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Papain: an enzyme of multiple applications

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Summary. Papain is a sulphydryl, proteolytic enzyme isolated from papaya latex and is readily available from a number of sources. This enzyme has been widely used in pharmaceutical, cosmetic and nutrition area.
It is used in topical formulations as a debriding agent for the healing of open, extensive wounds and burnings. It is also employed as an enhancer for cutaneous permeation of active molecules, chemical peeling and as a progressive depilatory agent. The stability of formulations containing enzymes is a difficult parameter, thus alternatives are required to improve papain's properties in order to allow the industry-scale manufacture of formulations in a stable manner that makes them viable for commercialization.

INDRODUCTION

Enzymes have been extensively employed in cosmetic formulations during the last years.

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As a part of our research program on anti-aging agents and peptides [1-6], we thought it would be of interest to review the properties of proteolytic enzymes, such as papain. Papain has been applied in cosmetology for chemical peeling, depilatory preparations and also as penetration enhancers [7,8].

Papain is derived from the latex of the adult green papaya leaves and fruit, *Carica papaya Linn*. It is a mixture of proteins that contains a combination of papain and chymopapain, starch and esters, especially in bondings involving alkaline amino acids, leucine or glycine, producing low molecular weight peptides. The term refers both to the dry rough latex, and also to the crystalline enzyme. Papaya trees are cultivated on most tropical countries [9, 10].

The active site structure and stability of papain have been studied by Butterfield DA and Lee J. [11].

One of the limiting factors for the incorporation of papain in topical formulations is its low chemical stability. The enzymatic activity of papain can be influenced by environmental conditions (e.g. temperature, light, oxygen, humidity and packing). The enzyme is more stable and active in pH 5.0-7.0 [12, 13]. Moreover, the stability of the enzyme (both as a solid and incorporated in semi-solid formulations) has been examined at different temperatures and results have showed

the decrease in its activity with the increase of temperature [14, 8, 15].

Kang & Warner investigated the effect of pH on the activity of papain. The combined actions of pH and temperature on the stability of this enzyme have suggested a small reduction at pH 5.0, some loss at pH 7.0 and significant reduction at pH 9.0, especially at the temperature of 70 °C [16].

In addition, thermal denaturation of papain was studied as a function of pH using high-sensitivity differential scanning—calorimetry by Ian G. Sumner et al [17].

Papain, as already referred, is a thiol proteolytic enzyme widely used in dermatology that found applications in wound treatment. Papain was used as absorption enhancer which can modify the peptide/ protein material in the bilayer domain. Moreover, papain safety was investigated using human skin that was exposed to papain *in vitro* at different times. The samples were examined using Light and Transmission Electron Microscopy (TEM) to study of the mechanisms involved in enhancer-skin interaction. Papain 0.2% (w/v) promoted a proteolytic digestion of stratum corneum first layers and induced a decreased crosslinking in corneosomes region. In the treated samples, after 48 h, the stratum corneum was recovered. The results of Lopes et al suggested that papain could be used safely used due to reversibility of barrier function of stratum corneum [18].

In addition, histological alterations in the skin and hair follicle of mice were evaluated following the application of gel and cream formulas containing papain as a safe treatment for hirsutism. Results showed that papain cream had a significantly higher depilatory effect than the papain gel [19].

According to K. Murugesh Babu, papain requires activation by a sulphydryl reagent. Activated papain attacks the peptide bonds between the carboxylic acid group of lysine or arginine and the adjacent amino acid residue. A small cleavage occurs at the carboxylic acid group of histidine and also of glycine, glutamic acid, glutamine, leucine tyrosine residues [20].

Modifying enzyme structure in order to protect its active hydrolysis site is an alternative to increase papain stability. There are many techniques to modify the stability of enzymes, considering their specific action. There have been many tries in order to stabilize papain structure, such as: covalent bondings, interaction with immobilized ion metal, insolubilization in glutaraldehyde, imobilization in agarose, biopolymer, covalent bondings with polyether sulphone, coupling with polymeric sucrose, modification with succinic anhydride, simple absorption in Celite®, ionic absorption in CM-cellulose (cationic ion-exchange resin) and QAE-Sephadex® (anionic ion-exchange resin), and cross-linked covalent bonding [13, 21, 22].

According to the study of Rajalakshmi N., the covalently modified papain retained > 80% intrinsic catalytic activity with no change in pH optima and kinetic constants, indicating that the gross tertiary structure was not altered by modification. However, the derivative exhibited better thermotolerance than native papain, while temperature optima being shifted by 10 degrees C [23].

Modifying enzyme structure may lead to alterations when it is added to pharmaceutical or cosmetic formulations. The behavior of free papain in topical formulations is reported in the literature. Velasco *et al.* (1999) have studied the stability of papain incorporated in gel formulations at different temperatures and they discovered that the formulation was more stable when kept under refrigeration. According to the Stability Test, the enzyme kept approximately 70% of its initial activity for almost two months [24].

The aim of the research of de Oliveira Pinto was to estimate the physical, physicochemical and chemical stability of free and modified papain incorporated in cosmetic formulations – gels (reference preparation) and emulsions with the aim to enable the industry-scale production of formulations in a stable manner that makes them applicable for commercialization. In this research, papain was modified with polyethylene glycol as a means to increase the stability of the formulations. The comparative Normal Stability Testing of the topical formulations containing unmodified and modified papain demonstrated that the modified variety showed a differentiated profile under the adopted temperature conditions The most suitable conditions for non-modified papain were 5.0 ± 1.0 °C, while for modified papain were 22.0 ± 2.0 °C. These results verified the higher stability of modified papain compared to free papain, as well as its potential to be used in topical formulations. The technique of papain modification with polyethylene glycol changed the release profile and the activity of the enzyme incorporated into the emulsion containing ammonium acryloyldimethyltaurate/VP copolymer, under all the estimated conditions. It was found that enzyme stability was higher than in the formulations containing the non-modified enzyme [25].

Liang YY, and Zhang LM performed the bioconjugation of papain on superparamagnetic nanoparticles decorated with carboxymethylated

chitosan. The conjugated papain exhibited enhanced enzyme activity, better tolerance to the variations of medium pH and temperature, and better storage stability and good reusability, comparing with the native papain. Due to the rapid, efficient, cost-effective and lacking of negative effect on biological activity technique, such a bioconjugate system may have potential applications in food, pharmaceutical, leather, cosmetic, and textile industries [26].

Yan-Yan Chen et al investigated the therapeutic effects of papain elastic liposomes (PEL) on hypertrophic scar through topical application. PEL were prepared via the reverse-phase evaporation technique and optimized by response surface methodology. The transdermal absorption of optimized PEL was tested by vertical Franz diffusion cells *in vitro*. The effects of PEL were investigated in rabbit model of hypertrophic scar *in vivo*, histological analysis and scar-related proteins were detected to reveal potential scar repair mechanism. After topical application, the scar elevation index, microvascular density, and collagen fiber were significantly decreased with regular arrangement. The expressions of TGF-β_1, P-Smad-3, P-NF-κB p65, and P-IKBa in hypertrophic scar were found notably down regulated contrary to those in model group. Thus, PEL were confirmed as an effective topical preparation for hypertrophic scar treatment [27].

In order to immobilize papain, hydrogel composites based on pineapple peel carboxymethyl cellulose, polyvinyl alcohol and mesoporous silica SBA-15 were synthesized by an environmental friendly technique of repeated freeze-thaw cycles. Optimization of the experiment was performed in order to achieve an efficient papain immobilization carrier. The conditions of immobilization such as enzyme concentration, pH, crosslinker concentration and cross-linking time were optimized as well. Results revealed that the immobilized papain had maximum activity at low reaction temperature of 40°C and showed pH-sensitivity by exhibiting a rapid decrease of activity within a minor range from pH 7.0 to pH 7.5. The immobilized papain revealed enhanced pH, thermal and storage stability comparing with the free papain. After 2h incubation at 80°C, the immobilized papain retained 56% of its initial activity while the free papain only retained 16%. Finally, 79% of the initial activity was retained for the immobilized papain while only 27% for the free papain, after 10days of storage [28].

The research of Holyavka et al investigated the photosensitivity of papain. The papain activity was decreased after the UV irradiation intensity of 453 $J \cdot m^{-2}$, and an increase of the papain globule size was detected at 755 $J \cdot m^{-2}$. It was shown that immobilization on chitosan matrix leads to the increase in the stability with respect to UV irradiation in comparison with the free enzyme. Therefore, it can be postulated that the chitosan matrix acts as photoprotector for immobilized papain [29].

The research of a new magnetic metal chelating carrier preparation for the immobilization of papain is described by Gu YJ et al. The new type of carrier was developed 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+}). In contrast to the free papain, the immobilized papain displayed higher enzyme activity, improved enzymatic properties, satisfactory stability and reusability as well. The novel carriers exhibited selectively biological adsorption capacity. The procedure employed is an efficient and economic way for the immobilization of enzyme [30].

Papain is the most common cysteine protease applied in acidic-alcoholic conditions The aim of the study of Milošević J et al was to compare the stability of papain and ficin (which is closely related to papain in terms of proteolytic activity and substrate specificity) in process conditions. Results showed that ficin has a broader range of stability in respect of pH and cold storage stability, in comparison to papain. Ficin retains about 70% of initial activity after 3-week cold storage at low pH and in the presence of ethanol, while papain loses about 70% of initial activity in the same incubation period as it is more prone to non-native aggregation as verified by FTIR analysis [31].

Papain can also be immobilized using cubic mesoporous silica nanoparticles (MSNs). Analytically, cubic mesoporous silica nanoparticles with the SBA-1 moiety, functionalized with carboxylic acid (COOH) groups and enlarged mesopores, were prepared using tetraethyl orthosilicate (TEOS) and carboxyethylsilanetriol sodium salt (CES) as silica sources, complexes formed by polyacrylic acid (PAA) and hexadecylpyridinium chloride (CPC) as templates, and trimethylbenzene (TMB) as pore expander. The MSNs display an adsorption capacity of 1138 $mg\,g^{-1}$ at pH=8.2 when employed to immobilize papain. Results show that the immobilized papain has better thermal stability, pH tolerance, and heat resistance than the free papain. Moreover, according to the study of Saikia D et al, these materials can also be applied for the selective adsorption of a single

protein (eg papain) from the binary mixture of two different types of proteins possessing different isoelectric points and shapes, such as papain and hemoglobin, merely by regulating the pH of the buffer [32].

DISCUSSION

The action of papain on the skin has been investigated by many researchers, who found positive results regarding hydration, penetration enhancement of active compounds and a decrease in hair growth velocity [33, 34, 18, 8].
The aim of this review was to summarize the applications of papain and the studies performed in order to improve the properties of this enzyme for potential appliance in food, pharmaceutical and cosmetic industries.

REFERENCES

1. Papagianni, P., Varvaresou, A., Papageorgiou, S., Panderi, I., Development and validation of an ion-pair RP-HPLC method for the determination of oligopeptide-20 in cosmeceuticals, Journal of Pharmaceutical and Biomedical Analysis, 56, 645-649, (2011).
2. Varvaresou, A., Papageorgiou, S., Mellou, F., Protopapa, E., Study in anti-wrinkle activity of a night cream containing a combination of antioxidants, phyto-steroids and acetyl-tetrapeptide-9 by biophysical methods and objective evaluation, Review of Clinical Pharmacology and Pharmacokinetics, Int Ed. 30, 67-70, (2016).
3. Giannakou, M., Varvaresou, A., Kiriazopoulos, E., Papageorgiou, S., Kavvalou, E., Tsirivas, E., Panderi, I., Quantification of oligopeptide - 20 and oligopeptide - 24 in cosmetic creams using hydrophilic interaction liquid chromatography with electrospray ionization mass spectrometry, Separation Science plus, 1, 159-167, (2018).
4. Mellou, F, Varvaresou, A., Papageorgiou, S., Renewable sources: Applications in personal care formulations, International Journal of Cosmetic Science, 41, 517-525 (2019).
5. Varvaresou, A., Papageorgiou, S., Protopapa, E., Katsarou, A., Efficacy and tolerance study of an oligopeptide with potential anti-aging activity cosmetics, Dermatological Sciences and Applications, 1, 133-140, (2011).
6. Mellou F., Gardiki V., Varvaresou A., Papageorgiou S., Protopapa, E., Kintziou H., Research and development of new products: Applications of Biotechnology in Cosmetic Science, Epitheorese Klinikes Farmakologias kai Farmakokinitikes Gr Ed., 38, 37-44, (2020).
7. Lopes, P.S., Yamamoto, J.K., Pinto, C.A.S.O., Takano, C.Y., Velasco, M.V.R., Consiglieri, V.O., Kaneko, T.M., Validação de metodologia analítica de avaliação da atividade da papaína, Rev. Bras. Cienc. Farm., 39, .214-216, (2003).
8. Traversa, E., Ottolenghi, A.T.B., Menegotto, C.P., Marcondes, C.P., Machado-Santelli, G.M., Kaneko, T.M., Consiglieri, V.O., Velasco, M.V.R., Desenvolvimento de formulações cosméticas contendo papaína e avaliação de sua eficácia depilatória, Rev. Bras. Cienc. Farm., 39, 201-203, (2003).
9. Merck Index. An encyclopedia of chemicals, drugs, and biological, 14ed., Whitehouse Station: Merck, 2564 p., (2006).
10. Martindale. The Complete Drug Reference, 36ed., London: Pharmaceutical Press, 1647-1648, (2009).
11. Butterfield D.A., Lee J., Active site structure and stability of the thiol protease papain studied by electron paramagnetic resonance employing a methanethiosulfonate spin label,] Arch Biochem Biophys., 310, 167-71, (1994).
12. Sasmito, T., Demeester, J., Bracke, M., Review on the production, properties and uses of papain, Pharm. Tijdschr. Belg., 59, 149-158, (1982).
13. Sim, Y.C., Lee, S.G., Lee, D.C., Kang, B.Y., Park, K.M., Lee, J.Y., Kim, M.S., Chang, I.S., Rhee, J.S., Stabilization of papain and lysozyme for application to cosmetic products, Biotechnol. Lett., 22, 137-140, (2000).
14. Arnon, R. Papain, Meth. Enzymol., 19, 226-244, (1970).
15. Szabó, A., Kotormán, M., Laczkó, I., Simon, M., Spectroscopic studies of stability of papain in aqueous organic solvents, J. Mol. Catal. B: Enzym., 41, 43-48, (2006).
16. Kang, C.K., Warner, W.D., Tenderization of meat with papayn latex proteases, J. Food Sci., 39, 812-818, (1974).
17. Sumner, G., Harris, G.W., Taylor, M.A. J., Pickersgill, R.W., Owen, A.J., Goodenough, P.W., Factors effecting the thermostability of cysteine proteinases from *Carica papaya* lan, Eur. J. Biochem., *214,* 129-134, (1993).
18. Lopes, P.S., Ruas, G.W., Baby, A.R., de Oliveira Pinto, C.A.S., Ii-sei Watanabe, Velasco, M.V.R., Kaneko, T.M., *In vitro* safety assessment of papain on human skin: A qualitative Light and Transmission Electron Microscopy (TEM) study, Revista Brasileira de Ciências Farmacêuticas Brazilian, Journal of Pharmaceutical Sciences, 44, (2008).
19. Traversa, E., Machado-Santelli, G.M., Velasco, M.V.R., Histological evaluation of hair follicle due to papain's depilatory effect, International Journal of Pharmaceutics, 335, 163-166, (2007).
20. Babu K.M., *Silk Processing*, Properties and Applications A volume in Woodhead Publishing Series in Textiles Book, ISBN 978-1-78242-155-9, p. 200, Woodhead Publishing Limited, (2013).
21. Afaq, S., Iqbal, J., Immobilization and stabilization of papain on chelating sepharose: a metal chelate regenerable carrier, Electron. J. Biotechnol, 4, (2001).
22. Li, M., Su, E., You, P., Gong, X., Sun, M., Xu, D., Wei, D., Purification and In Situ Immobilization of Papain with Aqueous Two-Phase System, 5, (2010).

23. Rajalakshmi N., Sundaram P.V., Stability of native and covalently modified papain, Protein Eng., 8, 1039-47, (1995).
24. Velasco, M.V.R., Rodrigues, L.B., Dazzi, C., YamamotoO, J.K., Kaneko, T.M., Avaliação da estabilidade da solução de papaína 2% p/v pelo método da coagulação do leite, Rev. Farm. Quim., 32, 8-13, (1999).
25. de Oliveira Pinto, C.A.S., Lopes, P.S., Sarruf, F.D., Polakiewicz, B., Kaneko, T.M., Baby, A.R., Velasco, M.V.R., Comparative study of the stability of free and modified papain incorporated in topical formulations, Brazilian Journal of Pharmaceutical Sciences, 47, (2011).
26. Liang Y.Y., Zhang L.M., Bioconjugation of papain on superparamagnetic nanoparticles decorated with carboxymethylated chitosan, Biomacromolecules, 8, 1480-6, (2007).
27. Chen, Y.Y., Lu, Y.H., Ma, C.H., Tao, W.W., Zhu, J.J., Zhang, X., A novel elastic liposome for skin delivery of papain and its application on hypertrophic scar Author links open overlay panel Biomedicine & Pharmacotherapy, 87, 82-91, (2017).
28. Dai H, Ou S., Liu Z., Huang H., Pineapple peel carboxymethyl cellulose/polyvinyl alcohol/mesoporous silica SBA-15 hydrogel composites for papain immobilization, Carbohydrate Polymers, 169, 504-514, (2017).
29. Holyavka M., Pankova S., Koroleva V., Vyshkvorkina Y., Lukin A., Kondratyev M., Artyukhov V., Influence of UV radiation on molecular structure and catalytic activity of free and immobilized bromelain, ficin and papain, J Photochem Photobiol B., (2019).
30. Gu YJ, Zhu ML, Li YL, Xiong CH. Research of a new metal chelating carrier preparation and papain immobilization, Int J Biol Macromol., 112, 1175-1182, (2018).
31. Milošević J., Janković B., Prodanović R., Polović N., Comparative stability of ficin and papain in acidic conditions and the presence of ethanol, Amino Acids, 51, 829-838, (2019).
32. Saikia D., Deka J.R., Wu C.E., Yang Y.C., Kao H.M., pH responsive selective protein adsorption by carboxylic acid functionalized large pore mesoporous silica nanoparticles SBA-1, Mater Sci Eng C Mater Biol Appl., 94, 344-356, (2019).
33. El-Kadi, K.N., Rawlings, A.V., Feinberg, C., Watkonson, A., Nunn, C.C., Battaglia, A., Chandar, P., Pocalyko, N.R.D.J., Broad specificity alkaline proteases efficiently reduce the visual scaling associated with soap-induced xerosis, Arch. Dermatol., 293, 500-507, (2001).
34. Sim, Y.C., Nam, Y.S., Shin, Y.H., Shin, E., Kim, S., Chang, I.S., Rhee, J.S., Proteolytic enzyme conjugated to SC-glucan as an enzymatic transdermal drug penetration enhancer, Pharmazie., 58, 252-256, (2003).