fruits of *Carica papaya* and bromelain from *Ananas comosus* stem are the most extensively used in food processing [11]. As an alternative to protein engineering, the screening of new plant sources can lead to the discovery of peptidases with novel and interesting properties to be applied in industrially useful reactions. As South America is well known for its highly diverse floras, the provision of resources is potentially huge [12].

Amino acid lipid conjugates are a class of bio-based surfactants with excellent adsorption and aggregation properties, high biodegradability, low potential toxicity, low environmental impact and broad antimicrobial activity [13–17]. Among them, arginine-based surfactants possess excellent self-assembling characteristics, low toxicity profile, high biodegradability and a broad antimicrobial activity. These properties make them compounds of interest for application as preservatives and antiseptics in pharmaceutical, food and dermatological formulations [18–22]. The enzymatic syntheses of these arginine lipid conjugates have focused our attention during the last decade. In most cases, the peptidase papain was the biocatalyst of choice for the synthesis of these compounds though its efficiency as amidation and esterification catalyst varied with the structure of the arginine derivative [23–28].

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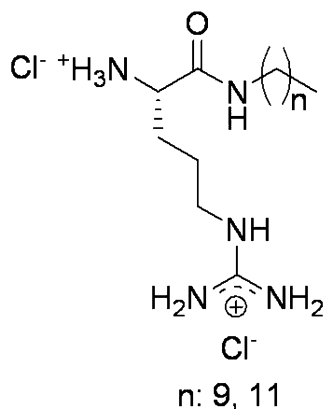


Fig. 1. Structure of arginine *N*-alkyl amide dihydrochloride derivatives.

The aim of this work was to screen cysteine plant proteases from native plant origin as biocatalysts for the synthesis of arginine-based surfactants of the *N*-alkyl amide type (Fig. 1). Species belonging to the *Asclepiadaceae* (*Araujia hortorum*, *Asclepias curassavica* and *Funastrum clausum*), and *Bromeliaceae* families (*A. comosus* and *Bromelia hieronymi*) were chosen as sources of potential biocatalyst for the proposed syntheses. Their catalytic performance was compared with that of papain from *C. papaya*, *Caricaceae*, considered in this case as the reference cysteine plant peptidase for these reactions.

2. Experimental

2.1. Chemicals

Papain (E.C. 3.4.22.2) from papaya (*C. papaya*) latex, crude powder (2.1 units/mg solid, one unit hydrolyzes 1.0 μmol of *N* $^{\alpha}$ -benzoyl-L-arginine ethyl ester hydrochloride per min at pH 6.2 at 25 $^{\circ}\text{C}$), bromelain (E.C. 3.4.22.4) from pineapple (*A. comosus*) stem (2.4 units/mg solid, 5.1 units/mg protein, one unit releases 1.0 μmol of *N* $^{\alpha}$ -carbobenzoxy-L-lysine *p*-nitrophenyl ester per min at pH 4.6 at 25 $^{\circ}\text{C}$), Coomassie Brilliant Blue G-250, casein from bovine milk, bovine seroalbumin (BSA), 1,4-dithio-D,L-threitol (DTT), *N* $^{\alpha}$ -benzoyl-D,L-arginine *p*-nitroanilide hydrochloride (BAPNA), *N* $^{\alpha}$ -carbobenzoxy-L-arginine *p*-nitroanilide hydrochloride (Z-Arg-pNA) and *N* $^{\alpha}$ -benzoyl-L-arginine ethyl ester hydrochloride (Bz-Arg-OEt-HCl) were purchased from Sigma. Molecular sieves (4 \AA), decyl- and dodecylamine were obtained from Fluka. *N* $^{\alpha}$ -carbobenzoxy-L-phenylalanyl-L-arginine *p*-nitroanilide hydrochloride (Z-Phe-Arg-pNA) was purchased from Bachem. Polyamide-6 (EP-700, particle size <800 μm , mean pore diameter 50–300 nm, specific surface area BET method 8.4 $\text{m}^2 \text{g}^{-1}$) was an Azko (Obernburg, Germany) generous contribution. L-Pyroglyutamyl-L-phenylalanyl-L-leucine *p*-nitroanilide (PFLNA) was synthesized in solid phase following the procedure described by Rivera et al. [29]. *N* $^{\alpha}$ -carbobenzoxy-L-arginine methyl ester hydrochloride (Z-Arg-OMe-HCl) was synthesized by the thionyl chloride method [30]. The rest of the chemicals and solvents used in this work were of analytical grade.

2.2. Crude enzymatic preparations

Crude proteolytic extracts from *Asclepiadaceae* sp. latex were prepared as follows: (1) latex from fruits of *A. hortorum*, was gathered on 0.1 M citric–citrate buffer pH 4.0 with 5 mM ethylene diamine tetraacetic acid (EDTA) and DTT. (2) Latex from leaf petioles of *A. curassavica* and *F. clausum*, was collected on 0.1 M citric acid–phosphate buffer pH 6.5, containing 5 mM EDTA and DTT. In

all cases, the latex suspensions were centrifuged at 10,000 $\times g$ for 30 min in to discard most gums and insoluble materials. Supernatants were lyophilized for further applications.

Crude extract from *B. hieronymi* (*Bromeliaceae*) was obtained by homogenizing frozen fruits in cold 0.1 M sodium phosphate buffer, pH 6.0, containing 5 mM EDTA and cysteine. The homogenate was filtered to remove plant debris and then centrifuged for 30 min at 16,000 $\times g$. Supernatant was collected, filtered when necessary, and lyophilized [31].

2.3. Determination of protein content and proteolytic activity of each extract

Protein content was determined according to Bradford's method [32] using a standard curve of BSA. Proteolytic activity was performed using 1% (w/v) casein as substrate in 0.1 M Tris–HCl or glycine–NaOH buffer containing different amounts of cysteine according to the enzyme. The pH, temperature, and reaction time depended on the proteolytic preparation [33–35]. Reactions were stopped by the addition of 5% (w/v) trichloroacetic acid. Absorbance of each supernatant was measured at 280 nm after centrifugation (2500 $\times g$, 20 min). Proteolytic activities were expressed in an arbitrary enzyme unit (Ucas) [36].

2.4. Enzyme adsorption onto polyamide

Lyophilized proteolytic extracts, as well as commercial papain and bromelain (100 mg), were dissolved in a buffer solution (1 mL of 0.1 M boric acid–sodium borate pH 8.5 containing 1 mM EDTA) with DTT (150 mg). The enzymatic solutions were mixed completely with the solid support (1 g) and dried under vacuum for 24 h at ca. 40 μbar [37].

2.5. Determination of the proteolytic activity of the adsorbed enzymatic preparations

The activity of the adsorbed enzymes was determined using PFLNA as substrate [38]. The adsorbed enzymes (100 mg) were suspended in phosphate buffer 0.1 M pH 6.5 containing KCl (0.3 M) and EDTA (0.1 mM). To this solution, PFLNA was added from a stock solution (1 mM) in dimethyl sulfoxide (DMSO) (i.e. 0.1 mM PFLNA final concentration). The mixture was incubated at 37 $^{\circ}\text{C}$ for 5 min on an orbital shaker (150 rpm). The reactions were stopped by the addition of 0.5 mL of 30% acetic acid and centrifuged (20 min at 2500 $\times g$) to separate the immobilized preparations. The absorbance of the *p*-nitroaniline released in the supernatant was measured spectrophotometrically at 410 nm. Blanks having the support without the enzymes were simultaneously made.

Similar determinations were made for the free enzymes. DTT was added to the reaction media in the same proportion contained in the immobilized preparations. Enzymatic international unit (IU) was defined as the amount of enzyme that released 1 μmol of *p*-nitroaniline per min in the assay conditions. The percentage of remaining activity of immobilized enzymes was calculated considering the activity of the enzyme solution prior to the adsorption step.

2.6. Determination of amidolytic activity of immobilized preparations

The enzymatic units of each proteolytic extract were determined using Z-Arg-pNA [39], BAPNA [40] and Z-Phe-Arg-pNA [41] as substrates. The adsorbed enzymes (100 mg) were suspended in phosphate buffer 0.1 M pH 7.4 containing 1 mM EDTA. To this mixture, stock solutions of Z-Arg-pNA (20 mM) in *N,N*-dimethyl

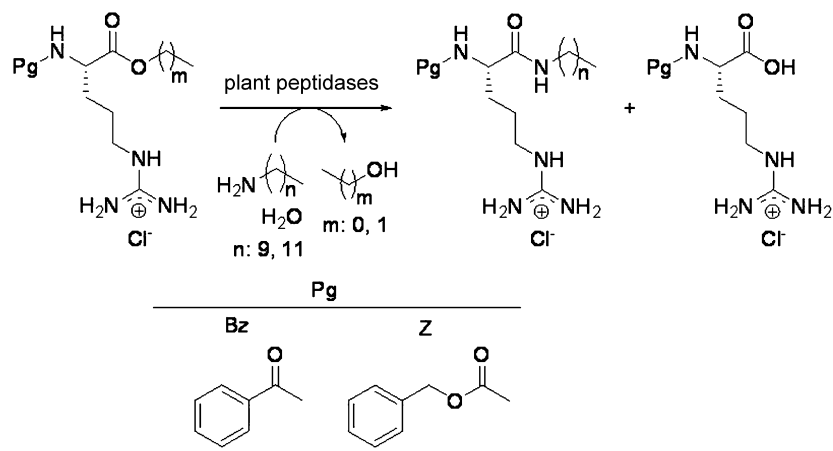


Fig. 2. Enzymatic condensation of N^α -protected Arg methyl ester derivatives with decyl- and dodecylamine.

formamide, and BAPNA (40 mM) and Z-Phe-Arg-pNA (1 mM) in DMSO were added giving final substrate concentrations of 2, 4 and 0.1 mM, respectively. The assays were carried out at 37 °C for 5 min placed on an orbital shaker (150 rpm). The reactions were stopped by the addition of 0.5 mL of 30% acetic acid and the immobilized preparations were separated by centrifugation (20 min, 2500 × g). Enzymatic units were calculated as described above.

2.7. Enzymatic syntheses of Z-Arg- and Bz-Arg-NH-alkyl amide derivatives

Reactions were performed in 4 mL closed flasks vessels under nitrogen atmosphere at 25 °C and placed on an orbital shaker at 150 rpm. Acetonitrile was dried previously over molecular sieves (4 Å) and stored under nitrogen atmosphere. Z-Arg-OME-HCl (0.04 mmol) or Bz-Arg-OEt-HCl (0.04 mmol) and the corresponding alkyl amine (decyl- and dodecylamine) (0.06 mmol) were dissolved in acetonitrile (2 mL) containing water (0.25%, v/v). To this solution the enzymatic immobilized preparation (450–550 IU of enzymatic activity) was added. Since no samples were withdrawn, six reaction vessels had to be used for each synthesis to monitor the reactions at 1, 3, 6, 24, 48 and 72 h. The reactions were ended by the addition of MeOH/AcOH 4:1 and washing the support with the same solvent mixture (3 × 2 mL). The extracts were centrifuged 20 min at 9000 × g and analyzed by HPLC–MS.

2.8. HPLC–MS analysis

The amount of condensation products, substrates, and hydrolysis by-products were measured by HPLC–MS (Agilent 1100 LC/MSD) using a Lichrosorb 100 propylcyano column (5 μm, 250 mm × 4 mm, Merck). The chromatographic conditions were: solvent A, 0.1% (v/v) TFA in H₂O; solvent B, 0.085% (v/v) TFA in CH₃CN/H₂O 80:20; flow rate 0.8 mL/min; detection at 215 nm. Quantification was made from peak areas according to the external standard method. Retention times for each compound were: Z-Arg-NHC₁₂, 16.7 min; Z-Arg-NHC₁₀, 15.5 min; Z-Arg-OME, 7.8 min; Z-Arg-OH, 6.7 min; Bz-Arg-NHC₁₂, 16.3 min; Bz-Arg-NHC₁₀, 15.0 min; Bz-Arg-OEt, 6.5 min; Bz-Arg-OH, 4.9 min. Characterization of each peak was made by ES-MS, positive mode. Z-Arg-NHC₁₂ (M+H⁺) = 476.3; Z-Arg-NHC₁₀ (M+H⁺) = 448.3; Z-Arg-OME (M+H⁺) = 323.1; Z-Arg-OH (M+H⁺) = 309.1; Bz-Arg-NHC₁₂ (M+H⁺) = 446.3; Bz-Arg-NHC₁₀ (M+H⁺) = 416.3; Bz-Arg-OEt (M+H⁺) = 307.1; Bz-Arg-OH (M+H⁺) = 279.1.

3. Results and discussion

The screening of novel cysteine plant proteases was performed on the condensation reaction of N^α -protected Arg methyl and ethyl ester derivatives with decyl- and dodecylamine (Fig. 2).

Prior the synthetic assays, the characterization of the proteolytic activity of each crude enzymatic preparation was carried out (Table 1). To this end, the use of an unspecific substrate such as bovine casein was considered to be the most appropriate as a first approach [42–45]. Araujain from *A. hortorum* showed the lowest specific activity towards casein, in contrast to those obtained for hieronymain from *B. hieronymi*, commercial papain and bromelain (Table 1). This may be due to differences in the extraction process for each preparation. Besides, it was found that latex of *A. hortorum* fruits has other hydrolases such as pectinases (unpublished data).

Deposited endopeptidases onto solid support were the biocatalyst configuration selected for all the condensations assays. Polyamide was the chosen support, which proved to be the best for these reactions [23]. Thus, the activity of the deposited enzymes onto polyamide was considered of utmost importance for the comparison of the different enzymes. In this case, casein was not considered due to its large size, which could limit intraparticle mass transfer and would result in an apparent low enzymatic

Table 1
Protein content and caseinolytic activity of peptidasic extracts

Enzyme	mg protein/mg solid	Ucas/mg solid	Specific activity (Ucas/mg protein)
Araujain	0.65	0.06	0.1
Asclepain c	0.05	0.02	0.4
Funastrain	0.05	0.08	1.5
Hieronymain	0.03	0.17	6.2
Bromelain	0.02	0.16	11.0
Papain	0.02	0.17	9.7

Table 2
Percentage of recovered amidolytic activity after enzyme adsorption onto polyamide

Enzyme	Remaining activity (%)
Araujain	21
Asclepain c	23
Funastrain	22
Hieronymain	18
Papain	24
Bromelain	26

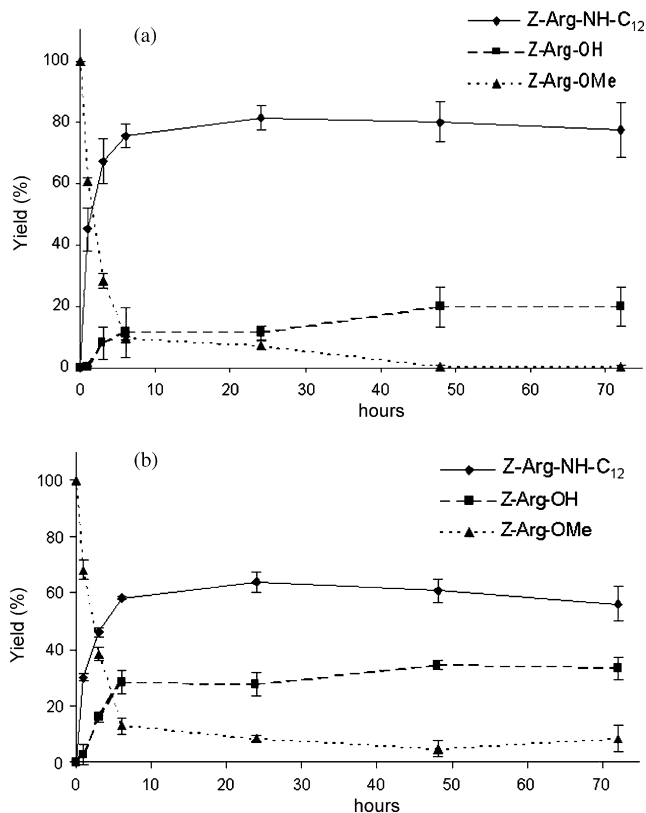


Fig. 3. Time-course reaction for the synthesis of Z-Arg-NH-dodecylamide (Z-Arg-NHC₁₂) using (a) papain; (b) araujiain as biocatalysts. All plant peptidases were adsorbed onto polyamide.

activity of the immobilized preparation [46]. Alternatively, PFLNA was demonstrated to be a good substrate [37], which has been developed specifically for cysteine plant peptidases [38]. As shown in Table 2, the remaining activity was in the range of 20–25% respect to the free enzymes. Although immobilization and deposition onto solid supports are generally considered to stabilize enzymes in organic media, lyophilization may involve strong denaturing processes that can damage them [47]. This could explain the low enzymatic activity that was recovered from the immobilized preparations.

To make the comparisons in a more accurate way, enzymatic activity should be determined towards substrates whose acyl ends were identical to those of the selected acyl donors for the condensation reactions. This is particularly important when the selectivity of the protease towards P2' may affect its activity. In this connection, Z-Arg-pNA and Z-Arg-OMe are homologous; the same can be said for BAPNA and Bz-Arg-OEt [48]. Hence, enzymatic units (as international units, IU) were calculated using Z-Arg-pNA as substrate for the syntheses of the Z-Arg-NH-alkylamides, whereas for the syntheses of Bz-Arg-NH-alkylamides BAPNA was chosen. Furthermore, Z-Phe-Arg-pNA, a more specific substrate for bromelain [41], was also considered. For the sake of comparison, the activity of immobilized papain was also proved with these substrates. As shown in Table 3, there are no significant differences among the results obtained for Z-Arg-pNA and Z-Phe-Arg-pNA for papain, bromelain and hieronymain. The activity values obtained for papain with BAPNA as substrate were close to those using Z-Arg-pNA and Z-Phe-Arg-pNA. Neither bromelain nor hieronymain showed the same behavior than papain. Good activities were recovered for both enzymes using Z-Phe-Arg-pNA, whereas no activity towards BAPNA was observed using adsorbed bromelain

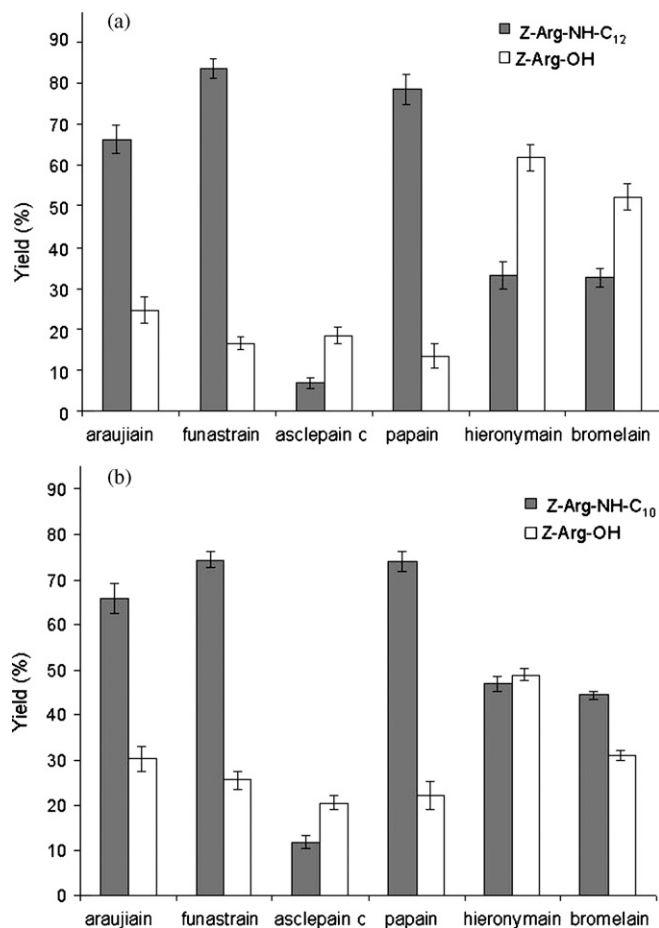


Fig. 4. Syntheses of (a) Z-Arg-NH-dodecylamide (Z-Arg-NHC₁₂) catalyzed by araujiain, papain, asclepain c, bromelain and hieronymain at 24 h of reaction; by funastrain at 72 h of reaction; (b) Z-Arg-NH-decylamide (Z-Arg-NHC₁₀) catalyzed by araujiain, papain and asclepain c at 24 h of reaction; by funastrain, bromelain and hieronymain at 72 h of reaction. The product of hydrolysis (Z-Arg-OH) is also shown in both cases. All plant peptidases were adsorbed onto polyamide.

and hieronymain. Both proteases showed low affinity for the Bz-group, which was confirmed by the poor yields in the synthesis of Bz-Arg-NH-alkylamides as to be discussed later in this section.

Once the characterization of immobilized proteases was thus determined, assays for the synthesis of Z-Arg-NHC₁₀ and Z-Arg-NHC₁₂ were performed. Acetonitrile with 0.25% (v/v) of aqueous boric acid–sodium borate buffer pH 8.5 as previously described [23] was used as reaction medium. The amount of enzymatic activity in each vial was adjusted into the range of 450–550 IU determined using Z-Arg-pNA as substrate.

As can be seen in Fig. 3, the maximum yield for Z-Arg-NHC₁₂ was attained after 24 h of reaction for both papain and araujiain (80% and 65%, respectively). However, in the case of bromelain and hieronymain, the highest yields for the synthesis of Z-Arg-NHC₁₀ were obtained after 72 h (45% and 47%, respectively); funastrain showed its maximum at 72 h of reaction for both products (84% for Z-Arg-NHC₁₂ and 72% for Z-Arg-NHC₁₀) (Fig. 4). Both, araujiain and funastrain gave more hydrolysis product than the reactions with papain.

Using dodecylamine, bromelain and hieronymain furnished Z-Arg-OH as the major product (Fig. 4a), whereas with decylamine, the hydrolysis and condensation conversions were almost in the same range (Fig. 4b). The behavior of these proteases could be due to their preference for polar amino acids in the S1' region (Schechter and Berguer notation) [49]. The hydrophobicity of the nucleophiles

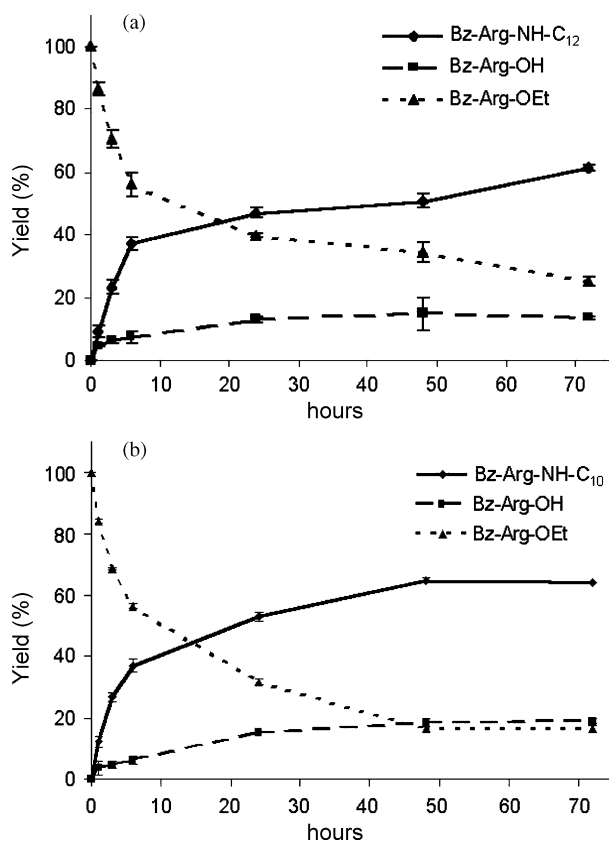


Fig. 5. Time-course reaction for the syntheses of (a) Bz-Arg-NH-dodecylamide (Bz-Arg-NHC₁₂); (b) Bz-Arg-NH-decylamide (Bz-Arg-NHC₁₀) using araujiain as biocatalyst in acetonitrile 0.25% (v/v) boric acid–sodium borate buffer pH 8.5. All plant peptidases were adsorbed onto polyamide.

used (i.e. alkylamines) did not fulfill this requirement, so their reactivity in front of these peptidases appears to be similar to that of water. Asclepain c had little activity in organic media probably due to an irreversible denaturation of the enzyme exposed to the solvent (Fig. 4b).

For the syntheses of the Bz-Arg-NH-alkyl amide derivatives, the commercial substrate Bz-Arg-OEt (BAEE) was used as acyl donor. The benzoyl N^α-protecting group fulfils the requirement of a hydrophobic residue at the P2 position necessary for the substrate selectivity of papain-like peptidases [50]. Acetonitrile with 0.25% (v/v) aqueous buffer was selected as reaction medium. Araujiain, funastrain, papain and bromelain immobilized onto polyamide were tested as biocatalysts. In this case, the best performance was obtained for araujiain, which showed 60 and 65% conversion to Bz-Arg-NHC₁₂ and Bz-Arg-NHC₁₀ (Fig. 5a and b) after 24

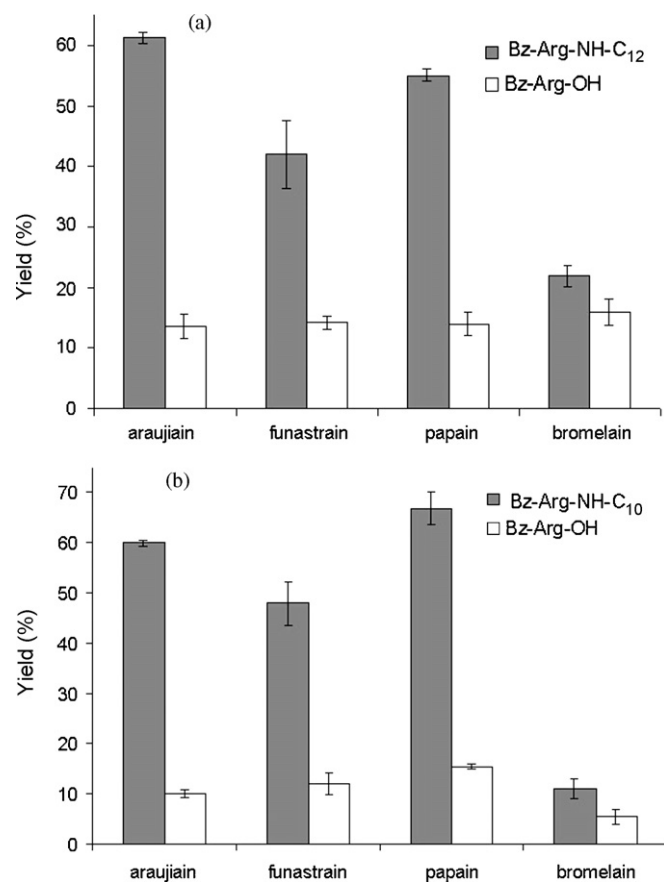


Fig. 6. Syntheses of (a) Bz-Arg-NH-dodecylamide (Bz-Arg-NHC₁₂) and (b) Bz-Arg-NH-decylamide (Bz-Arg-NHC₁₀) at 72 h of reaction. The product of hydrolysis (Bz-Arg-OH) is also shown in both cases. All plant peptidases were adsorbed onto polyamide.

and 72 h, respectively. Moderate conversions were obtained with funastrain, i.e. 45% and 50% for each arginine derivative, respectively (Fig. 6a and b). When bromelain was assayed as catalyst, 22% and 11% conversion to Bz-Arg-NHC₁₂ and Bz-Arg-NHC₁₀, respectively, were obtained after 72 h of reaction (Fig. 4a and b). These results were in a good agreement with the activity measurement with BAPNA. Interestingly, some conversion was observed with adsorbed preparations of bromelain and hieronymain in low-water content medium, although practically no activity was detected using BAPNA as substrate (Table 3). Crude stem bromelain has at least three distinct cysteine proteases: stem bromelain (E.C. 3.4.22.32), the major component, up to 90% of the total protein, ananain (E.C. 3.4.22.31) (5%) and comosain (≤5%, not yet included in

Table 3
Enzymatic international units determined for each enzyme and each substrate

Enzyme	Z-Arg-pNA		BAPNA		Z-Phe-Arg-pNA	
	Activity (IU/mg immobilized preparation)	Specific activity ^a (IU/mg immobilized protein)	Activity (IU/mg immobilized preparation)	Specific activity ^a (IU/mg immobilized protein)	Activity (IU/mg immobilized preparation)	Specific activity ^a (IU/mg immobilized protein)
Araujiain	3.3	51	2.8	43	n.d.	n.d.
Papain	2.7	1350	2.5	1250	2.5	1250
Funastrain	2.3	460	2.4	480	n.d.	n.d.
Asclepain c	2.4	480	Not detectable	n.d.	n.d.	n.d.
Bromelain	2.3	1150	Not detectable	n.d.	2.4	1200
Hieronymain	2.3	767	Not detectable	n.d.	2.3	767

References: n.d.: non-determined.

^a Specific activity was calculated assuming that all the protein present in the extract was adsorbed.

the IUBMB recommendations) [51,52]. It has been found that stem bromelain (E.C. 3.4.22.32) and comosain could only act efficiently by hydrolyzing Arg-Arg in synthetic substrates, whereas ananain showed a stronger affinity for Z-Phe-Arg fragment in synthetic substrates [53,54]. Bz-Arg- residues, such as benzoyl-argininamide (BAA) and BAEE have been described as to determine amidasic and esterolytic activity of crude stem bromelain preparations [55,44,40] until more specific substrates were designed [38,53,54]. The absence of enzymatic activity towards BAPNA found in the immobilized preparations of commercial stem bromelain, could be due to the lower selectivity of these crude material for this substrate in addition to the negative effect of the immobilization process on the enzymatic activity. Thus, when using a more selective substrate such as Z-Phe-Arg-pNA some activity was observed. Hieronymain activity towards BAPNA has never been proved before, while one of its main proteases, hieronymain II, showed a substrate preference towards Z-Phe-Arg-pNA [56]. It is likely that the distance between the nitrogen and the phenyl groups, shorter in the benzoyl moiety (Bz-) than that in the Z-, may potentially have non-productive interaction in the S2 subsite of the peptidases.

4. Conclusions

The syntheses of Z-Arg-NHC₁₀, Z-Arg-NHC₁₂, Bz-Arg-NHC₁₀ and Bz-Arg-NHC₁₂ were proved with plant cysteine peptidases belonging to the papain family obtained from native South American plant species of *A. hortorum*, *F. clausum*, *A. curassavica* and *B. hieronymi*, as well as with a commercial preparation of stem bromelain. Their performance were compared to those of commercial papain as the reference biocatalyst.

The crude proteolytic extracts from the latex of the milkweeds belonging to the *Asclepiadaceae* family *A. hortorum* (i.e. araujiain) and *F. clausum* (*Asclepiadaceae*) (i.e., funastrain), showed a very similar performance (65–84% conversions) to that obtained for papain for the synthesis of the two Z-Arg-NH-alkylamide surfactants. Moreover, araujiain and papain were the biocatalysts which rendered the best conversions (60–65%) for the synthesis of the two novel Bz-Arg-NHC₁₂ and Bz-Arg-NHC₁₀. All these assays demonstrated the importance of the screening to find alternative sources of enzymes with improved selective for the syntheses of new products with interest in pharmaceutical and food industries.

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