Proteolytic activity of cosmetic enzyme peel products*

Karol Białkowski✉

Department of Clinical Biochemistry, Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

Our goal was to verify the proteolytic mode of action and activity levels among several commercial cosmetic facial peels advertised by manufacturers as “enzymatic”. Eleven enzyme peels were analyzed for their proteolytic activity against casein as a generic substrate and compared to the activity found in pineapple and papaya fruits. The highest specific protease activity was observed in the flesh of a pineapple (5.88 U/g). Only two products demonstrated sufficient activity (0.924 and 0.238 U/g) to be called “enzyme peels”. Three products showed trace activity (0.023–0.125 U/g), albeit too low to exert a significant exfoliating effect. Six preparations had no detectable enzyme activity.

Keywords: enzyme peels, cysteine proteases, proteolytic activity, spectroscopy, formulation/stability

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✉e-mail: karolb@cm.umk.pl

*This work is dedicated to the memory of the great musician, Wojciech Karolak.

Abbreviations: CDSN, corneodesmosin; CTSD, aspartate protease cathepsin D; CTSV, cysteine protease cathepsin L2; DSC1, desmocollin 1; DSG1, desmoglein 1; EDTA, ethylenediaminetetraacetic acid; KLK, kallikrein family serine peptidase; PEG, polyethylene glycol; TCA, trichloroacetic acid; ZO-1, Zonula Occludens Protein 1 (product of TJP1 gene)

INTRODUCTION

Enzymatic facial peels are increasingly popular because they reduce abrasion and redness, which often occur with mechanical or chemical peeling agents. Enzymatic skin exfoliation is promoted as safe for most skin types and recommended for individuals with sensitive skin who do not tolerate the α-hydroxy and β-hydroxy acids found in many chemical peels. Most enzyme peels exploit plant-based cysteine proteases, i.e., papain and bromelains. They cleave the extracellular domains of cell adhesion proteins constituting corneodesmosomes, the structures that bind the epidermal corneocytes together (Lopes et al., 2008).

The extracellular parts of corneodesmosomal plaques are composed of at least three cell adhesion proteins, i.e., desmoglein 1 (DSG1), desmocollin 1 (DSC1), and corneodesmosin (CDSN) (Ishida-Yamamoto & Igawa, 2015). A few cell layers above the level at which the cells change from the granular to cornified phenotype, the cell surfaces become covered with corneodesmosomes (Naoe et al., 2010). During cornocyte maturation, corneodesmosomes are progressively decomposed and are preserved only at the lateral rims of the cells, where they are protected from proteolysis by the cell-to-cell tight-junction structures (Igawa et al., 2011).

The physiological degradation of corneodesmosomal proteins is controlled mainly by a cascade composed of the kallikrein family of serine peptidases. KLK5 cleaves CDSN, DSC1, and DSG1; KLK7 degrades CDSN and DSC1 (Caubet et al., 2004); whereas the KLKs 1, 6, and 14 hydrolyze the DSG1 cadherin (Borgoño et al., 2007). The aspartate protease cathepsin D (CTSD) (Igarashi et al., 2004) and cysteine protease cathepsin L2 (CTSV) (Bernard et al., 2003) are involved in desquamation processes by decomposing CDSN. The above proteases, along with their physiological polypeptide inhibitors, control the balance between the formation and desquamation of the outer layers of the stratum corneum (reviewed by Ishida-Yamamoto & Igawa, 2015).

Application of a peel enriched in external endopeptidases to the skin mimics natural enzymatic exfoliation and accelerates the process. For example, papain degrades the proteins of the tight junctions of human keratinocytes, i.e., ZO-1, claudin 4, and occludin (Stremnitzer et al., 2015), as well as the proteinaceous components of corneodesmosomes (Lopes et al., 2008). Likewise, the bromelain proteases, i.e., fruit bromelain, stem bromelain, and ananain, hydrolyze a vast array of skin cell-surface proteins (Hale et al., 2005).

Cosmetologists frequently ask how to judge which of the commercial peel products act by proteolytic degradation of the cell adhesion proteins. We could not find any peer-reviewed publications addressing this issue directly. It is hard to answer this question, considering that revealing the specific activity of the enzyme peels is not required by EU regulations (Regulation (EC) No 1223, 2009; Commission Regulation (EU) No 655, 2013), nor is it voluntarily supplied by most manufacturers. Therefore, we decided to pursue our investigation by measuring the proteolytic activity of eleven representative facial peel products marketed as “enzymatic peels” that were available on the Polish market at the time.

MATERIALS AND METHODS

Chemicals and materials

Anhydrous sodium carbonate, L-tyrosine, potassium dihydrogen phosphate, proteinase K from Tritirachium album (lyophilized, >3 U/mg), sodium dihydrogen phosphate, sodium hydroxide, and Triton X-100, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine Hammarsten casein was from LOBA Chemie (Fischamend, Austria). Folin-Ciocalteu reagent and trichloroacetic acid were from POCH (Gliwice, Poland). High-purity water (18.3 MΩ/cm resistance) was produced with a Hydrolab HLP 10UV purification system (Straszyn, Poland).
| No. | Product’s form                  | Product’s composition specified by manufacturer                                                                                                                                                                                                                                                                                                                                                     |
|-----|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1   | Light brown thick paste         | Prunus Amygdalus Dulcis Oil, Elaeis Guineensis Oil, Theobroma Cacao Seed Oil, Butyrospermum Parkii Butter, Glyceryl Stearate, Lauryl Glucoside, Papain, Bromelain, Hydroxyecaric Acid, Cymbopogon Schoenanthus Oil, Tocopherol Acetate, Pelargonium Graveolens Oil, Citrus Limonum Peel Oil, Allantoin, Benzyl Alcohol, Dehydroacetic Acid, Geraniol.                                                                                     |
| 2   | Light brown thin cream          | Aqua, Prunus Amygdalus Dulcis Oil, Glycerin, Simmondsia Chinensis Seed Oil, Sodium Acryloyldimethyl Taurate/acylamide/vp Copolymer, Glycyrrhiza Glabra Root Extract, Humulus Lupulus Cone Extract, Rheum officinale Baill, Spirea Ulmaria Extract, Salix Alba Bark Extract, Hibiscus Rosa-sinensis Flower/leaf Extract, Tropaeolum Majus Extract, Benzyl Alcohol, Bromelain, Lactobionic acid, Parfum, Ethylhexylglycerin, Lactic Acid. |
| 3   | Light yellow thin gel           | Aqua, Papain, Glycine, Macadamia Ternifolia Seed Oil, Persea Gratissima (Avocado) Oil, Vitis Vinifera Seed Oil, Olea Europaea (Olive) Fruit Oil, Hydroxypropyl Starch Phosphate, Panthenol, Sodium Hyaluronate, Sorbitan Stearate, Carbomer, Phenoxethanol, Sodium Polyacrylate, Propylene Glycol, Dipotassium Glycyrrhizate, Chlorophesins, Polysorbate 20, Fragrance, Xanthan Gum, Propylparaben, Ethylhexylglycerin, Stearic Acid, Sodium Chloride, Sodium Citrate. |
| 4   | White powder (lyophilisate)     | Talc, Onyza Salvia (Rice) Starch, Sodium Carrageenan, Algin, CI 77891 (Titanium Dioxide), Sodium Carboxymethyl Starch, Lactose, Parfum (Fragrance), Ananas Sativus Extract (Bromelain), Papain, Silica, Ascorbic Acid, Maris Aqua (Sea Water).                                                                                                                                         |
| 5   | White thick cream               | Sea Salt, Caprylic/Capric Triglyceride, Cetyl Alcohol, PEG-75 Lanolin, Zea Mays (Corn) Seed Flour, Aluminum Starch Octenylsuccinate, Laureth-4, Beeswax, Hydrogenated Castor Oil, Glycerol Oleate, Ananas Sativus (Pineapple) Fruit Juice, PEG-45/Dodecyl Glycol Copolymer, Glycerin, Lecithin, Tocopherol, Hydrogenated Palm Glycerides Citrate, Ascorbul Palmitate, Benzyl Alcohol, Quaternium-90 Bentonite, Salicylic Acid, Retinol, Lecithin, Rosmarinus Officinalis Leaf Extract, Iron Oxides, Benzyl Alcohol, Dehydroacetic Acid, Benzoic Acid, Sorbic Acid. |
| 6   | Light yellow thin gel           | Water, Polyquaternium-6, Polyethylene, Propanediol, Ananas Sativus (Pineapple) Fruit Extract, Glycolic Acid, Trehalose, Hydrogenated Starch Hydrolysate, Carbomer, Phenoxethanol, Sodium Polyacrylate, Propylene Glycol, Dipotassium Glycyrrhizate, Chlorophesins, Polysorbate 20, Fragrance, Xanthan Gum, Glycerin, Papain, Calcium Pantothenate, Caprylyl Glycol, Urea, Magnesium Lactate, Ethylhexylglycerin, Potassium Lactate. Serine, Alanine, Proline, Magnesium Chloride, Sodium Citrate, CI 19140. |
| 7   | White thin cream                | Aqua (Water), Ammonium Acryloyldimethyltaurate/VP Copolymer, PEG-7 Glyceryl Cocoate, Phenoxyethanol, Panthenol, Niacinamide, Octyldodecanol, Passiflora Edulis Fruit Extract, Citrus Limon Fruit Extract, Sodium Hyaluronate, Sorbitan Tristearate Propanediol, Alcohol Denat., Parfum (Fragrance), Ethylhexylglycerin, Glycerin, Stearate, Maltodextrin, Agar, Scierotium Gum, C20-24 Alkyl Dimethicone, Papain, Allantoin, Sodium Bisulphite, Potassium Sorbate, Disodium EDTA, Methylparaben, Propylparaben, Ethylhexylglycerin, Xylitilglycosides, Anhydroxylitol, Xylitol, Glycol. |
| 8   | White thin cream                | Aqua, Hydrogenated Polycadecane, Isopropyl Myristate, Glycerin Stearate, Hydrogenated Polydecane, Glycerin, Glycerin, Stearyl Alcohol, Papain, Carbomer, Algin, Aloe Barbendensis Leaf Juice Powder, Lecithin, Sorbitol, Ascorbyl Glucoside, Panthenol, Glucose, Tri laureth-4 Phosphate, Ascorbyl Tetraisopalmitate, Alcohol, Termina l Ferdandiana Fruit Extract, Xanthan Gum, Dimethicone, Ceteareth-20, Ceteareth-25, Sodium Hydroxide, Disodium EDTA, Phenoxethanol, Hydroxyacetophenone, Octadeyl Di-T-Butyl-4-Hydroxyhydrocin namate, Benzyl Salicylate, Citronellol, Hexyl Cinnamal, Hydroxycitronellal, Limonene, Linolool, Parfum, CI 15985, CI 19140. |
| 9   | White thin cream                | Aqua (Water), Prunus Amygdalus Dulcis (Sweet Almond) Oil, Polyacrylamide, Parfum (Fragrance), C13-14 Isoparaffin, Panthenol, Laureth-7, Alcohol, Papain, Ethylhexylglycerin, Propylene Glycol, Lecithin, Guar Hydroxypropyltrimonium Chloride, Hydrogenated Starch Hydrolysate, Hydroxyethylcellulose, Malpigia Punicifolia (Acerola) Fruit Extract, Bambusa Vulgaris (Bamboo) Shoot Extract, Nelumbo Nucifera Flower Extract, Nymphaea Alba (Water Lily) Root Extract, Phenoxyethanol, Methylparaben, Mica, CI 77891 (Titanium Dioxide), CI 16035 (FD&C Red No. 40), CI 17200 (D&C Red No. 33). |
| 10  | White thin cream                | Aqua, Cetearyl Alcohol, Glycine, Caprylic/Capric Triglyceride, Glycerin, Octyldodecanol, Glycerin Stearate, Cetearyl Glucoside, Sodium Hydroxide, Hydroxyethylcellulose, Angelica Archangelica Root Water, Prunus Armeniaca Kernel Oil, Mangifera Indica Seed Butter, Macadamia Ternifolia Seed Oil, Olea Europaea Fruit Oil, Sodium Stearyl Glutamate, Benzyl Alcohol, Alcohol, Papain, Ethylhexylglycerin, Propylene Glycol, Lecithin, Guar Hydroxypropyltrimonium Chloride, Hydrogenated Starch Hydrolysate, Hydroxyethylcellulose, Malpigia Punicifolia (Acerola) Fruit Extract, Bambusa Vulgaris (Bamboo) Shoot Extract, Nelumbo Nucifera Flower Extract, Nymphaea Alba (Water Lily) Root Extract, Phenoxyethanol, Methylparaben, Mica, CI 77891 (Titanium Dioxide), CI 16035 (FD&C Red No. 40), CI 17200 (D&C Red No. 33). |
| 11  | Pink thin cream                 | Aqua (Water), Glycerin, Prunus Amygdalus Dulcis (Sweet Almond) Oil, Polyacrylamide, Parfum (Fragrance), C13-14 Isoparaffin, Panthenol, Laureth-7, Alcohol, Papain, Ethylhexylglycerin, Propylene Glycol, Lecithin, Guar Hydroxypropyltrimonium Chloride, Hydrogenated Starch Hydrolysate, Hydroxyethylcellulose, Malpigia Punicifolia (Acerola) Fruit Extract, Bambusa Vulgaris (Bamboo) Shoot Extract, Nelumbo Nucifera Flower Extract, Nymphaea Alba (Water Lily) Root Extract, Phenoxyethanol, Methylparaben, Mica, CI 77891 (Titanium Dioxide), CI 16035 (FD&C Red No. 40), CI 17200 (D&C Red No. 33). |
Naturally ripened pineapples (Ananas comosus) and papaya (Carica papaya) were bought in the local supermarket. The facial peel products were popular, widely distributed in Poland, clearly labeled as "enzymatic," and analyzed well within the specified periods of their minimum durability. The products were assigned consecutive numbers without revealing the identities of the peel preparations or their producers. Their forms and declared compositions are listed in Table 1. The author has had no relationships with the peel-producing companies or their employees.

**Determination of L-tyrosine release**

The L-tyrosine assay used the Folin and Ciocalteu method (Folin & Ciocalteu, 1927). A stock solution was prepared by dissolving 20 mg of L-tyrosine in 100 mL H₂O at 30°C. To determine a precise molar concentration of this solution, it was diluted three times with 20 mM phosphate buffer, pH 7.0. Its UV absorbance at 200–340 nm was measured against the same buffer. The absorbance at 274.5 nm was used to calculate the L-tyrosine concentration (1000.8 μM) based on its molar absorption coefficient (ε=1405 M⁻¹·cm⁻¹) (Fig. 1a). Calibration solutions were prepared by diluting the L-tyrosine stock with water to the concentrations of 30, 60, 120, 240 and 300 μM.

The 0.5 mL aliquots of calibration solutions or enzymatic reaction samples were reacted at 37°C for 30 min with 1.25 mL of 0.5 M sodium carbonate and 0.25 mL of 4-times water-diluted Folin-Ciocalteu reagent. All samples were centrifuged for 10 min at 3850×g (room temperature), the resulting supernatants were analyzed spectrophotometrically. To generate a calibration curve, the absorbance values of the calibration samples were measured at 660 nm (Fig. 1b) against a blank containing a water equivalent in the place of the tyrosine solution (Fig. 1c). For enzymatic reaction mixtures, the measurements were performed against blank samples prepared as described below.

**Preparation of samples for the proteolytic activity assay**

Four-gram portions of each enzyme peel, or the fruit flesh samples, were homogenized on ice with 12 mL of cold 50 mM KH₂PO₄-NaOH buffer, pH 7.5, containing 1% Triton X-100, in a motor-driven 50-mL Potter-Elvehjem homogenizer. The lyophilized product No. 4 was suspended in the manufacturer-specified amount of water. The homogenates were centrifuged for 15 min at 3850×g (10°C) in a 50-mL screw-capped polypropylene tube. The water phases of the supernatants (referred to hereafter as the "extracts") were gently aspirated and immediately analyzed for proteolytic activity.

**Determination of the proteolytic activity**

The proteolytic activity assay was based on the method developed by Maeno et al. (Maeno et al., 1959). Casein, 0.65% (m/v), in 50 mM KH₂PO₄-NaOH buffer, pH 7.5, was used as a substrate. To prepare the substrate solution, 650 mg of casein was gently stirred into 100 mL of the above buffer while gradually increasing the temperature to 80°C. The substrate solution, 14 mL in 50-mL screw-capped polypropylene tube, was pre-incubated at 37°C in a circulating water bath. The enzymatic reaction was initiated by adding 0.7 mL of peel extract. The progress of the reaction was monitored after 10, 20, 30, 60, 90, and 120 min by transferring a 2.1 mL aliquot of the reaction mixture to a 15-mL screw-capped polypropylene tube containing 2 mL of 110 mM trichloroacetic acid (TCA). The samples were vigorously vortexed and then incubated at 37°C for at least 30 min to precipitate non-hydrolyzed casein. A blank sample was prepared by the sequential mixing of 2 mL of 110 mM TCA, 2 mL of the substrate solution, and 0.1 mL of the analyzed extract (TCA inhibits the activity of the proteases) followed by incubation at 37°C for 120 min. All TCA-treated samples were centrifuged for 30 min (10°C) at 3850×g and the released L-tyrosine in the collected supernatants was determined as described above. The absorbance values at 660 nm were measured in the enzymatic reaction samples against the corresponding blank samples containing the same peel or fruit extract.

**Determination of the specific activity**

Enzymatic activity was calculated from the initial rate of the reaction (measured 10 min after initiation) and expressed as micromoles of L-tyrosine released per min in one mL of the reaction mixture. The enzymatic activity unit (U) was defined as the amount of activity liberating 1 μmol of L-tyrosine per min in a 14.7-mL reaction mixture under the reaction conditions defined above. The specific activity was expressed as the activity units contained in one gram of peel product or fruit flesh.

**RESULTS**

To confirm the accuracy of the well-established assay that we used for general proteolytic activity (Folin & Ciocalteu, 1927; Maeno et al., 1959), we applied it to follow the progress of casein hydrolysis by a commercial preparation of proteinase K from Tribinichium album (1 mg/mL) under our standard reaction conditions.

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Figure 1. An assay of general proteolytic activity based on the liberation of tyrosine from a casein substrate:

(a) The UV absorbance spectrum of an L-tyrosine stock solution and the determination of its molar concentration based on its absorbance at 274.5 nm. (b) The visible light absorbance spectra of the Folin-Ciocalteu reagent before (1) and after the reaction with tyrosine (2), both measured against water. (c) The calibration curve for L-tyrosine determination with the Folin-Ciocalteu method. (d) The time course of the casein hydrolysis by proteinase K, expressed as liberated tyrosine.
We tested the proteolytic activity of eleven common commercial cosmetic facial products clearly labeled as “enzymatic peels”. Table 1 shows nine of the products listed protease(s), i.e., papain, bromelain, or both, in their preparation. Product No. 5 contained pineapple juice as the sole source of proteases, whereas the list of ingredients for product No. 10 surprisingly had no potential protease source. In our opinion, only two of the eleven commercial peels demonstrated a proteolytic activity sufficient to qualify as an “enzyme peel”. Our findings do not mean that the remaining nine products were unable to have positive peeling effects, just that these effects could not be attributed to proteolytic causes. Five peels (No. 2, 3, 5, 6, 10) contained alpha and/or beta hydroxy acids that induce chemical exfoliation. Additional product ingredients may improve the general condition of the skin - but not by the measurable action of proteases.

Since the manufacturers do not specify the originally intended units of the proteolytic activity of their peel products, it is impossible to determine the cause of the poor or absent activity, i.e., whether the proteolytic activity was negligible to begin with or was lost post-production. Cysteine proteases are known to have limited stability in water solutions. For instance, the activity of an unconjugated papain solution was shown to drop by more than 90% after one month at 25°C (Sim et al., 2000). Bromelain in oil/water emulsions and gels was significantly more stable, but it still lost up to 70% of its initial activity after six months at 25°C. Moreover, certain gel formulations have been demonstrated to immediately inhibit up to 50% of bromelain’s activity (Lourenço et al., 2016).

Papain has better depilatory efficacy in creams than in gel formulations (Traversa et al., 2007). Two of the peel products we investigated (No. 3 and 6) were in gel form (Table 1). As shown in Fig. 2a and 3, product No. 3 had low but detectable proteolytic activity, whereas No. 6 was inactive. Since the other five inactive peels (No. 7–11) were in the form of an emulsion cream, it would suggest that the amount of active enzyme added to the products during manufacture was what most likely determined their measured proteolytic performance. The exceptionally low activity of peel No. 4, sold in a lyophilized form, normally highly stable, further emphasized the need for strict regulation of the enzymatic activity introduced during the production process. Product No. 1, the most enzymatically active of the peels tested, was the only water-free product. It was based on a mixture of oils and meant to be applied to wet skin. Our study found this formulation advantageous for this specific enzyme peel product, most likely by producing a longer shelf-life, although we have not conducted a shelf-life analysis.

Within the limited number of peel products investigated, most were labeled incorrectly as enzymatic. Since quality control of enzyme-based cosmetics is not mandatory under current commercial regulations, a consensus recommendation for verifying the effectiveness of commercial enzyme peels is needed from cosmetology and dermatology professionals. In our opinion, manufacturers should verify whether the right amount of active enzyme is added to their preparations and how other ingredients, further processing, and storage conditions affect the stability of the cysteine protease activities of their products.

Cysteine proteases extracted from papaya (papain, chymopapain, glycyl endopeptidase, caracain) and pineapple (stem bromelain, fruit bromelain, ananain, comedosin)
have a broad substrate specificity (Choe et al., 2006) and are able to cleave multiple peptide bonds in most proteins. Therefore, a proteolytic activity of the peel product toward cascin is a good measure of its potential to cleave cell adhesion proteins of the skin. The enzymatic activity assay used in this study is simple, cheap, and capable of being performed in any biochemical laboratory with basic equipment. The two most commonly used enzyme exfoliants are papain and bromelain, and bovine cascin is degraded at the neutral pH by both, as well as by fresh extracts of papaya and pineapple fruits (Barbosa et al., 2004; Corzo et al., 2012; Martin 2017). Tyrosine release determination with Folin-Ciocalteu reagent is sensitive and suitable for the detection of proteolysis. This analytical procedure can be easily reproduced by the manufacturers to verify the proteolytic potential and relative shelf-life of their cosmetic peel products.

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REFERENCES

Barbosa CM, Mozais HA, Delvivo FM, Mansur HS, De Oliveira MC, Silvestre MP (2004) Papain hydrolysates of casein: molecular weight profile and encapsulation in lipospheres. J. Sci. Food Agric. 84: 1891–1900. https://doi.org/10.1002/jsfa.1855

Bernard D, Méhul B, Thomas-Colignon A, Simonetti I, Remy V, Bernard MA, Schmidt R (2003) Analysis of proteins with cascinolytic activity in a human stratum corneum extract revealed a yet unidentified cysteine protease and identified the so-called “stratum corneum thiol protease” as cathepsin L2. J. Invest. Dermatol. 120: 592–600. https://doi.org/10.1046/j.1523-1747.2003.12086.x

Borgoño CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GJ, Sotropoulou G, Diamandis EP (2007) A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J. Biol. Chem. 282: 3640–3652. https://doi.org/10.1074/jbc.M607567200

Caubet C, Jonca N, Brattsand M, Guerin M, Bernard D, Schmidt R, Egérdul T, Simon M, Serre G (2004) Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5, kK5 and SCCE/KLK7, kK7. J. Invest. Dermatol. 122: 1235–1244. https://doi.org/10.1111/j.0022-202X.2004.22512.x

Choe Y, Leonetti F, Greenbaum DC, Locatelli F, Boygo M, Brömme D, Ellman JA, Craik BS (2006) Substrate profiling of cysteine proteases using a combinatorial peptide library identifies functionally unique specificities. J. Biol. Chem. 281: 12824–12832. https://doi.org/10.1074/jbc.M513331200

Commission Regulation (EU) No 655/2013 of 10 July 2013 laying down common criteria for the justification of claims used in relation to cosmetic products. Of J. L 190 11.07.2013: 31–34

Corzo CA, Waliszewski KN, Wolti-Chanes J (2012) Pineapple fruit bromelain affinity to different protein substrates. Food Chem. 133: 631–635. https://doi.org/10.1016/j.foodchem.2011.05.119

Folin O, Ciocalteu V (1927) On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 73: 627–650. https://doi.org/10.1016/S0021-9258(18)84277-6

Hale LP, Greer PK, Trinh CT, James CL (2005) Protease activity and stability of natural bromelain preparations. Int. Immunopharmacol. 5: 785–793. https://doi.org/10.1016/j.intimp.2004.12.007

Igarashi S, Takizawa T, Takizawa T, Yasuda Y, Uchiwa H, Hayashi S, Bysk H, Robinson JM, Yamamoto K, Bysk MM, Honkoshi T (2004) Cathepsin D, but not cathepsin E, degrades desmosomes during epidermal desquamation. Br. J. Dermatol. 151: 355–361. https://doi.org/10.1111/j.1365-2133.2004.06061.x

Igawa S, Kishibe M, Murakami M, Honma M, Takahashi H, Izzuka H, Ishida-Yamamoto A (2011) Tight junctions in the stratum corneum explain spatial differences in corneodesmosomal degradation. Exp. Dermatol. 20: 53–57. https://doi.org/10.1111/j.1600-0625.2010.01170.x

Ishida-Yamamoto A, Igawa S (2015) The biology and regulation of corneodesmosomes. Cell Tissue Res. 360: 477–482. https://doi.org/10.1007/s00441-014-2037-z

Lopes PS, Bass GW, Babu AR, Pinto CAS de O, Watanabe I, Velasco MVR, Kaneko TM (2008) In vitro safety assessment of papain on human skin: A qualitative Light and Transmission Electron Microscopy (TEM) study. Rev. Bras. Cienc. Farm. 44: 151–156. https://www.scielo.br/j/rbcf/a/phRqMMBDPTydshxmb9xnGFN/?format=pdf&lang=en

Lourenço CB, Ataide JA, Cefalí LC, Novais LCDL, Moriel P, Silveira E, Tambourgi EB, Mazza PG (2016) Evaluation of the enzymatic activity and stability of commercial bromelain incorporated in topical formulations. Int. J. Cosmet. Sci. 38: 535–540. https://doi.org/10.1111/jics.12308

Macno H, Mitsuhashi S, Sawai Y, Okonogi T (1959) Studies on Habu snake venom. 2. Enzymic studies on the proteinase of Habu snake venom. Jpn. J. Med. Sci. 3: 131–138. https://doi.org/10.1111/j.1348-0421.1959.tb00190.x

Martin H (2017) Protease Activities of Kiwifruit, Pineapple and Papaya Using Ovalbumin, Soy Protein, Casein and Bovine Serum Albumin as Substrates. J. Food Nutr. Res. 5: 214–225. https://doi.org/10.12691/jfnr-5-4-3

Naoe Y, Hata T, Tanigawa K, Kimura H, Masunaga T (2010) Bidimensional analysis of desmoglein 1 distribution on the outermost corneocytes provides the structural and functional information of the stratum corneum. J. Dermatol. Sci. 57: 192–198. https://doi.org/10.1016/j.jdermsci.2009.12.014

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Of J. L 342 22.12.2009: 59-209

Sim YC, Lee S-G, Lee D-C, Kang B-Y, Park K-M, Lee J-Y, Kim M-S, Chang I-S, Rhee J-S (2000) Stabilization of papain and lysozyme for application to cosmetic products. Biotechnol. Lett. 22: 137–140. https://doi.org/10.1023/A:1005670323912

Sremrmitzer C, Manzano-Szalai K, Willemsdorfer A, Starkl P, Pieper M, Künig P, Mädner M, Tschachler E, Reichhart U, Jensen-Jarolim E (2015) Papain degrades tight junction proteins of human keratinocytes in vitro and sensitizes C57BL/6 mice via the skin independent of its enzymatic activity or TLR4 activation. J. Invest. Dermatol. 135: 1764–1770. https://doi.org/10.1016/j.jid.2015.05.006

Traversa E, Machado-Santelli GM, Velasco MVR (2007) Histological evaluation of hair follicle due to papain’s depilatory effect. Int. J. Pharm. 335: 163–166. https://doi.org/10.1016/j.ijpharm.2007.01.020