Pharmacognosy Reviews [Phcog Rev.]
Vol 2, Issue 4, Jul-Dec, 2008
Page 266 - 270

PHCOG REV.
An official Publication of Phcog.Net

PHCOG REV.: Review Article

Potentiality of Papain as an Antiaging Agent in cosmetic formulation

Madhuri Banchhor[1], Swarnalata Saraf[2]*

[1] *Columbia Institute of Pharmacy, Raipur (C. G.), India*
[2]**Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C. G.), India*
*Author for Correspondence: * Swarnlatasaraf2007@rediffmail.com*

ABSTRACT

Safe proteolytic enzymes in cosmetic formulation can reduce the signs of aging by improving natural defense systems and by protecting the skin from the ravages of time and environmental attack. Cosmetic compositions comprising proteolytic enzymes removes the stratum corneum of the skin, thus enhances the penetration of other active components, including biological components, into the underlying layers of the skin. Study of proteolytic enzyme papain have become fertile and exitic field of basic as well as applied research because of its pharmaceutical and industrial applications. It has also been implicated in the design and synthesis of therapeutic target as well as in the food processing industry. This paper has discussed at length the cosmetic attributes of papain which could be utilized in cosmetic formulations as a potential antiaging and exfoliative agent.

Key Words: Antiaging, Exfoliant, Papain, Proteases, Cosmeceuticals

INTRODUCTION

Skin, the largest organ in the body that separates and protects the internal environment from the external one, can be considered to be a mirror of the soul. Skin aging can be attributed to photo aging (extrinsic) and chronological (intrinsic) aging which cause cumulative damage to building blocks of skin DNA, collagen, and cell membranes. Approximately 80 % of skin aging associated changes can be attributed to extrinsic factors such as ultraviolet (UV) light (1). Long-term sun exposure has been linked to many aging related skin changes, including loss of elasticity (*elastosis*), skin growths (*keratoacanthomas*), thinning of the skin, blotchy pigmentation, *actinic keratosis* (precancerous skin changes), skin cancers, wrinkling and scaling. Cosmeceuticals improve the functioning/texture of the skin by encouraging collagen growth by combating harmful effects of free radicals, thus maintaining keratin structure in good condition and making the skin healthier (2).

Exfoliants are the compounds which remove the dead cell layer (stratum corneum) of the skin, and are used, in the treatment of aging skin, photo damaged skin, acne, dry skin, and other skin conditions. Currently commercially available cosmetic formulations (exfoliants) for topical use typically include alpha hydroxy acids, beta hydroxy acids and retinoids, which may cause adverse reactions. Thus, there is a need for cosmetic preparations comprising exfoliants which are effective and non-irritating, especially in individuals with sun damaged skin. One preferred enzyme is papain, a proteolytic enzyme obtained from latex of unripe papaya (*Carica papaya*). Papain is wonderful exfoliant (3,4). It also helps reduce and attenuate freckles or brown spots due to exposure to sunlight, smoothing the skin and creating a healthier looking skin. Proteolytic enzymes have been used for skin peeling and soothing (5). It has been used and researched extensively for the positive effect it has on skin ulcers (6,7), as well as helping patients with severe burn wounds (8). Not only does it have great antibacterial and wound healing abilities (9) but is also extensively used in wound care (10) and is of particular help in removing damaged and dead skin. As an antiaging and exfoliative, products containing proteolytic enzymes like papain are commom choice since being natural it is devoid of skin problems associated with the use of synthetics and harsh chemicals. Here, we presented the cosmetic importance of papain that could be utilized for development of new potential antiaging cosmeceutical.

Papain

Papain was the first sulfhydryl enzyme discovered and has been the subject of mechanism and structural studies for many years (11,12). Papain consists of 212 amino acids stabilised by 3 disulfide bridges. Its 3D structure consists of 2 distinct structural domains with a cleft between them. This cleft contains the active site (Figure 1), which contains a catalytic triad that has been likened to that of chymotrypsin. The enzyme is very stable at neutral pH, even at elevated temperatures. It contains six sulfhydryls and one free cysteine, which is part of the active site. It hydrolyses amides of arginine, lysine readily and glutamine, histidine, glycine and tyrosine at reduced rates. Besides papain, papaya latex also contains chymopapain (13), and papaya peptidase A now known as Caricain. Another fraction has been detected that demonstrated activity against the glycine ester but not against Bz-Arg-Pna, is papaya peptidase B. This enzyme is now called proteinase IV or glycyl endopeptidase (14). The mechanism by which papain breaks peptide bonds involves deprotonation of Cys-25 by His-159. Asn-158 helps to orient the imidazole ring of His-159 to allow this deprotonation to take place. Cys-25 then performs a nucleophilic attack on the carbonyl carbon of a peptide backbone. This frees the amino terminal of the peptide, and forms a covalent acyl-enzyme intermediate. The enzyme is then deacylated by a water molecule, and releases the carboxy terminal portion of the peptide.

Other papain-like cysteine proteases includes, bromelain, human cysteine cathepsins (B, H, L, S, C, K, O, F, V, X, W), and parasite proteases cruzain and falcipains, that have been

Table 1. Proteolysis of various IgG species with non-immobilized and Immobilized Papain as reported in the literature

Species IgG	Digestion Method	Purification Method
Human		
Monoclonal IgG1 Polyclonal IgG Anti-*Schistosoma mansoni* soluble egg antigens Cohn fraction II	Papain	DEAE Cellulose
	Crystalline Papain, Papain	DEAE Cellulose, Protein A
	Immobilized Papain	Immobilized Antigen
	Mercuripapain	DEAE
Mouse		
IgG2a from myeloma	Papain	Protein A
Polyclonal IgG	Crystalline Papain, Papain, Mercuripapain	DEAE, Protein A
Rat		
Monoclonal IgG1, IgG2a, IgG2b	Mercuripapain	DEAE
Polyclonal IgG	Papain	DEAE
Polyclonal IgG subclasses	Papain	DEAE Cellulose or Protein A
IgG subclasses	Papain or Mercuripapain	Electrophoresis used to monitor digestion
Rabbit Polyclonal IgG	Immobilized Papain, Crystalline Papain, Mercuripapain	Protein A, CM Cellulose
Sheep		
Antidigoxin	Crystalline Papain	gel filtration, affinity chromatography
Polyclonal IgG	Crystalline Papain	DEAE Cellulose
Goat	Crystalline Papain, Papain, Mercuripapain	DEAE, Protein A, ion exchange
Horse, Cow, Guinea Pig, Chicken	Crystalline Papain	DEAE

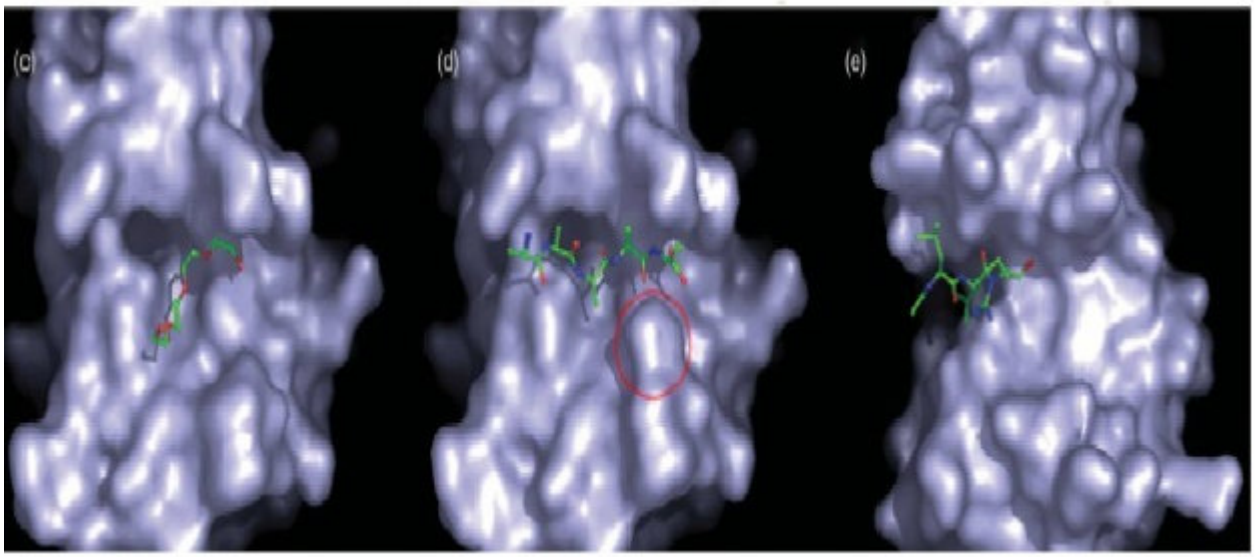

Figure 1: The Binding Surface of PEG, the Peptide Fragment in SPE 31 & Leukopeptin in papain is shown from (c-e). The binding surface is represented by red circle.

characterized as key enzymes in many biological and pathological events (15, 16). The major source of these proteases are microorganisms but Plant proteases have not been extensively examined and found to be occurring in plant seeds and beans including Wheat (17), Barley (18), Sorghum (19), Corn (20), Potato tubers (21), Mung Beans (22), Soybean (23), Sunflower seeds (24), Rice seeds (25), Moringa Oleifera seeds (26) and Lotus seeds (26).

Papain Improves Collagen Content

Collagen and elastin production within the aging skin slows down and therefore the skin loses its firmness and elasticity. It does not snap back as readily as it used to and it is much thinner and susceptible to damage. Type-I collagen is the main structural component of the extra cellular matrix (ECM) which is known to perform a pivotal function in the maintenance of the structure of the skin dermis. Ageing of

PHCOG REV.
An official Publication of Phcog.Net

the skin is primarily related to reductions in the level of Type-I collagen (27). Papain treated samples shows a slight higher collagen content, which might be due to effect of papain on proteolysis of collagen. This suggests that papain might improve the skin firmness and elasticity.

As hydrolyting agent

The age related increase in concentration of oxidized proteins is partly due to cell's decreased capacity to degrade them. Normal human fibroblasts undergo a limited number of divisions in culture and progressively they reach a state of irreversible growth arrest, a process termed as replicative senescence. Senescent fibroblasts are viable and fully functional however they exhibit several morphological and biochemical changes as compared to their young counterparts (28). They have irregular shape, do not line up parallel arrays, have larger nuclei, shorter telomerase, altered gene expression, and accumulate damaged proteins and connective tissue. Accumulation of damaged proteins and connective tissue gives lifeless appearance to the skin. Papain is very powerful in hydrolyzing fibrous protein and connective tissue (29). Kang and Rice reported that papain solubilized 15 % of connective tissue protein; Scientific evidences shows that this may improve the appearance of the skin.

Skin Hydration

The water holding capacity value also increased with increasing concentration of papain, this might be due to increased PH. Increased PH improved the water holding capacity (30). This improves the stratum corneum hydration level and water holding capacity which is thought to locate 90-100% of water intracellulary (31). One may presume that hydrating power of papain gives healthier looking skin.

Papain in Immunology

Papain has been used successfully to overcome the allergies associated with certain diseases. The proteins can passively diffuse into the bloodstream and provoke an all-out immune response. Indeed one study showed papain to have significant analgesic and anti-inflammatory activity. Papain encourage the complete proteolysis of offending proteins found in wheat, soy and milk products in the bowel that accounts for its anti-allergic action so often seen in unpredictable conditions like Autism and Leaky Gut Syndrome. According to research conducted at Case Western Reserve University, Cleveland in the U.S. enzyme combinations including papain may be acting as immune system regulators in autoimmune conditions like MS (32). Papain breaks and clears out circulating immune "clots" which is responsible for a slowing in the progressive myelin deterioration seen in MS (33). These phenomenons improve circulation, enhance tissue repair and bring required nutrients to disease sites whilst waste products removal aids immune response.

Immobilized Papain is convenient for producing Fab and Fc fragments from a variety of IgG species. By following the interaction of Fc receptors and immunoglobulins, the phenomenon of cell surface recognition mediation and activation can be studied (34). Fab fragments are useful in immunohistochemical studies. Proteolysis of various IgG species with non-immobilized and Immobilized Papain is reported in the literature (Table 1)

Skin Rejunavation

Young skin has naturally a faster rate in which the skin produces new skin cells. Aged skin cells do not regenerate quickly. Therefore, dead skin cells do not shed as quickly leading to rough dull looking skin. Papain degrades damaged, misfolded and potentially harmful proteins and provides free amino acids required for the synthesis of new proteins (35). This helps in tissue repair and encourage the new growth of skin cells which might be associated with the rejunvation effect of papain on skin.

As Adjunct in the Treatment of Skin Cancer

Chronic and possibly acute exposure to UV radiation increases the risk of skin cancer (36). Animal studies indicate that papain in combination with other enzymes like trypsin and chymotrypsin have astonishing radio-protective and antitumour effects in mice (37). The Fab fragment prepared from purified mAb using papain proteolysis provides a basis for further genetic manipulation of the binding site to improve tumor targeting (38). Papain has found value as an adjunct in the treatment of cancers of the skin and even to mitigate the effects of radiotherapy and chemotherapy. It supports the skin by increasing the absorption of nutrients like vitamin C and E and essential fatty acids from the diet. Anyone with dull skin, eczema, acne, wrinkles, stretch marks, fungal infections like athlete's foot and even skin cancer can benefit from papain supplementation.

Papain as an Exfoliant

Skin is living tissue, but as it ages it goes through a replacement cycle. It keratinizes and forms the horny layer - also referred to as the Stratum corneum. Exfoliation is the removal of dead skin cells from the uppermost part of the skin. The dead cells shed naturally over a period of time, but should the cells not naturally and totally shed, a build-up of dead skin cells results, which then forms dull ashy patches on the skin, or can cause an all-over lack-luster look. To restore the skin's appearance manual or chemical removal of these dead cells are necessary. Accumulation of abnormal proteins in cells impedes cellular function and can lead to cellular apoptosis (39).

Chemical exfoliation helps loosen the bond between dead skin cells and new, so that they come off more easily. Products containing certain enzymes like papain and bromelin are common choices in this category since harsh chemicals can cause problems to the living cells as well. One particularly preferred form of papain is Linked Papain (papain carbomer, as described in CTFA, the International Cosmetic Ingredients Dictionary) in which papain is covalently immobilized to 1% polyacrylic acid. In a preferred embodiment, the enzyme is present in the formulation in an amount between 2% and 5%. The ability of papain to act as an exfoliant allows enhancement of penetration of any desired medicinal agent beneficial to the skin, such as, for example, biological additives and moisturizers. The activity of papain is greatest at a pH of 6, although the enzyme retains about 75% of its activity between pH 5 and 7. The cosmetic compositions preferably have a pH that is basic relative to the pH of skin, and preferably have a pH of about 7.0 (40).

Compositions of topical use may be combination of enzymes with at least one/two/three/more, biological additive that are preferably selected from the group consisting of *Echinacea angustifolia* extract, *mimosa tenuiflora* extract, *hydrocotyl* extract, *gingko biloba* extract, tea tree oil, *Matricaria chamomila* extract, *Hypericum perforatum* extract, and *Aloe barbedensis* extract, *caterndiele* extract.

Papain in Chemical debridement

The exfoliative property of papaya has extensively been used in medical conditions to debribe the skin from abnormal skin growth and skin diseases. Chemical debridement is the application of a topical agent (enzymatic or nonenzymatic), which chemically disrupts or digests devitalized extra cellular material present in the wound. Most of the research in the field of chemical debridement has focused on the use of enzymes with proteolytic action. Theoretically, the combination of chemical agents, which are nonenzymatic and enzymatic, rather than a single enzyme preparation may offer additional efficacy in the debridement process. Papain-Urea is the combination of a papain and a chemical agent, which denatures nonviable protein (urea). Papain-Urea Chlorophyllin Copper Complex Sodium is the papain, chemical activator-urea, and non-specific inhibitor of wound digestion products-chlorophyllin copper complex sodium. Both complexes are suggested for debridement of necrotic tissue and liquification of slough in acute and chronic lesions The published data suggest some improvement in pressure ulcer healing. Papain is also used as an ingredient in various enzymatic debriding preparations, notably Accuzyme (41), Panafil (42), Kovia (43), Ethenzyme (44), Galadase (45) and Ziox (46).

Papain as Antiaging

Aging skin can regain a youthful glow with their regular exfoliation. The exfoliation treatment is pleasant, relaxing and radiant. Exfoliation also helps enhances the elimination of waste products from the skin and epidermis layer. It also stimulates the blood circulation in the skin, which nurtures the new skin cells improving the skin's elasticity and firmness. According to recent work papain also acts as a powerful shield against the damaging effects of radiation on skin. One may presume that the collective effects (Hydration. Hydrolyting, Rejunvation, Immune response, debidment and exfoliative property) of papain on skin reduces the signs if aging skin.

CONCLUSION

Extrinsic factors reduces the body's ability to protect itself so there is an ideal opportunity to do more to promote healthy, youthful looking skin by using proteolytic enzymes in cosmetic formulations. Papain is well known to treat several disorders, but new research is showing that it is an excellent exfoliant, possessing a protective antioxidant action against UV radiation, good hydrolyzing and hydrating property, improves the functionality of collagen and thereby reduces the signs of aging. The inventions relates an enzyme combination for increasing the function of proteases safe to the skin and suggest to use papain in combination with one or more biological additives to increase a keratin exfoliation effect and, at the same time, maintain skin safety. For a more fundamental antiaging formulation, not only the exfoliation of keratin on the epidermis, but also the production and differentiation of new cells in the granular layer and basal layer of the skin, should be activated such that skin turnover can be promoted and skin homeostasis can be maintained. The combination of papain with other botanicals may prove to have considerable value as Antiaging cosmeceutical and apart from its internal use, papain will probably come into extensive use as a potential Antiaging and Exfoliative agent in cosmetic formulations.

ACKNOWLEDGEMENT : One of the authors thank University grant commission [F. No.32-133/2006(SR)] for financial support.

REFERENCES

1. D.E Godar. UV doses worldwide. *Photochem Photobiol.* 81: 736-749 (2005).
2. H. Dureja, D. Kaushik and M. Gupta. Cosmeceuticals: An emerging concept. *Indian J Pharmacol.* 37: 155-159 (2005).
3. B. Laidet and M. Letourneur. Enzymatic debridement of leg ulcers using papain. *Ann Dermatol Venereol.* 120: 248 (1993).
4. W.C. Beck and D. Gent. Enzymatic debridement with topical papain. *Guthrie Clin Bull.* 23: 149-51 (1953).
5. L. M.Lods, C. Dres and C. Johnson. The Future of Enzymes in Cosmetic. *International Journal of cosmetic science.* 22: 85-94 (2000).
6. H. Hewitt, S. Whittle and S. Lopez. Topical use of papaya in chronic skin ulcer therapy in Jamaica. *West Indian Med J.* 49: 32-33 (2000).
7. J.E. Morgan. Topical therapy of pressure ulcers. *Surg Gynecol Obstet.* 141: 945-7 (1975).
8. I.F. Starley, P. Mohammed, G. Schneider and S.W. Bickler. The treatment of paediatric burns using topical papaya. *Burns*. 25: 636-9 (1999).
9. G. Dawkins, H. Hewitt and Y. Wint. Antibacterial effects of *Carica papaya* fruit on common wound organisms. *West Indian Med J.* 52: 290-292 (2003).
10. B. Pieper and M.H. Caliri. Nontraditional wound care: A review of the evidence for the use of sugar, papaya/papain, and fatty acids. *J Wound Ostomy Continence Nurs.* 30: 175-83 (2003).
11. J. Drenth, J.N. Jansonius, R. Koekoek and B.G.Wolthers. The crystal structure of papain C. I. Two dimensional fourier synthesis. *Adv. Protein Chem.* 25: 79-115 (1971).
12. A.N. Glazer and E.L. Smith. Papain and other sulfhydryl proteolytic enzymes. In: "The Enzymes" (Boyer PD Eds.) Vol 3. Academic Press New York; 501-546 (1971).
13. E.F. Jansen and A.K. Balls. Chymopapain: a new crystalline proteinase from papaya latex. *J. Biol. Chem.* 137: 459 (1941).
14. D.J. Buttle, A.A. Kembhavi and S. Sharp. Affinity purification of the novel cysteine proteinase papaya proteinase IV and papain from papaya latex. *Biochem. J.* 261: 469- 476 (1989).
15. F. Lecaille Kaleta and J. Brömme. D. *Chem. Rev.* 102: 4459-4488 (2002).
16. D. Brömme and J. Kaleta. *Curr. Pharm. Des.* 8: 1639-1658 (2002).
17. J. Skupin and J. Warchalewski. Isolation and properties of protease A from wheat grain. *J Sci Food Agric.* 22:11-15 (1971).
18. W.C. Bunger. Multiple forms of acidic endopeptidase from germinated barley. *Plant Physiol.* 51:1015-1021 (1973).

19. G.K. Garg and T.K. Virupaksha. Acid protease from germinated sorghum, Purification and characterization of the enzyme. *Eur J Biochem.* 17:4-12 (1970).
20. M. Abe, S. Arai and M. Fujimaki. Purification and characterization of a protease occurring in endosperm of germinating corn. *Agric Biol Chem.* 41: 893-899 (1977).
21. N. Kitamura and Y. Muruyama. Cysteine endopeptidase activity in sprouting potato tubers. *Agric Biol Chem*. 49: 1591-1597 (1985).
22. B. Baumgartner and M.J. Chrispeels. Purification and characterization of vicilin peptidohydrolase, the major endopeptidase in the cotyledons of mung bean. *Eur J Biochem.* 77: 223-233 (1977).
23. J. Weil, A. Pinsky and S. Grossman. The proteases of soybean. *Cereal Chem.* 43: 392-399 (1966).
24. P. Walde, PL. Luisi and S. Palmieri. Proteolytic activity in sunflower seeds (*Helianthus annulus L*). *J Agric food Chem*. 32: 322-329 (1984).
25. S. Arai, H. Hosoyama and K. Abe. Gibberellin induced cysteine proteinase occurring in germinating rice seeds and its specificity for digesting oxidized insulin B-chain. *Agric Biol Chem*. 52: 2957-2959 (1988).
26. M.U. Dahot, S.A. Ali and A.R. Memon. Proteolytic enzymes of *Moringa oleifera* seeds. *J Pharm Pb Univ* Lhr. 6: 1-9 (1985).
27. J. Eunsun, L. Jongsung and B. Jihoo. Effect of *camellia japonica* oil on human type I procollagen production and skin barrier function. *Journal of ethnopharmacology*. 112: 127-131 (2007).
28. J. Campisi. *Exp. Gerantol.* 36: 607-618 (2001).
29. C.K. Kang and E.E. Rice. Degradation of various meat fractions by tenderizing enzymes. *Journal of Food Science*. 35: 563-565 (1970).
30. C.A. Milesand and R.A. Lawrie. Relationship between PH and tenderness in cooked muscle. *Journal of Food Science.* 35: 325 (1970).
31. L. Norlen. Stratum Corneum Keratin Structure, Function and Formation- A Comprehensive Review. *International Journal of Cosmetic Science.* 28: 397-425 (2006).
32. P.V. Lehmann. Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. *Nature.* 358: 155-157 (1992).
33. K. Ransberger and W. Van Schaik. Enzymtherapie bei multipler sklerose. *Der Kassenarz.* 41: 41-45 (1986).
34. J. Rousseaux. Optimal conditions for the preparation of Fab and F(ab') fragments from monoclonal IgG of different rat IgG subclasses. *J. Immunol. Meth.* 64:141-146 (1983).
35. A Schaller. A cut above the rest: the regulatory function of plant proteases. *Planta.* 220: 183-197 (2004).
36. M.S. Ashawat, A. Gupta, S. Saraf and S. Saraf. Role of Highly Specific and Complex Molecules in Skin Care. 3: 191-195 (2007).
37. G. Barth and H. Graebner. Zurfrage der therapie des letalen Strahlenschadens. *Deutsche Medizinische Forschung.* 2: 143-44 (1963).
38. K. Ute, O. Lise-Lotte and M. Carlos. Structure and Molecular Interactions of a Unique Antitumor Antibody Specific for *N*-Glycolyl GM3*. *The Journal Of Biological Chemistry.* 279: 5597–5603 (2004).
39. M.Y. Sherman and A.L.Goldberg. Cellular defenses against unfolded proteins: A cell biologist think about neurodegerative diseases. *Neuron*. 29: 15-32 (2001).
40. V. Jacob. Cosmetic Compositions Comprising Exfoliating Enzymes And Uses Thereof WO 2001/066066-20010913 (http://www.wipo.int/pctdb/en/wo.jsp?wo=2001066066&IA=US2001040227&DISPLAY=STATUS , Accessed in July 2008)
41. Accuzyme® package insert. Healthpoint®. San Antonio. Texas (July 2002).
42. Panafil® package insert. Healthpoint®. San Antonio. Texas (July 2002).
43. Kovia™ package insert. Stratus Pharmaceuticals. Inc. Miami. Florida. (2001)
44. Ethezyme 830™ package insert. Ethex Corporation. Saint Louis. Missouri. (May 2001).
45. Gladase® package insert. Smith & Nephew. Inc. Largo Florida (2001).
46. Ziox™ package insert. Stratus Pharmaceuticals. Inc. Miami. Florida. (2001).

****