What is Dental Plaque?
Dental plaque is defined as the soft deposits that form the bio film adhering to the tooth surface. Plaque is composed of organic, inorganic materials derived from saliva, gingival crevicular fluid & bacterial products.

The organic constituents of plaque include polysaccharides, proteins, glycoproteins & lipid material.

The inorganic constituents of plaque include primarily of calcium & phosphorus & traces of sodium, potassium.

Types of Dental Plaque:
Dental plaque is broadly classified based on its position as:

a) Supragingival plaque: is found at or above the gingival margin
b) Sub gingival plaque: is found below the gingival margin.

The process of plaque formation can be divided into three phases:

a) Formation of Dental Pellicle
b) Initial colonization of bacteria
c) Secondary colonization & plaque maturation.

What is Dental Pellicle?
Pellicle is a glycoprotein, derived from components of saliva and crevicular fluid as well as from bacteria and host tissue cell products and debris. Pellicle is formed on all surfaces of the oral cavity, including all tissue surfaces as well as surfaces of teeth and fixed and removable restorations if any.

Pellicle functions as a protective barrier but pellicle provides a substrate on which bacteria progressively accumulate to form Dental Plaque. i.e.…
Pellicle provides a medium or base on which bacteria in the oral cavity attach. Pellicle gets easily stained & may display many colors ranging from white to dark brown due which the teeth appear discolored.

Mechanism of Pellicle formation:

The enamel surface has predominance of negatively charged phosphate groups that interact directly or indirectly with positively charged components of salivary & crevicular fluid macromolecules. Within few hours bacteria are found on the pellicle. These initial bacteria colonizing the pellicle-coated tooth surface are predominantly gram-positive microorganisms such as Actinomyces viscosus & Streptococcus sanguis.
These initial colonizers adhere to the pellicle through specific molecules termed adhesins, on the bacterial surface that interact with receptors in dental pellicle.

For example, cells of Actinomyces viscosus possess fibrous protein structures called Fimbriae that extend from the bacterial cell surface. Protein adhesins on these Fimbriae specifically bind to proline-rich proteins that are found in dental pellicle, resulting in the attachment of the bacterial cell to the pellicle-coated tooth surface.

The plaque mass then matures through the growth of attached species as well as colonization & growth of additional species.

Plaque may be readily visualized on teeth after 1 to 2 days with no oral hygiene measures. Plaque is white, grayish or yellow & has a globular appearance.

With good oral hygiene practices one can remove this pellicle & plaque layers. There are different types of treatments which are extensively aim at removal of plaque & stains; patient friendly methods are practically more acceptable than clinical treatment.

Certain chemical toothpastes remove stains by altering the surface environment of the teeth thereby inhibiting the adherence of plaque.
For example hydrogen peroxide in the toothpaste acts by releasing nascent oxygen, abrasive characteristic of baking soda or silica is said to cleanse the tooth surface.

Some toothpaste contains specific enzymes which are believed to remove plaque, stain & thereby improving the gingival health. These toothpastes are biologically compatible with the tooth surface & the surrounding soft tissues.

What is Proteins?
Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds.

What are enzymes?
An enzyme is a protein that catalyzes and regulates all biochemical reactions within the body. Enzymes function by temporarily binding to reactants (precursors) and lowering the amount of activation energy needed to form the product, thus speeding up the reaction.

Types of Enzymes:

a) Metabolic Enzymes: these enzymes drive our metabolism and provide the basic necessities of the body such as hearing, seeing, feeling, thinking and moving. They are found in every living cell in the body and speed up the reactions of energy production and detoxification.
b) Digestive Enzymes: are produced within the digestive system and are secreted to break down the food substances to produce nutrients and wastes. The following enzymes are Digestive Enzymes:
    - Pepsin
    - Trypsin
    - Lipase
    - Protease
        - ✓ Papain
        - ✓ BROMELAiN
    - Amylase
c) Food Enzymes: are found within the food substance itself and are introduced into the body through the foods we eat.

What are Proteases?
Proteases are enzymes responsible for breaking down protein consumed in the diet into absorbable parts. Protease enzymes are useful supplements for digestion, anti-inflammatory properties, and immune, cardiovascular, and general nutrition support. Proteases are also known as proteolytic enzymes.

What are Proteolytic enzymes?

Proteolytic enzymes (or proteases) refer to the various enzymes that digest protein (break down into smaller units). These enzymes include the BROMELAiN (pineapple enzyme), Papain (papaya enzyme), pancreatic proteases chymotrypsin and trypsin, fungal proteases.

A proteolytic enzyme conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain.

BROMELAIN:

BROMELAiN is a proteolytic enzyme derived from the ripe and unripe fruit, as well as stem and leaves of Pineapple (Ananas comosus). Surface stains stick to the Pellicle first so BROMELAiN helps break down the protein pellicle on the tooth surface.

BROMELAiN have been used extensively both commercially as meat tenderizers and medically to assist both the digestive process and the immune system, reduce inflammation, and support the cardiovascular system and general health. It acts by blocking pro-inflammatory metabolites that propagate the inflammatory process. It has been used to treat sports injuries, traumas, arthritis, and other forms of inflammation. The unit of measure of activity is PU (Papain unit) or GDU (Gelatin Digesting Units).

BROMELAiN is a mixture of sulfur-containing protein, digesting enzymes called Proteolytic enzymes and several other substances in smaller quantities. The two main enzymes are as follows:

a) Stem BROMELAiN
b) Fruit BROMELAiN.

Source:

BROMELAiN is present in all parts of the Pineapple plant, but the stem is the most common commercial source, presumably because it is readily available after the fruit has been harvested. Pineapples have had a long tradition as a medicinal plant among the natives of South and Central America.

Surface stains stick to the Pellicle first, so BROMELAiN helps to break down the protein pellicle on the tooth surface.

Authoritative body in Germany called Commission E (similar to U. S. Food and Drug Administration) approved the use of BROMELAiN.

Both Papain & BROMELAiN belong to the group of proteases. “Proteases are catalytic and their function is to hydrolyze the peptide bonds of Proteins (Pellicle)”.

Papain & BROMELAiN are hydrolyzing the pellicle and thereby preventing the bacteria from adhering to the tooth surfaces.

References on BROMELAiN:

There are numerous studies which demonstrate that BROMELAiN is a proteolytic enzyme:

1) Clin Immunol. 2005 Jun 1; [Epub ahead of print] : Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease.
Hale LP, Greer PK, Trinh CT,Gottfried MR.
Department of Pathology, DUMC 3712, Duke University Medical Center, Durham, NC 27710, USA.

BROMELAiN is a mixture of proteinases derived from pineapple stem that is marketed in health food stores as a "digestive aid".

DETAILED DESCRIPTION OF THE INVENTION

The invention provides some improvements introduced into the object of the Spanish patent application P9401832 relating to a whitening toothpaste which is anti-plaque and anti-tartar of low abrasion, consisting of the development of a whitening toothpaste which is anti-plaque and anti-tartar of low abrasion and which is suitable for treating sensitive teeth, that is characterized by the lack of detergents and whose formulation comprises:

Component % by weight with respect to total
Papain 0.1-1
Xylitol 5-10
abrasive system based on silica 16-18
a buffer consisting of:
i) tetra-potassium pyrophosphate 4-5
ii) potassium dihydrogen phosphate 1-3
additives/recipients s.q.
[s.q.: sufficient quantity to make up 100%]

Papain is a proteolytic enzyme with low specificity, suitable for cleaning the salivary protein plaque that has a whitening effect on the tooth surfaces. A valued enzymatic extract can be used, whose proteolytic activity has been adjusted to a constant value, from a latex obtained from unripe fruits of the Carica papaya (papaya). Papain hydrolysed proteins, amides and amino acid esters, and its activity is associated with the presence of free sulphyl (--SH) groups in its active center. To produce the toothpaste of this invention, an enzymatic extract is preferably used that contains papain with a proteolytic activity of, approximately, 6,000 U-USP/mg [Units of United States Pharmacopea]. The optimum working temperature for this enzyme lies between 40 and 65° C. Due to the low substrate specificity, papain can act on multiple protein products, in over pH range of from 3 to 9. Outside these values the enzyme is inactive. In general, the toothpaste that contains papain conveniently has a pH near to neutral, that is to say, approximately 7, with a view to guaranteeing the activity of the enzyme without de-mineralising the enamel. Papain has a cleaning action on the bacterial plaque and tartar by breaking the glycoprotein and lipoprotein chains from the saliva fluid as well as

acting on the bacterial excretory activity of mucylaginose substances (capsule) that attach themselves to the enamel allowing colonisation by bucal flora (bacterial plaque) and the fixing of calcium salts to these structures that act as supports (tartar). Therefore, by attacking these structures, the processes associated with plaque and tartar excess is improved such as tooth decay and periodontal disease.

Papain:

Papain is naturally occurring enzyme, obtained from latex of fruit of PAPAYA (Carica papaya Latex). The enzyme Papain is obtained by drying papaya latex by suitable method viz; sun drying, tray drying, spray drying etc. spray drying gives a fine powder, which dissolves easily in water to provide a solution of high proteolytic activity.
The crude Papain obtained from the crude latex is extracted, filtered, concentrated and finally spray dried into a very fine & soluble powder. All processing of Papain is carried out in aseptic conditions to suit for the pharmaceutical applications.

Papain is a mixture of proteolytic enzymes derived from the juice of the unripe fruit of the tropical plant Carica papaya, commonly known as papaya. Papain hydrolyzes proteins to form oligopeptides and amino acids. Papain also contains the proteolytic enzyme chymopapain which differs from Papain in electrophoretic mobility, solubility and substrate specificity. The molecular weight of chymopapain is approximately 27,000 daltons. Papain is used as a digestive aid. It is also used as a meat tenderizer. Papain has putative anti-inflammatory activity. The activity of Papain is expressed in Papain units or PU. The assay of Papain activity is based on the hydrolysis of casein.

Mode of Action:
Papain is a proteolytic (protein degrading) enzyme, characterized by the ability to hydrolyze large proteins into smaller peptides and amino acids. Its broad substrate specificity and ability to hydrolyze small peptides as well as large proteins make Papain an ideal enzymatic supplement.
Papain has a mild, soothing effect on the stomach and is an excellent aid in protein digestion.

References on Papain:

1) J Dent Child (Chic). 2007 May-Aug;74(2):93-7. Links (The Journal of dentistry in Children)

Effect of a papain-based gel for chemomechanical caries removal on dentin shear bond strength.

Lopes MC, **Mascarini RC**, **da Silva BM**, **Flório FM**, **Basting RT**.

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PURPOSE: The aim of this study was to assess the shear bond strength of an adhesive restorative system on sound and demineralized dentin after the use of a papain-based agent. METHODS: Forty human dentin slabs were randomly distributed into 4 groups: (1) sound dentin slabs that received an application of papain-based gel (N=10); (2) sound dentin slabs that did not receive a papain-based gel application (N=10); (3) demineralized slabs that received an application of the agent (N=10); and (4) demineralized slabs that did not receive an application of the agent (N=10). After manual excavation and use of the chemomechanical agent, the slabs were restored with a total etch adhesive system and microhybrid resin composite. The test specimens were individually stored in a damp environment for 7 days, and the shear bond strength test was performed using a universal test machine at a speed of 0.5 mm/min. The fragments were observed under a stereoscopic microscope to assess the fracture mode. RESULTS: Fracture mode assessment showed adhesive and

cohesive type fractures in resin for all the groups. The analysis of variance and the Tukey test showed that there were no differences in the shear bond strength means among the groups (P>.05). CONCLUSIONS: The use of a papain-based gel to remove dental caries did not interfere in the bond strength of restorative materials to dentin.

PMID: 18477426 [PubMed - in process]

2) J Clin Pediatr Dent. 2005 Winter;30(2):115-9. Links (The Journal of clinical Pediatric Dentistry)

Papain gel: a new chemo-mechanical caries removal agent.

Bussadori SK, **Castro LC**, **Galvão AC**.

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The chemo-mechanical caries removal method has been a solution for treatment of patients seeking alternatives to conventional methods. Among different kinds of chemo-mechanical caries removal systems, Papacarie--a papain gel--was found to be easy to manipulate, simple and cheap, as well as effective in removing infected tissues.

PMID: 16491964 [PubMed - indexed for MEDLINE]

3) **According to Cancer Chemotherapy & pharmacology:** 2001 Jul; 47 Suppl: S10-5..
Concludes that BROMELAiN & Papain are proteolytic enzymes. Thus BROMELAIN & PAPAIN the natural proteolytic enzymes derived from pineapple & papaya respectively are effective in removing enamel Pellicle thereby preventing bacteria, stain accumulation on tooth surface.

4) **Oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood.**

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Therapy with oral proteolytic enzymes (OET) with combination drug products containing papain, bromelain, trypsin, and chymotrypsin has been shown to be beneficial in clinical settings such as radiotherapy-induced fibrosis, bleomycin pneumotoxicity and immunosuppression in cancer, all of which are nowadays known to be accompanied by excessive transforming growth factor-beta (TGF-beta) production. It has been demonstrated that proteolytic enzymes reduce TGF-beta levels in serum by converting the protease inhibitor alpha2 macroglobulin (alpha2M) from the "slow" form into the "fast" form, whereby the "fast" form binds and inactivates TGF-beta irreversibly. In this study we have investigated the effect of OET on the concentration of TGF-beta1 in serum of patients with rheumatoid arthritis (RA) (n = 38), osteomyelofibrosis (OMF) (n = 7) and herpes zoster (HZ) (n = 7). Seventy-eight healthy volunteers served as controls. TGF-beta1 levels in serum were assessed by enzyme-linked immunosorbent assay (ELISA). We have demonstrated that in healthy volunteers and in patients there exists a correlation between active and latent TGF-beta1 in serum ($r=0.8021$; $P<0.0001$). Treatment with OET had no significant effect on TGF-beta1 concentration in healthy volunteers or patients with a normal level of TGF-beta1. In patients with elevated TGF-beta1

concentration (> 50 ng/ml serum), OET reduced TGF-beta1 in RA ($P < 0.005$), in OMF ($P < 0.05$) and in HZ ($P < 0.05$). Conclusion: These results support the concept that OET is beneficial in diseases characterized in part by TGF-beta1 overproduction.