ORIGINAL ARTICLE

Formulation of Antimicrobial Toothpaste Containing Bromelain Enzyme

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ABSTRACT

Introduction: Incorporating bromelain into toothpaste is crucial due to its exceptional bioactive properties, which include anti-inflammatory and antimicrobial effects. These attributes enable bromelain to promote faster healing of oral ulcers, significantly improving gum health and overall oral hygiene. With the growing consumer demand for natural ingredients in toothpaste, the medicinal benefits of bromelain make it a promising additive. A key challenge lies in developing toothpaste formulations that maintain the stability of the bromelain while ensuring effective antimicrobial activity. Therefore, this study aims to address the issue of bromelain stability in toothpaste formulations and determine its antimicrobial potential. The bromelain toothpaste was developed to meet all the necessary criteria for maintaining oral freshness and preventing tooth decay caused by bacteria. It was then compared to commercially available toothpaste. **Materials and methods:** The toothpaste developed was characterized in terms of pH value, moisture content, viscosity and antimicrobial activity. **Results:** The results indicated that the component extracted from pineapples possesses antimicrobial properties. The color of the substance is white with a slight yellowish tint. It has a smooth texture and a pH value of 8.63 ± 0.05. A lower viscosity value results in better spreadability of the toothpaste. The assessment of the antimicrobial effects on Staphylococcus aureus indicates that the prepared bromelain toothpaste demonstrated significant activity, as evidenced by a zone of inhibition (ZOI) of 18 ± 0.5 mm. This study demonstrates that the development of toothpaste formulation, is equally effective in terms of results when compared to commercially available formulations. **Conclusion:** This study demonstrates that the development of toothpaste formulation containing bromelain, is equally effective in terms of results when compared to commercially available formulations.
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INTRODUCTION

Enzymes are widely regarded as the most crucial components in industrial processes. Enzymes constitute approximately 70% of the total global commercial products. It is extensively utilized in the food, pharmaceutical, and certain detergent industries. Bromelain is an enzyme and one of the protease enzymes present in the pineapple plant. The pineapple is the predominant consumable member of the Bromeliaceae family [1], is commonly referred to as "nanas" throughout southern Asia, particularly in Malaysia. The main component that contributes to the high value of pineapple is Bromelain which is a proteolytic enzyme and has been scientifically identified as a therapeutic agent. Bromelain has been proven to have great oral bioavailability and has been found to be safe even at high doses over extended periods [2].

Toothpaste primarily serves the purpose of maintaining oral hygiene by acting as an abrasive agent that prevents

the buildup of dental plaque and food debris on the teeth. It also assists in the elimination or masking of bad breath, and contains active ingredients like fluoride that help in the prevention of tooth and gum diseases. The toothbrush primarily relies on mechanical action, assisted by the additives included in toothpaste, to carry out the majority of the cleaning process [3-5]. Typical toothpaste contains detergent, binder, colorants, flavoring agent, fluoride, tartar control agent, desensitizing agent, abrasive, humectant and water [6]. Cleansing abrasive materials are the substances that protect the surface of teeth without scratching it. This abrasive is effectively removing surface stains from your teeth and make them appear whiter. A study done by a group of researchers indicated that both toothpastes had equivalent efficacy in removing extrinsic stains; however, the enzymatic paste exhibited superior outcomes compared to the abrasive toothpaste [7]. On the other hand, it was reported commercial toothpaste typically includes sodium lauryl sulfate, which has the potential to cause irritation and inflammation when brushing [8]. Different bleaching agents had different effects on dental enamel. Considering that the appropriate concentration of bleaching gel, the initial and final roughness, the combination of whitening toothpaste and bleaching agent could be changes by the usage of different ingredients.

Dental caries, if not properly monitored in any age group, can lead to common health issues by damaging tooth tissues and compromising the tooth surface. In normal practice, the source of carries which is contribute by the food consume can be removed when brushing with toothbrush with a toothpaste. The toothpaste with Bromelain extract is having advantages over commercial products. Therefore, this study was to develop a toothpaste incorporated with bromelain as an abbrasive ingredients to lessen the drawback besides highlight the usage of natural ingredients with therapeutic properties. The toothpaste was then analyzed and evaluated for its antibacterial properties and physical characteristics, including color, pH level, taste, and spreadability.

MATERIALS AND METHODS

Materials

Sodium benzoate was obtained from sigma Aldrich, Malaysia. Virgin coconut oil, stevia solution, pineapple flavor, Xanthan gum and peppermint essential oil were obtained from Agrins Chemical, Tampoi, Johor Bharu. Bromelain powder from MD2 has been obtained from Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai.

Formulation of toothpaste

Sodium benzoate, virgin coconut oil, Stevia solution, pineapple flavor, peppermint essential oil and Bromelain powder being weighed based on the best formulation from twelve recipes. The homogenizer was used to make the solution mixed perfectly. Xanthan gum was added as a stabilizer.

Anti-microbial analysis using disc diffusion method

In-vitro anti-bacterial study of formulated toothpaste was performed by disc diffusion method in triplicate manner by using Mukker Hinton Agar mediam against a pathogenic bacteria strain Staphyloccus aureus (S. aureus, MTCC 3160). S. aureus was initially cultured cells were tend to multiple in the Muller Hington agar plates. Then the formulated paste containing discs were placed over the bacterial plates and incubated at 37°C for the 24 hours, comparing ciprofloxacin as the positive control [8] . The diameter of zone of inhibition (ZOI) was measured in milimeters (cm). The antimicrobial analysis carried out triplet to check the standard deviation of the diameter of the ZOI.

Physical Evaluation

The visual color was assessed using colorimeters, namely the Chroma meter Minolta CR410 and the Konica Minolta colorimeter, both manufactured by Sensing in Japan. The meter was calibrated in line with the CIELAB system. The meter was calibrated using a white background. The sample was inserted into the cuvette and the measurement was obtained in three replicates. The mean and standard deviations were then calculated. The color properties were derived based on the color coordinates. The odor was verified by scenting the goods. The taste of the formulation was assessed using manual sensory evaluation. The smoothness was assessed by frictionally manipulating the paste formulation between the fingertips.

Viscosity Test

The viscosity test was conducted utilizing Brookfield viscometers manufactured by Brookfield AMETEK, located in Middleboro, MA 02346 USA. The experiment involves testing the speeds of 25 RPM, 50 RPM, 75 RPM, and 100 RPM. The 100 ml samples were placed in a glass beaker, ensuring that there were no air bubbles or gaps in the sample. A rotational speed type with a disk located at the bottom of the spindle (size 07) was utilized for viscosity measurement. The measurement was determined every two minutes, with triplicates taken for each measurement. The mean and standard deviations were then calculated. The scientific unit of measurement known as centipoise (cP) is commonly used [9].

pH Analysis

pH of sample toothpaste was determined by using pH meter (Milwaukee pH meter, MI 180-US). 10 g of toothpaste placed in 150 ml of beaker. Diluted the sample with 100 ml distilled water and stir vigorously to make a suspension. The diluted sample was tested using pH meter.

Moisture Content Analysis

Moisture content is usually assessed using a thermal gravimetric method called loss on drying. This involves heating the sample and measuring the weight loss caused by the evaporation of moisture. Five gram of the toothpaste sample was weighed in crucible and heated in an oven at 105 °C for about six hours [9]. After six hours, the crucible with the leftover's toothpaste sample was weight again. The moisture content then was calculated from the different between the initial and final weight not including the crucible weight. This method undergoes triplet repetition to determine the mean and standard deviations.

RESULTS

Formulation and viscosity of the toothpaste

According to the value in Table I, the statistics of the moisture content measured by the thermogravimetric method, moisture contents ranging from 60% to 62% were obtained, which was higher than the typical moisture content for conventional toothpaste. Typical moisture content for conventional commercial toothpastes, as measured by TGA, generally ranges from approximately 27% to 52%. Gel formulations, based on their composition, may contain up to approximately 55% water [10, 11]. The development of high-moisture toothpastes, potentially leveraging natural ingredients like bromelain extract more extensively as suggested by some patent concepts [12], could represent an innovative direction in oral care. It has been found that the faster the speed, the lowest the viscosity, therefore, the toothpaste is easily spread on the toothbrush once the force acts on it (Fig. 1). A lower cP value indicates higher performance, which in turn affects the spreadability of the toothpaste.

Table I: Characteristics of Formulated Bromelain Toothpaste

Parameters	Results
pH	8.63 (±0.05)
Moisture Content (%)	61.57% (±0.73%)
Abrasiveness	Good
Spreadibility	Good
Colour	White Yellowish

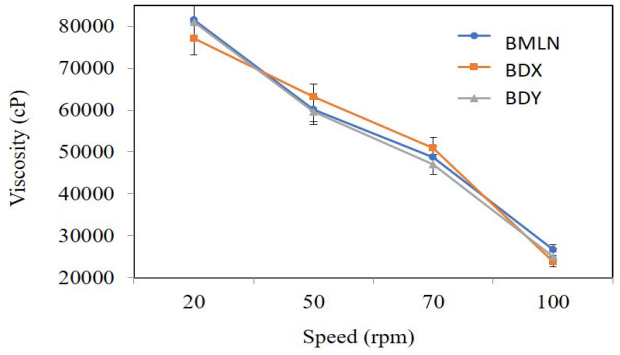

Fig. 1: The viscosity of toothpaste at various speeds using viscometer

Antimicrobial analysis of different percentage of Bromelain enzyme

Following a 24-hour period of incubation, each plate was inspected. The diameter of the zone of inhibition (ZOI) was measured using a ruler, and the measurement was recorded in millimeters (mm). Fig. 2 shows the inhibition zone of different percentages of Bromelain enzyme (1%, 2.5% and 5%) that been added in toothpaste formulation in order to determine the best percentage for antimicrobial activity. The sample with zero bromelain enzyme added acts as control. From the study, it was found that the size of the inhibition zone with 5% bromelain enzyme was the biggest with 18 ± 1 mm compared to the 1 and 2.5%, while the positive controls only give 10.0 ± 1 mm. The ZOI diameter for the 2.5% Bromelain enzyme was measured to be 14.3 ± 1 mm, while the ZOI diameter for the 1% Bromelain enzyme was measured to be 12 ± 1 mm. The findings indicate that increasing the concentration of bromelain enzyme in the toothpaste formulation enhances its antimicrobial efficacy, demonstrating a stronger ability to inhibit pathogenic microorganisms. The antibacterial activity was assessed by measuring the diameter of the zones of inhibition formed against the tested bacteria [13-15] .

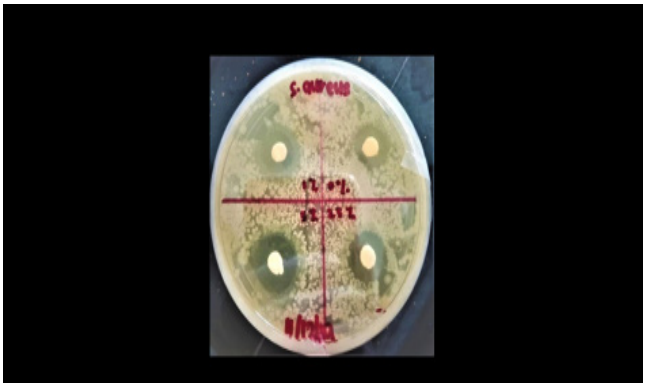

Fig. 2: The antimicrobial result of different percentage of bromelain enzyme used in sample by using Staphyloccus aureus

Comparison between pH, viscosity and moisture of the toothpaste.

The comparison between pH, viscosity and moisture content between Bromelain toothpaste and other two common toothpaste brands in industry is shown in Figure 3. From the result it is observed that pH and viscosity value of bromelain toothpaste gave small differences with common brand toothpaste. The typical pH range for toothpaste is often between 6 and 9, which corresponds to the pH levels found in natural substances. The triplet test on the pH of this Bromelain toothpaste indicates a value within the specified range. The toothpaste should have a pH that is either natural or somewhat basic to ensure compatibility with the pH of the mouth, hence preventing any potential side effects or internal issues within the oral cavity. The moisture content of Bromelain toothpaste had the lowest percentage compared to the other two common brands (Fig.3). From Fig. 3 the bromelain toothpaste

(BMLN) is having an equal or nearly result with other two toothpastes (BD X and BD Y).

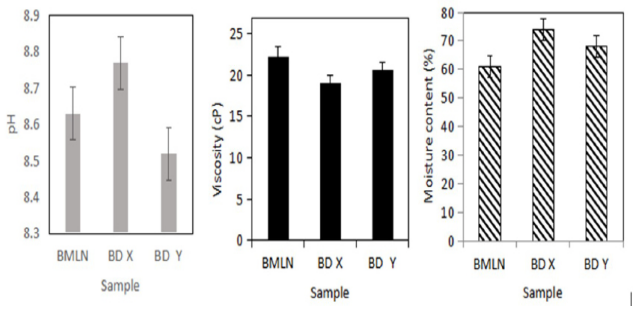

Fig. 3: The comparison between (a) pH, (b) viscosity, and (c) moisture content between Bromelain toothpaste (BMLN) with two others common toothpaste brands (BD X and BD Y) in industry.

DISCUSSION

The Bromelain toothpaste recipe was created using primary natural ingredients, with a minimal addition of chemicals. At the trial phase of formulation twelve formulas were performed and characterized for their structure, smell, spreadability, and taste. From twelve formulations, one best formulation was selected due to the features of common toothpaste and being used for the experiment.

Formulation and viscosity of the toothpaste

The best formulated bromelain toothpaste shows white yellowish in colour and showed good spreadability, high moisture content, taste, smell; pH is alkaline, and smooth with good anti-microbial activity. Other researchers also found that the pH of the herbal toothpaste was at 7.5, placing it within the acceptable range for oral pharmaceutical preparations. This near-neutral pH is optimal for maintaining the physiological pH of the oral cavity and helps minimize the potential for enamel erosion and irritation of the oral mucosa [16]

Antimicrobial analysis of different percentage of Bromelain enzyme

The oral cavity has also been identified as a potential reservoir for Staphyloccus aureus despite it being regarded transient and not permanent in nature [17, 18]. Increasing the concentration of Bromelain enzyme in toothpaste yields the most effective antibacterial outcome, enabling it to effectively combat germs.

Comparison between pH, viscosity and moisture of the toothpaste.

In general, the moisture content of toothpaste affects both its physical properties and its quality. The moisture in toothpaste aids in the prevention of xerostomia, also known as dry mouth. Xerostomia can often result in malodorous breath [19]. The moisture content of Bromelain toothpaste had the lowest percentage compared to the other two common brands due to the others brand contain chemicals substance for preservatives such as ethyl paraben, polyethylene glycol while bromelain toothpaste used sodium benzoate as preservatives. Sodium benzoate is also a chemical substance, but it consists of less toxicity and is less harmful compared to paraben.

CONCLUSION

A natural toothpaste formulated with bromelain enzyme and minimal chemical additives was successfully developed. It matches commercial toothpaste in key quality parameters such as moisture content, pH, viscosity, and sensory attributes including color, texture, odor, and taste. The formulation effectively promotes oral hygiene and exhibits antibacterial activity against Staphylococcus aureus. By utilizing natural ingredients, this toothpaste offers a safer and more effective alternative for daily dental care, with strong potential for widespread consumer and clinical use.

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REFERENCES

1. Britos, S. A., Orsingher, O. A., & Fulginiti, S. (1978). Effects of venom gland extract of the black widow spider on rat brain and heart levels of noradrenaline, 5-hydroxytryptamine and acetylcholine. Toxicon, 16(4), 393-395.
2. Gani, A. M., Chigurupati, S., Manik, L. G., ZG, S., & Byragani, S. (2019). The Impact of Bromelain on Health Outcomes: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Frontiers in Physiology, 9, 1899. doi:10.3389/fphys.2018.01899.
3. Nema RK, Rathore KS and Dubey BK. A Textbook of Cosmetics. CBS Publisher and distributor. 2009; 1st Ed: pp.
4. Mangilal T and Ravikumar M. Preparation and Evaluation of Herbal Toothpaste and Compared with Commercial Herbal Toothpastes: An In-vitro Study. International Journal of Ayurvedic and Herbal Medicine. 2016; 6: 2266 –2251.
5. Dange VN, Magdum C.S, Mohite SK and Nitlikar M. Review on Oral Care Product: formulation of toothpaste from various and extracts of tender twigs of neem, J of Pharm Res. 2008; 1(2): 148-152.
6. Sahu, B & Mohanty, R. Herbal Toothpaste: A

Comprehensive Overview. Indian Journal of Public Health Research & Development (2019) 10. 1283. 10.5958/0976-5506.2019.03700.8.
7. Gautam D, Palkar P, Maule K, Singh S, Sawant G, Kuvalekar C, Rukari T, A. Jagtap V. Asian Journal of Pharmacy and Technology. 10(3): July- September, 2020
8. Patil PA, Ankola AV, Hebbal MI, Patil AC. Comparison of effectiveness of abrasive and enzymatic action of whitening toothpastes in removal of extrinsic stains - a clinical trial. Int J Dent Hyg. 2015 Feb;13(1):25-9. doi: 10.1111/idh.12090. Epub 2014 Jul 21. PMID: 25046241.
9. Karunakaran, C. 1999. Modeiling Safe Storage Time of Eigh (17 and 19%) Moisture Content Wheat, Master in Science Thesis, Department of Biosystems Engineering University of Manitoba Wimipeg, ManitobaDeshmukh. P., Roshan.T & Mahendra.G (2017). Research Article Formulation and Evaluation of Herbal Toothpaste. International Journal of Pharmaceutics & Drug Analysis Vol.5 Issue 10, 2017; 406 – 410; http://ijpda.com; ISSN: 2348-8948
10. Toothpaste Manufacturing Process - Silverson, accessed May 11, 2025, https://www.silverson.com/us/resource-library/application-reports/manufacture-of-toothpaste
11. Paul, Chanchal., khan Abu., Sarkar, Probir., Hakim, Md. & Waliullah, Md. Assessment_of_the_Level_and_Health_Risk_of_Fluoride_and_Heavy_Metals_in_Comercial_Toothpastes_in_Bangladesh. Vl (20). Indonesian Journal of Chemistry.
12. CN103405367A - Toothpaste containing bromelain component, accessed May 11, 2025, https://patents.google.com/patent/CN103405367A/en
13. Divya S, Suresh J, Meenakshi S. Comprehensive review on herbal toothpaste. J Oral Health Comm Dent. 2021;25(4):9509–18.
14. Mulla JA, Palkar MB, Maddi VS, Khazi IA. Rational design of antibacterial thienopyrimidines by 2D-QSAR study. J. Drug Delivery Ther. 2012;2(2):55-66.
15. Panchamukhi SI, Mulla JA, Shetty NS, Khazi MI, Khan AY, Kalashetti MB, Khazi IA. Benzothieno[3,2-e][1,2,4]triazolo[4,3- c]pyrimidines: synthesis, characterization, antimicrobial activity, and incorporation into solid lipid nanoparticles. Arch Pharm. 2011; 344(6):358-65.
16. Chalke, P. M., Mulla, J. A. S., & Kapse, M. V. (2024). Development and evaluation of herbal toothpaste with antibacterial and anti-inflammatory properties. World J Biol 2024: 1(1): 1-5
17. Arshad, M. S., et al. (2020). Bromelain: A review on its sources, purification and therapeutic uses. Biotechnology Letters, 42, 845–858.
18. Pithayanukul, P., et al. (2009). In vitro antimicrobial activity of extracts from pineapple fruit, peel, and crown. Songklanakarin J. Sci. Technol., 31(1), 103–107.
19. de Lencastre Novaes, L. C., Jozala, A. F., Lopes, A. M., de Carvalho Santos-Ebinuma, V., Mazzola, P. G., & Pessoa Junior, A. (2016). Stability, purification, and applications of Bromelain: A review. Biotechnol Prog, 32(1), 5-13. doi:10.1002/btpr.2190