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Exploring the Industrial Application of Xylitol Derived from Over-Ripe Fruit Fermentation by Formulation of Xylitol-Based Toothpaste

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KEYWORDS

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ABSTRACT

Xylitol, a naturally occurring five-carbon sugar alcohol, is recognized for its anticariogenic and antimicrobial properties. The present study investigates a sustainable biotechnological route for xylitol production through fermentation of over-ripe fruits using xylose-assimilating yeast strains, followed by its application in toothpaste formulation. Yeast isolates, identified as *Debaryomyces hansenii* and *Meyerozyma guilliermondii* were obtained, optimized for fermentation parameters and evaluated for xylitol yield. The fermentation process achieved a xylitol yield of over 50% with efficient xylose utilization confirmed via HPLC. Formulated toothpaste incorporating the obtained xylitol underwent physicochemical characterization, pH stability testing, spectrophotometric analysis and antimicrobial efficacy studies against common oral pathogens including *Streptococcus mutans, Escherichia coli, Streptococcus agalactiae, Candida albicans* and *Enterococcus faecalis*. The toothpaste showed a neutral pH, high absorbance at 240 nm and significant zones of inhibition demonstrating its potential in oral hygiene applications. This work effectively links green production of xylitol to a downstream product with proven antimicrobial and dental health benefits, highlighting a scalable biorefinery approach for oral care industries.

1. INTRODUCTION

Dental caries is one of the most prevalent non-communicable diseases worldwide, affecting both children and adults. It is primarily caused by acidogenic and aciduric microorganisms such as *Streptococcus mutans* and *Lactobacillus* species, which ferment dietary carbohydrates to produce acids that demineralize tooth enamel. According to the Global Burden of Disease Study, dental caries affects over 2.3 billion people with permanent teeth and 560 million children with deciduous teeth [1].

Xylitol, a five-carbon sugar alcohol, has emerged as an important natural sweetener with anticariogenic properties [2]. Unlike sucrose, xylitol cannot be metabolized by *S. mutans*, leading to reduced acid production and maintenance of a neutral oral pH. Furthermore, xylitol interferes with bacterial metabolism through the "futile cycle," wherein the sugar is phosphorylated and expelled, draining bacterial energy reserves and reducing virulence [3]. It also promotes remineralization of enamel and enhances salivary flow, making it a valuable component in oral hygiene products [4-5].

Industrial xylitol is commonly produced via chemical hydrogenation of D-xylose, a process that is costly and energy-intensive. Biological production through microbial fermentation offers an eco-friendly and cost-effective alternative [6-7]. Over-ripe fruits are rich in fermentable sugars, including xylose and represent an underutilized biomass resource. Previously, our work demonstrated the isolation of xylose-assimilating yeasts from guava, banana, apple, chikoo and papaya with optimized fermentation yielding over 50% xylitol [8]. The identified yeast species *Debaryomyces hansenii* and *Meyerozyma guilliermondii* showed efficient xylose utilization [9], confirming their biotechnological potential.

Xylitol has wide applications in oral care, food and pharmaceutical industries. In oral hygiene, it is incorporated into chewing gums, lozenges and toothpastes due to its anticariogenic and antimicrobial effects [10]. Multiple studies have documented xylitol's ability to reduce plaque formation, inhibit *S. mutans* and stabilize oral pH [11]. In addition to *S. mutans*, other microbes like *S. agalactiae, C. albicans, E. faecalis* and *E. coli* are associated with oral or

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opportunistic infections and were therefore selected for antimicrobial testing in this study [12].

The present study integrates upstream bioprocessing (fermentation-based xylitol production) with downstream application (formulation of xylitol toothpaste and evaluation of its safety and antimicrobial efficacy). This approach demonstrates a circular bioeconomy model that converts fruit waste into a high-value dental care product, aligning with sustainable development goals and green chemistry principles [8-9]

2. MATERIALS AND METHODS

2.1 Xylitol Production from Over-Ripe Fruits

2.1.1 Yeast Isolation and Identification: Over-ripe fruits (Banana, Guava, Chikoo, Papaya and Apple) were sourced locally. Samples were enriched in Peptone–Xylose medium [13], plated on Rose Bengal agar and purified. Morphological, biochemical and molecular characterization identified the strains as *Debaryomyces hansenii* (S2) and *Meyerozyma guilliermondii* (S4) [14-15].

2.1.2 Optimization of Fermentation Conditions: Fermentation parameters including pH (3.0–5.5), temperature (25–37°C) and substrate concentration (2–20%) were optimized [16-17]. Biomass was quantified via dry cell weight and CFU counts over six days. Maximal xylitol yield was achieved at pH 4.5, 30°C and 5% substrate concentration on day 4.

2.1.3 Fermentation and Quantification: Cultures were grown in YP medium with 6% xylose. HPLC quantified xylose utilization and xylitol production [18-19].

2.2 Toothpaste Formulation

Xylitol obtained from fermentation was incorporated into toothpaste according to standard FDA guidelines [1,3]. Components included xylitol (sweetener), calcium carbonate (abrasive), sodium lauryl sulphate (foaming agent), gelatin (binder), glycerol (humectant), strawberry extract (coloring and flavoring agent) and distilled water (solvent). The mixture was homogenized and stored in sterile tubes.

2.3 Physicochemical Testing

For pH measurement, samples were measured using a calibrated pH meter at 25°C. For spectrophotometric analysis study, optical density was recorded from 200 to 600 nm to determine the characteristic absorption spectrum of xylitol [18-19].

2.4 Antimicrobial Testing

The different test microbes used in the study included *Streptococcus mutans, Streptococcus agalactiae, Escherichia coli, Candida albicans* and *Enterococcus faecalis* [10-12]. Standardized inocula (Brain Heart Infusion-BHI broth for *S. mutans*, *S. agalactiae* and *E. faecalis*, LB broth for *E. coli* and Yeast Glucose Chloramphenicol Agar- YGC Agar for *C. albicans*) were spread on agar plates to study the zone of inhibition. Wells were loaded with toothpaste samples and incubated at 37°C for 24 h. ZOI was measured with Vernier calipers. Azithromycin (30 µg/mL) served as the positive control.

3. RESULTS

3.1 Xylitol Fermentation

HPLC confirmed >99% xylose utilization with xylitol yields of 50.85% (S2) and 51.05% (S4). Optimal conditions (pH 4.5, 30°C, 5% substrate) produced maximum yields. The fermentation profile demonstrated rapid and efficient xylose utilization by both *D. hansenii* and *M. guilliermondii*. The initial xylose concentration in the medium was 60 g/L, with nearly 50% of the sugar consumed within the first 48 hours. By day 4, over 99% of xylose was utilized. Maximum xylitol yield was recorded at this point with 50.85% for S2 (*D. hansenii*) and 51.05% for S4 (*M. guilliermondii*) [Figure 1, Table 1]. The residual sugar content was minimal, indicating efficient conversion without significant by-product accumulation. The fermentation time course revealed that productivity peaked between 48 and 96 hours, aligning with the exponential growth phase of the yeasts. Xylitol accumulation plateaued beyond 96 hours, suggesting substrate limitation and metabolic saturation. The yields are consistent with previously reported ranges for wild-type yeast strains grown on xylose-based substrates. The fermentation performance reflects the inherent metabolic efficiency of these strains in converting pentose sugars to polyols.

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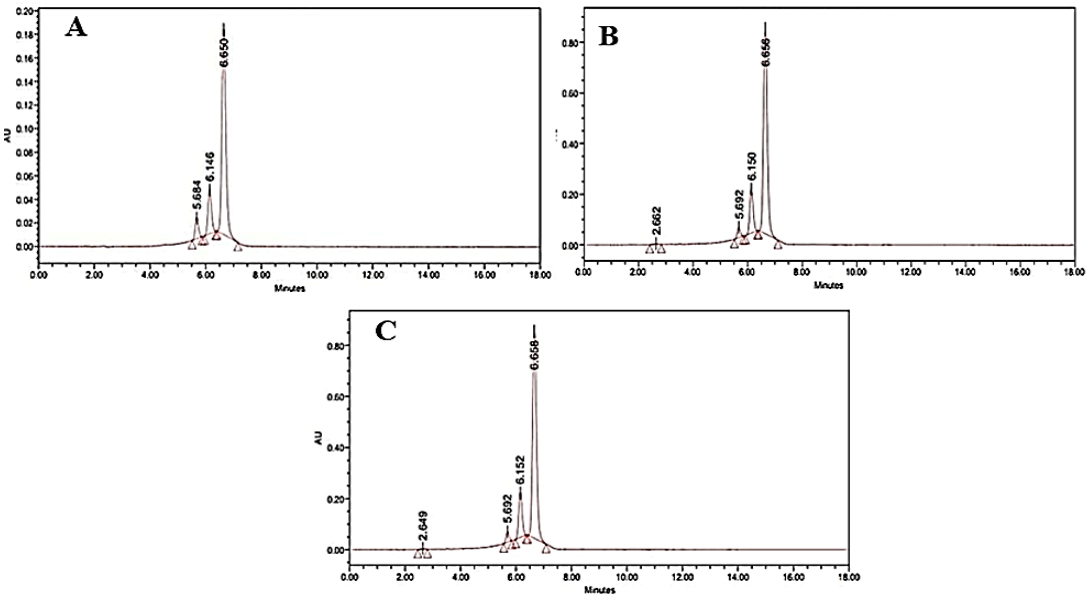

Figure 1: HPLC chromatogram of A) Standard Xylitol B) Xylitol curve for S2 isolate C) Xylitol curve for S4 isolate.

Sample	RT	Area	Concentration	% xylitol production
S2	6.658	7853130	508.5	50.85
S4	6.656	7878771	510.5	51.05

Table 1: HPLC results showing amount of xylitol produced by yeast isolates S2 and S4

3.2 Toothpaste Formulation

The xylitol toothpaste formulated in this study exhibited desirable physical characteristics for a standard oral care product. The mixture was homogenous with a smooth and pinkish-red appearance (because of strawberry extract used as coloring and flavoring agent) indicating proper dispersion of all excipients. Its consistency was semi-solid, non-sticky and easy to spread on the toothbrush, demonstrating good compatibility between xylitol and other components such as calcium carbonate (abrasive), sodium lauryl sulphate (foaming agent), gelatin (binder), glycerol (humectant) and water (solvent). The formulation showed no phase separation or air entrapment after preparation and its texture remained stable upon storage at ambient temperature for several weeks. Such stable physical characteristics are crucial for ensuring ease of use and consumer acceptability. Additionally, the visual and textural uniformity supports the suitability of xylitol for direct incorporation into personal oral care formulations. The prepared formulation is shown in Figure 2, illustrating the smooth texture and stable matrix.

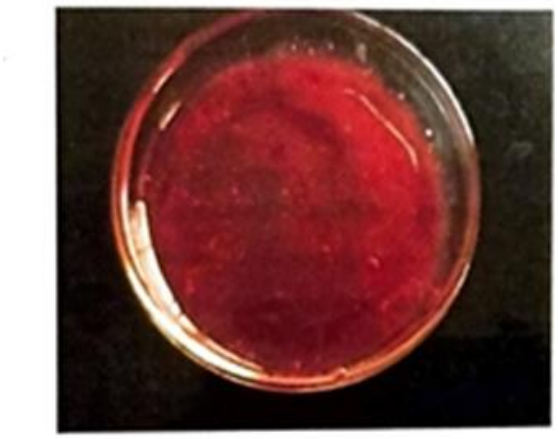

Figure 2: Formulated xylitol toothpaste

3.3 Physicochemical Properties of Toothpaste

The pH of xylitol toothpaste was 7.32, indicating a neutral formulation suitable for oral care products. Neutral pH plays a key role in maintaining oral microbial balance and

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preventing enamel demineralization. When exposed to acidic or basic conditions (1 mL of 0.1N HCl or NaOH), the formulation demonstrated predictable buffering behavior- shifting to pH 1.0 and 14.0 respectively- without destabilization or precipitation. This confirms the formulation's chemical stability across physiological pH variations.

UV-VIS spectroscopy revealed a peak absorbance at 240 nm corresponding to xylitol [Table 2], which is a characteristic of xylitol's electronic transitions. The peak remained stable across replicate measurements, indicating structural integrity of xylitol in the formulation. This spectral stability can be used as a marker for quality control of xylitol-containing formulations.

Sl. No.	Wavelength (in nm)	Absorbance
1	200	0.62
2	210	0.88
3	220	1.27
4	230	1.5
5	**240**	**1.59**
6	250	1.52
7	260	1.42
8	270	1.37
9	280	1.32
10	290	1.31
11	300	1.28
12	310	1.22
13	320	1.16
14	330	1.09
15	340	1.05
16	350	1.13
17	360	1.15
18	370	1.04
19	380	1.35
20	390	1.43
21	400	1.49
22	410	1.51
23	420	1.52
24	430	1.50
25	440	1.46
26	450	1.37
27	460	1.45

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28	470	1.48
29	480	1.51
30	490	1.52
31	500	1.54
32	510	1.54
33	520	1.55
34	530	1.54
35	540	1.53
36	550	1.52
37	560	1.53
38	570	1.45
39	580	1.41
40	590	1.48
41	600	1.49

Table 2: Absorbance data recorded between 200–600 nm

3.4 Antimicrobial Activity

The antimicrobial assay results revealed significant inhibitory effects of xylitol toothpaste against all tested organisms. The largest zone of inhibition (ZOI) was observed for *S. agalactiae* (20 mm), followed by *S. mutans* (19 mm), *C. albicans* (17 mm), *E. coli* (16 mm) and *E. faecalis* (12.5 mm) [Figure 3, Table 3]. This gradient of inhibition aligns with known xylitol susceptibility patterns, especially for cariogenic streptococci.

Xylitol is known to disrupt carbohydrate metabolism in *S. mutans*, leading to energy loss through a futile cycle and reduced acid production. This mechanism likely underlies the observed high inhibition against streptococcal species. The moderate activity against *C. albicans* and *E. faecalis* suggests broader antimicrobial potential beyond strictly cariogenic bacteria. Such a spectrum is advantageous for oral hygiene formulations aimed at maintaining balanced oral microbiota.

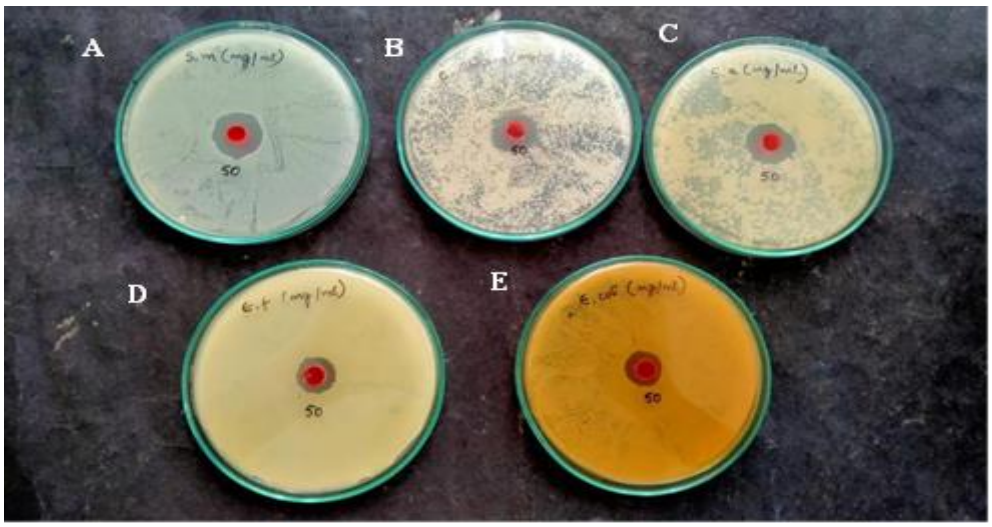

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Figure 3: Antimicrobial activity for test organisms

(**A-** *S. mutans*, **B-** *S. agalactiae*, **C-** *C. albicans*, **D-** *E.faecalis*, **E-** *E.coli*)

Plate	Test microorganism	ZOI (in mm.) (50 µg/mL of sample)	ZOI (in mm.)- Standard (30 µg/mL of Azithromycin)
A	*S. mutans*	19	24
B	*S. agalactiae*	20	26
C	*C. albicans*	17	20
D	*E. faecalis*	12.5	16
E	*E.coli*	16	18

Table 3: Zone of inhibition observed (in mm.) for test organisms

4. DISCUSSION

This study provides a sustainable, low-cost biotechnological route to produce xylitol from fruit waste using *D. hansenii* and *M. guilliermondii* [6,8]. The obtained xylitol was successfully formulated into toothpaste with favorable physicochemical properties and broad antimicrobial activity. Previous reports have highlighted xylitol's efficacy in disrupting *S. mutans* metabolism [20-21] and inhibiting biofilm formation [22]. Our results are very consistent with these findings and extend the application to include activity against *S. agalactiae*, *C. albicans* and *E. faecalis*, which can be opportunistic pathogens in oral and systemic infections. The combination of neutral pH, specific UV signature and measurable antimicrobial action indicates that biologically derived xylitol is a viable ingredient in oral formulation. This integration of upstream fermentation with downstream formulation exemplifies a circular bioeconomy model and supports scaling of sustainable oral care products.

5. CONCLUSION

This work demonstrates an integrated approach for xylitol production from over-ripe fruits and its application in toothpaste formulation. The product exhibits neutral pH, stable physicochemical properties and broad antimicrobial activity. This green production strategy provides a sustainable pathway for developing value-added oral care products.

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