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Effect of laundry detergent formulation on the performance of alkaline phytoproteases

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Abstract

Background: Proteases constitute the largest product segment in the global industrial enzymes market; they are used in food, pharmaceutical, leather, textile, wood and detergent industries. Alkaline proteases improve the cleaning efficiency of detergents and represent one of the most successful applications of modern industrial biotechnology. The aim of this work was to study the performance of two alkaline phytoproteases, *araujiain* (*Araujia hortorum* Fourn*.)* and *asclepain* (*Asclepias curassavica* L.), for their potential application as additive in laundry detergent formulations.
Results: The effect of pure non-ionic and ionic surfactants on proteolytic activity of *araujiain* and *asclepain* was analyzed measuring the remaining activity after 1 hr of incubation of those enzymes in aqueous solutions of surfactants at different concentrations (0.1, 0.4 and 1% v/v) and temperatures (25, 40 and 60ºC). Besides, the compatibility of the enzymes with six commercial laundry detergents was also studied measuring the remaining proteolytic activity at 37ºC after 1 hr. Commercial detergent components influenced in different ways on *araujiain* and *asclepain*, in spite of the similar behaviour of the two enzymes in buffer. In commercial detergent solutions, *araujiain* expressed between 60% and 140% of its remaining proteolytic activity in buffer (pH 8.5) at 37ºC after 1 hr, while *asclepain*, was practically inactivate in most of them at the same conditions.
Conclusions: Proteolytic extract of *Araujia hortorum* fulfilled all the requirements for its application as additive for laundry detergents: high stability in a broad temperature range (25-70ºC), high activity in alkaline pH (7.5-9.5) and very good compatibility with the commercial detergent additives. Nevertheless, in spite of its high stability and activity in buffer, the proteolytic extract of *Asclepias curassavica* did not show the same performance than *araujiain*.

Keywords: *asclepain*, *araujiain*, detergents formulations, alkaline plant proteases.

INTRODUCTION

The global market for industrial enzymes is forecast to reach US$ 3.74 billion by 2015. Key factors promoting market growth include new enzyme technologies improving cost efficiency and productivity, and the growing interest among consumers in substituting petrochemical products and harsh chemicals for other organic compounds like enzymes. Other factor propelling market growth is the surging demand from textile manufacturers, animal feed producers, detergent manufacturers, pharmaceutical and cosmetic companies (Reportlinker.com, 2012).

Proteases constitutes the largest product segment in the global industrial enzymes market, representing about the 40% of the total industrial enzymes market with an important place in food, pharmaceutical and detergent industries, as well as in the preparation of leather, textile and wool, among others (Doran, 2002; Gupta et al. 2002; Abidi et al. 2008; Sellami-Kamoun et al. 2008; González-Rábade et al. 2011).

In detergent formulations, proteases act on protein-based stains (blood, grass and food stains, for example); the amylases assist in the removal of starch-based stains from many types of food products, and the lipases help to remove fat and oil-based stains from greasy food and human sebum (Smulders et al. 2002). Enzymes must exhibit some important properties to be well suited for use in detergents, namely: i) optimum activity at alkaline pH; ii) effectiveness at low wash temperatures of 20-40ºC; iii) stability at up to 60ºC wash temperatures; iv) stability in the presence of other detergent ingredients, such as surfactants, builders and activated bleach, both during storage and use; and v) broad specificity, enough to enable the degradation of a large variety of proteins, starches, and triglycerides (Smulders et al. 2002). From the different classes of known proteases, alkaline proteases are suitable for industrial applications based on their properties such as high stability and activity under harsh conditions (Banerjee et al. 1999; Haki and Rakshit, 2003; Sellami-Kamoun et al. 2008; Arunachalam and Saritha, 2009).

Available detergents in the international market contain proteolytic enzymes, mostly produced by members of the genus *Bacillus*. Subtilisins have been the enzymes of choice in detergent formulations due to their widespread distribution, availability and broad substrate specificity (Donlon, 2007; Araújo et al. 2008). However, new sources of enzymes are required. In this sense, the number of industrial enzymes from plants is small, but growing fast (González-Rábade et al. 2011).

New species of cysteine phytoproteases isolated from the latex of climbing plants from South America that grow in southern Brazil, Paraguay, Uruguay and Argentina, have been studied (López et al. 2000; Pardo et al. 2000; Morcelle et al. 2004; Morcelle et al. 2009). Some examples of them are *araujiain* from the latex of fruits of *Araujia hortorum* Fourn. (*Asclepiadaceae*) and *asclepain* from the latex of stem and petiole of *Asclepias curassavica* L. (*Asclepiadaceae*) (Priolo et al. 2000; Obregón et al. 2001; Liggieri et al. 2004; Liggieri et al. 2009). Both enzymes have been characterized and their efficiency to catalyze hydrolytic and synthetic reactions has been proved (Priolo et al. 2001; Barberis et al. 2002; Guzmán et al. 2007; Barberis et al. 2008; Quiroga et al. 2008; Illanes et al. 2009a; Illanes et al. 2009b; Morcelle et al. 2009). Their uses in detergents have not been reported yet. So, the main objective of this work was to study the performance of two alkaline phytoproteases, *araujiain* (*Araujia hortorum* Fourn.) and *asclepain* (*Asclepias curassavica* L.) for their potential application as additive in laundry detergent formulations.

MATERIALS AND METHODS

Materials

Araujiain is a partially purified enzymatic preparation obtained from the latex of fruits of *Araujia hortorum* Fourn. (*Asclepiadaceae*). This preparation contains three cysteine proteases (*araujiain* hI, hII and hIII) belonging to the papain family. Such proteases have been biochemically studied in detail (Priolo et al. 2000; Obregón et al. 2001). The enzyme extract, prepared according to Priolo et al. (2000), was kindly provided by the Laboratorio de Investigación de Proteínas Vegetales (LIPROVE).

Asclepain is an enzymatic preparation obtained from stem and petiole latex of *Asclepias curassavica* L. (*Asclepiadaceae*), 'scarlet milkweed', which is an erect, evergreen perennial subshrub with woody base and milky sap. Latex was obtained from plants grown in Rosario (Santa Fe, Argentina) and it was processed according to Liggieri et al. (2004). A voucher specimen (UNR 1130) has been deposited at the UNR herbarium (Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina) (Liggieriet al. 2004; Liggieri et al. 2009).

All analytical grade chemicals used in this work were supplied by Sigma (St. Louis, USA). Surfactants and detergents used in this work were selected based on the previous studies (Barcia et al. 2009; Barcia et al. 2010).

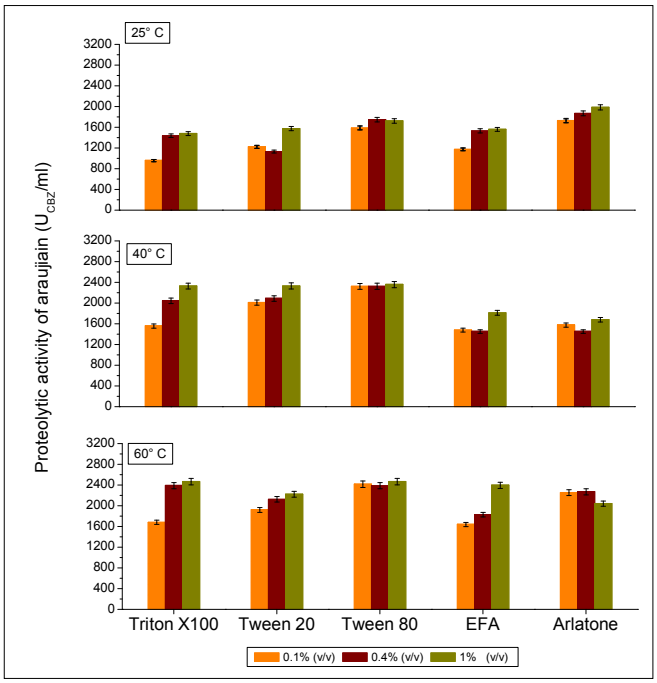

Fig. 1 Effect of different concentrations (0.1, 0.4 and 1% v/v) of some ionic and non-ionic surfactants on the proteolytic activity of *araujiain*, after 1 hr at 25, 40 and 60ºC. Z-Ala-*p*No (5 mg/ml) was used as substrate.

Proteolytic activity assays

Proteolytic activity was measured using 5 mg/ml of N-(benzyloxycarbonyl)-L-alanine *p* nitrophenyl ester (Z-Ala-*p*No) as substrate. After 5 min of incubation at 25, 37, 40 and 60ºC, the absorbance of the *p*-nitrophenol released was measured spectrophotometrically at 405 nm. Enzymatic units (U_{CBZ}) were obtained by performing a standard curve of *p*-nitrophenol (Priolo et al. 2001).

Effect of surfactants on the proteolytic activity of *araujiain* and *asclepain*

The effect of some non-ionic surfactants (Triton X100, Tween® 20 and Tween® 80) and ionic surfactants: -cationic (Phospholipid® EFA) and -anionic (Arlatone® MAP 230) on the proteolytic activity of both *araujiain* and *asclepain* was assessed at different concentrations (0.1, 0.4 and 1% v/v). Each enzyme was incubated in the surfactant solutions at 25, 40 and 60ºC, for 1 hr. Then, the enzyme residual activity was determined using Z-Ala-*p*No (5 mg/ml) as substrate and the absorbance of the released p-nitrophenol was spectrophotometrically measured at 405 nm (Priolo et al. 2001). The variation coefficient ((Sdmean^{-1}) 100) of reported values after the activity assays performed by triplicate was lower than 2.5%.

Effect of commercial detergent on the proteolytic activity of *araujiain* and *asclepain*

Compatibility of *araujiain* and *asclepain* with different commercial laundry detergents (Woolite®, Ariel Hydro Gel Max and Ace (Procter & Gamble), Skip Intelligent and Ala Matic (Unilever) and Enzimax (TVB)) was assessed. The detergents were diluted in distilled water (7 mg/ml) to simulate washing conditions (Phadatare et al. 1993). Both enzymes were incubated in the detergent solutions for 1 hr at 37ºC. Then, the residual proteolytic activity was measured using Z-Ala-*p*No (5 mg/ml) as substrate under the conditions above described. Control samples without detergent and incubated under similar conditions were taken at 100%. The variation coefficient ((Sdmean^{-1}) 100) of reported values, calculated in each case by triplicate, was lower than 2.5% after activity assays.

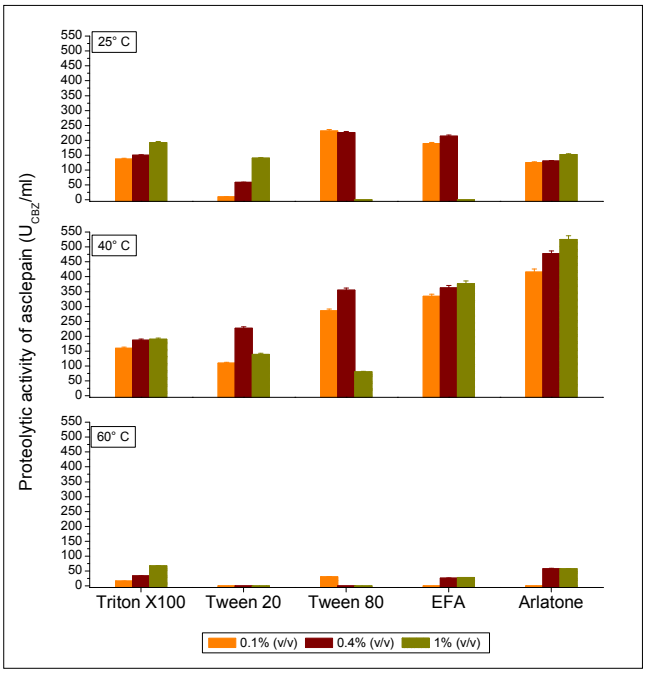

Fig. 2 Effect of different concentrations (0.1, 0.4 and 1% v/v) of some ionic and non-ionic surfactants on the proteolytic activity of *asclepain*, after 1 hr at 25, 40 and 60ºC. Z-Ala-*p*No (5 mg/ml) was used as substrate.

RESULTS AND DISCUSSION

In the present study, the performance of *araujiain* and *asclepain* was evaluated as additive of laundry detergent formulations.

The biochemical characterization of *araujiain* and *asclepain* has been previously reported. Both enzymes showed high proteolytic activity within a pH range from 7.5 to 9, while maximum activity was obtained at pH 8.5. Besides, they were highly active and stable at 40, 50 and 60ºC (Priolo et al. 2000; Liggiere et al. 2004; Quiroga et al. 2011).

Thermostability, optimum pH and compatibility with commercial detergent components, *e.g.* surfactants, perfumes and bleaches, are important parameters for selecting proteases as additives of laundry detergents (Gupta et al. 1999; Kumar and Takagi, 1999).

In this sense, it is important to point out that the high activity and thermostability of *araujiain* and *asclepain* at temperatures close to 40ºC is desirable for laundry purposes from ecological and economic points of view mainly because of energy saving.

On the other hand, the pH of a detergent solution in which proteases will work should be close to the optimum pH of the selected enzyme (Gupta et al. 2002). Since the pH of laundry detergents is alkaline, proteases and other enzymes currently used in detergents should be also alkaline in nature with a high pH optimum (Banerjee et al. 1999).

In order to evaluate the effect of pure non-ionic and ionic surfactants on the studied proteases, *araujiain* and *asclepain* were assayed in aqueous solutions at different concentrations and temperatures. Besides, the compatibility of the enzymes with six commercial laundry detergents was also studied as it was previously described in Materials and Methods section.

According to Figure 1, proteolytic activity of *araujiain* increased or remained constant when increasing of the ionic and non-ionic surfactant concentration. This behaviour was independent of temperature and the nature of the surfactant. Besides, the proteolytic activity of *araujiain* was usually higher than in buffer (1609 U_{CBZ}/mL) at 40 and 60ºC, and similar or slight lower than in buffer at 25ºC. The good performance of *araujiain* in surfactant solutions was probably due to the presence of an interfacial area which favored enzyme performance as was previously observed (Morcelle et al. 2006; Quiroga et al. 2008).

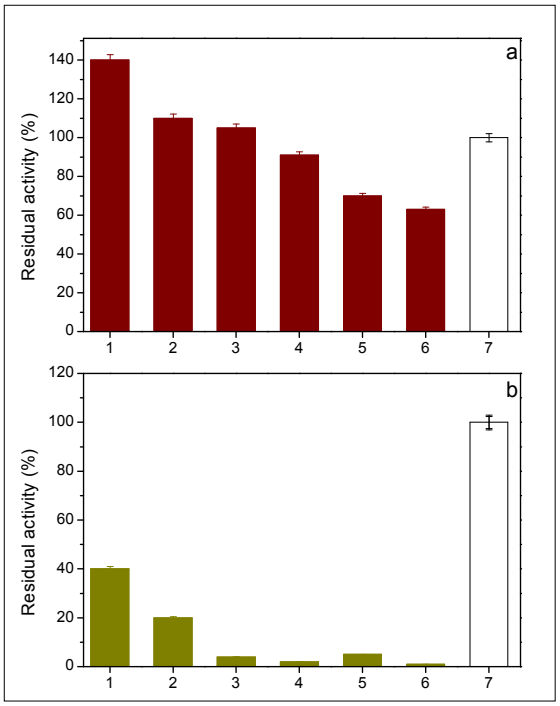

Fig. 3 Effect of different commercial detergents. (1: Woolite; 2: Ariel; 3: Ace: 4: Skip: 5: Ala Matic; 6: Enzimax; 7: None) on the proteolytic activity of (a) *araujian* and (b) *asclepain*, after 1 hr of incubation at 37ºC. Z-Ala-*p*No (5 mg/ml) was used as substrate.

According to Figure 2, proteolytic activity of *asclepain* was affected by the temperature and the concentration and nature of the surfactants. Proteolytic activity of *asclepain* was negligible at 60ºC, while the best values were obtained at 40ºC. Particularly, ionic surfactants as EFA and Arlatone allowed expressing the highest proteolytic activity of *asclepain* at the latter temperature (335-514 U_{CBZ}/mL). These values were between 87% and 187% higher than the proteolytic activity of *asclepain* in buffer (179 U_{CBZ}/mL).

On the other hand, *araujiain* was able to express between 60% and 140% of its proteolytic activity in buffer (pH 8.5), when incubated with six commercial detergents for 1 hr at 37ºC (Figure 3).

In spite of *asclepain* retaining high activity after 1 hr in buffer (pH 8.5) at 37ºC, its residual proteolytic activity was poor when incubated with different commercial detergent solutions at that temperature. Only two commercial detergents (Woolite and Ariel) allowed expressing between 20 and 40% of its proteolytic activity in buffer solution, under the same conditions.

The activity decrease observed on *araujiain* and *asclepain* activities was attributed to detergent components other than the typical laundry detergent proteases as they were previously inactivated by freezing (24 hrs) and overheating (95ºC, 1 hr).

In brief, commercial detergent components influenced *araujiain* and *asclepain* activities to different extents despite the similar behaviour of the two enzymes in buffer. *Araujiain* showed a good stability and compatibility with commercial laundry detergents while *asclepain* was practically inactivated.

CONCLUDING REMARKS

Enormous efforts over the past two decades to screen new detergent proteases and improve existing ones have been unsuccessful in bringing forth a single new enzyme as versatile and universally applicable as the high-alkaline protease *subtilisin*. At this moment, the research is focused on finding new suitable enzymes to be used in specific environments such as low-temperature automatic washing detergents because of the necessity to save energy and to adapt to new fragile clothing materials (Maurer, 2004). Proteolytic extracts from *Araujia hortorum* fulfilled all the requirements for its application in those detergent formulations: high stability in a broad temperature range (25-70ºC), high activity in alkaline pH (7.5-9.5) and very good compatibility with the commercial detergent additives. Nevertheless, in spite of its high stability and activity in buffer, the proteolytic extract of *Asclepias curassavica* did not show the same performance than *araujiain*.

By last, this work reports a new plant protease with potential application to the laundry detergent industry, from a renewable natural resource (an autochthonous plant) of the Latin American region. In this sense, we believe that this research has novelty and in addition, the obtained enzymatic extract is very simple and cheap, and it avoids the use of fermentation and genetic engineering.

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REFERENCES

ABIDI, F.; LIMAM, F. and NEJIB, M.M. (2008). Production of alkaline proteases by *Botrytis cinerea* using economic raw materials: Assay as biodetergent. *Process Biochemistry,* vol. 43, no. 11, p. 1202-1208. **[CrossRef]**

ARAÚJO, R.; CAVACO-PAULO, A. and CASAL, M. (2008). Strategies towards the functionalization of *Subtilisin* E from *Bacillus subtilis* for wool finishing applications. *Engineering in Life Science,* vol. 8, no. 3, p. 238-249. **[CrossRef]**

ARUNACHALAM, C. and SARITHA, K. (2009). Protease enzyme: An eco-friendly alternative for leather industry. *Indian Journal of Science and Technology,* vol. 2, no. 12, p. 29-32.

BANERJEE, U.C.; SANI, R.K.; AZMI, W. and SONI, R. (1999). Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. *Process Biochemistry*, vol. 35, no. 1-2, p. 213-219. **[CrossRef]**

BARBERIS, S.; QUIROGA, E.; ARRIBÉRE, M.C. and PRIOLO, N. (2002). Peptide synthesis in aqueous-organic biphasic systems catalyzed by a protease isolated from *Morrenia brachystephana* (*Asclepiadaceae*). *Journal of Molecular Catalysis B: Enzymatic*, vol. 17, no. 1, p. 39-47. **[CrossRef]**

BARBERIS, S.; GUZMAN, F.; ILLANES, A. and LOPEZ-SANTIN, J. (2008). Study cases of enzymatic processes. In: ILLANES, A. ed. *Enzyme biocatalysis: Principles and applications*. Berlin, Heidelberg, New York; Springer Science + Business Media B.V., p. 253-273.

BARCIA, C.; QUIROGA, E.; ARDANAZ, C.; QUIROGA, G. and BARBERIS, S. (2009). *Acacia caven* (Mol.) Molina pollen proteases. Application to the peptide synthesis and to laundry detergents. In: DUMONT, F.E. and SACCO, J.A. eds. *Biochemical Engineering*. Hauppauge, NY, Nova Science Publisher, p. 293-316.

BARCIA, C.; QUIROGA, E.; QUIROGA, G. and BARBERIS, S. (2010). Pollen proteases of *Acacia caven* (Mol.) Molina for laundry detergents application. In: HAGEN, E.T. ed. *Detergents: Types, Components and Uses.* Hauppauge, NY, Nova Science Publisher, p. 143-160.

DONLON, J. (2007). Subtilisin. In: POLAINA, J. and MACCABE, A.P. eds. *Industrial enzymes. Structure, function and applications.* Valencia, España, Springer, p. 196-206.

DORAN, P.M. (2002). Properties and applications of hairy-root cultures. In: OKSMAN-CALDENTY, K.-M. and BARZ, W.H. eds. *Plant biotechnology and transgenic plants.* New York, Mercel Dekker Inc., p. 143-162.

GONZÁLEZ-RÁBADE, N.; BADILLO-CORONA, J.A.; ARANDA-BARRADAS, J.S. and OLIVER-SALVADOR, M.C. (2011). Production of plant proteases *in vivo* and *in vitro* - A review. *Biotechnology Advances,* vol. 29, no. 6, p. 983-996. **[CrossRef]**

GUPTA, R.; GUPTA, K.; SAXENA, R.K. and KHAN, S. (1999). Bleach-stable, alkaline protease from *Bacillus* sp. *Biotechnology Letters,* vol. 21, no. 2, p. 135-138. **[CrossRef]**

GUPTA, R.; BEG, Q.K. and LORENZ, P. (2002). Bacterial alkaline proteases: Molecular approaches and industrial applications. *Applied Microbiology Biotechnology,* vol. 59, no. 1, p. 15-32. **[CrossRef]**

GUZMÁN, F.; BARBERIS, S. and ILLANES, A. (2007). Peptide synthesis: Chemical or enzymatic. *Electronic Journal of Biotechnology,* vol. 10, no. 2. **[CrossRef]**

HAKI, G.D. and RAKSHIT, S.K. (2003). Developments in industrially important thermostable enzymes: A review. *Bioresource Technology,* vol. 89, no. 1, p. 17-34. **[CrossRef]**

ILLANES, A.; GUZMAN, F. and BARBERIS, S. (2009a). Aplicaciones emergentes en reacciones de síntesis. In: CAFFINI, N. ed. *Enzimas Proteolíticas de Vegetales Superiores. Aplicaciones Industriales.* Buenos Aires, Argentina, Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED), p. 201-219.

ILLANES, A.; GUZMAN, F. and BARBERIS, S. (2009b). Proteases as powerful catalysts for organic synthesis. In: HUGHES, A.B. ed. *Amino Acids, Peptides and Proteins in Organic Chemistry.* Weinheim, Germany, Wiley-VCH, vol. 2, part 3: Enzymes, p. 341-361.

KUMAR, C.G. and TAKAGI, H. (1999). Microbial alkaline proteases: From a bioindustrial viewpoint. *Biotechnology Advances,* vol. 17, no. 7, p. 561-594. **[CrossRef]**

LIGGIERI, C.; ARRIBÉRE, M.C.; TREJO, S.A.; CANALS, F.; AVILÉS, F. and PRIOLO, N.S. (2004). Purification and biochemical characterization of *Asclepain* c I from the latex *Asclepias curassavica* L. *The Protein Journal,* vol. 23, no. 6, p. 403-411. **[CrossRef]**

LIGGIERI, C.; OBREGÓN, W.; TREJO, S. and PRIOLO, N. (2009). Biochemical analysis of a papain-like protease isolated from the latex of *Asclepias curassavica* L. *Acta Biochimica et Biophysica Sinica,* vol. 41, no. 2, p. 154-162. **[CrossRef]**

LÓPEZ, L.M.I.; SEQUEIROS, C.; NATALUCCI, C.L.; BRULLO, A.; MARAS, B.; BARRA, D. and CAFFINI, N.O. (2000). Purification and characterization of *Macrodontain* I, a cysteine peptidase from unripe fruits of *Pseudananas* macrodontes (Morr.) Harms (Bromeliaceae). *Protein Expression and Purification,* vol. 18, no. 2, p. 133-140. **[CrossRef]**

MAURER, K.H. (2004). Detergent proteases. *Current Opinion in Biotechnology*, vol. 15, no. 4, p. 330-334. **[CrossRef]**

MORCELLE, S.R.; CAFFINI, N.O. and PRIOLO, N. (2004). Proteolytic properties of *Funastrum clausum* latex. *Fitoterapia,* vol. 75, no. 5, p. 480-493. **[CrossRef]**

MORCELLE, S.R.; BARBERIS, S.; PRIOLO, N.; CAFFINI, N.O. and CLAPÉS, P. (2006). Comparative behaviour of proteinases from the latex of *Carica papaya* and *Funastrum clausum* as catalysts for the synthesis of Z-Ala-Phe-OMe. *Journal of Molecular Catalysis B: Enzymatic,* vol. 41, no. 3-4, p. 117-124. **[CrossRef]**

MORCELLE, S.R.; BARBERIS, S. and CLAPES, P. (2009). Aplicaciones de peptidasas vegetales en reacciones de síntesis. In: CAFFINI, N. ed. *Enzimas Proteolíticas de Vegetales Superiores. Aplicaciones Industriales.* Buenos Aires, Argentina, Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED), p. 317-331.

OBREGÓN, W.D.; ARRIBÉRE, M.C.; MORCELLE DEL VALLE, S.; LIGGIERI, C.; CAFFINI, N. and PRIOLO, N. (2001). Two new cysteine endopeptidases obtained from the latex of *Araujia hortorum* fruits. *Journal of Protein Chemistry,* vol. 20, no. 4, p. 317-325. **[CrossRef]**

PARDO, M.F.; LÓPEZ, L.M.I.; CANALS, F.; AVILÉS, F.X.; NATALUCCI, C.L. and CAFFINI, N.O. (2000). Purification of Balansain I, an endopeptidase from unripe fruits of *Bromelia balansae* Mez (*Bromeliaceae*). *Journal of Agricultural and Food Chemistry,* vol. 48, no. 9, p. 3795-3800. **[CrossRef]**

PHADATARE, S.U.; DESHPANDE, V.V. and SRINIVASAN, M.C. (1993). High activity alkaline protease from *Cornidiobolus coronatus* (NCL 86.8.20): Enzyme production and compatibility with commercial detergents. *Enzyme and Microbial Technology,* vol. 15, no. 1, p. 72-76. **[CrossRef]**

PRIOLO, N.; MORCELLE DEL VALLE, S.; ARRIBÉRE, C.M.; LÓPEZ, L. and CAFFINI, N. (2000). Isolation and characterization of cysteine protease from the latex of *Araujia hortorum* fruits. *Journal of Protein Chemistry,* vol. 19, no. 1, p. 39-49. **[CrossRef]**

PRIOLO, N.; ARRIBÉRE, C.M.; CAFFINI, N.; BARBERIS, S.; VÁZQUEZ, R.N. and LUCO, J.M. (2001). Isolation and purification of cysteine peptidases from the latex of *Araujia hortorum* fruits: Study of their esterase activities using partial least-squares (PLS) modeling. *Journal of Molecular Catalysis B: Enzymatic,* vol. 15, no. 4-6, p. 177-189. **[CrossRef]**

QUIROGA, E.; PRIOLO, N.; OBREGÓN, D.; MARCHESE, J. and BARBERIS, S. (2008). Peptide synthesis in aqueous-organic media catalyzed by proteases from latex of *Araujia hortorum* (*Asclepiadaceae*) fruits. *Biochemical Engineering Journal,* vol. 39, no. 1, p. 115-120. **[CrossRef]**.

QUIROGA, E.; ILLANES, C.O.; OCHOA, N.A. and BARBERIS, S. (2011). Performance improvement of *araujiain*, a cystein phytoprotease, by immobilization within calcium alginate beads. *Process Biochemistry,* vol. 46, no. 4, p. 1029-1034. **[CrossRef]**

Reportlinker.com (2012). World Enzyme Industry. http://www.reportlinker.com/d012544125/World-Enzyme - Industry-Overview.html

SELLAMI-KAMOUN, A.; HADDAR, A.; EL-HADJ ALI, N.; GHORBEL-FRIKHA, B.; KANOUN, S. and NASRI, M. (2008). Stability of thermostable alkaline protease from *Bacillus licheniformis* RP1 in commercial solid laundry detergent formulations. *Microbiological Research*, vol. 163, no. 3, p. 299-306. **[CrossRef]**

SMULDERS, E.; RÄHSE, W.; VON RYBINSKI, W.; STEBER, J.; SUNG, E. and WIEBEL, F. (2002). Detergent Ingredients. In: SMULDERS, E. ed. *Laundry detergent*. Wiley-VCH, Verlag GmbH & Co. p. 38-98.

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