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Evaluating the Effects of Chewing Gum Ingredients on the Oral Microbiome

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Abstract
As a result of the diverse active ingredients in different chewing gums, the effect of the active ingredients was evaluated on the oral microbiome of consumers. Following this aim, three commonly, gums based on their active ingredient were investigated. The gums namely: azonbin-based banana gum, xanthan-based centre fruit gum and sorbitol-based centre fresh gums were purchased and chewed by ninety (90) adult volunteers. Samples were obtained by swabbing the oral cavity of the volunteer consumers before and after chewing the gums (ten minutes). The samples thereafter were prepared (10^{-2} dilution) and analyzed using standard microbiological techniques. Results showed the mean counts of heterotrophic bacteria were significantly, different before and after chewing of azonbin and sorbitol-based gums at a probability level of 0.05, while volunteers who chewed azonbin-based gums showed no significant difference in counts obtained. Heterotrophic fungal counts were not different before and after volunteers chewed sorbitol and xanthan-based gums at a probability level of 0.05, however, azonbin-based gums differed. Staphylococcal counts showed no significant difference. No counts were reported for total coliform. A total of 20 microbial isolates belonging to four genera were identified namely; *Staphylococcus aureus* (40%), *Proteus* spp. (30%), *Candida* spp. (20%) and *Bacillus* spp. (10%). The study showed that the active ingredients in the gums had an effect on the oral microbiome of consumers. Hence, gum consumers should consider the active ingredients before purchasing.

Keywords: Active ingredients. Chewing-Gum, Oral, Microbiome

Introduction

The increase in the production/manufacture of different types and brands of chewing gums has continued to flood the market space with slight knowledge on the effect of the active ingredient of the gum on the oral microbiome of consumers (Choudhury,2024). Studies have shown that the active ingredients are not the same for all gums (Nieburg, 2013). According to Takeuchi et al. (2018) the gums are produced by different manufacturing industries with diverse chemical agents which may distort the oral microbiome of the consumer and thereby result in infections by opportunistic pathogens. These infections may include; bad breath, periodontitis, mouth injury or mouth odour as noted by Aslani and Rostami (2015). Consequently, the economic importance of the sale of chewing gums continues to increase (Konar et al., 2016). According to Konar et al. (2016), chewing gum is a form of mouth exercise, that stimulates and increases the flow of saliva in the mouth. It could help protect the teeth and reduce or control mouth odour/breath. Consequently, the active ingredient such as sugar, gumbase, glucose, syrup, aspartine, lecithin, mannitol, flavour etc. present in these gums sold in public spaces may distort the normal flora of the mouth (Takeuchi et al.,2018), as the side effect have not been properly reported (Allen et al., 2014). The mouth also called the oral cavity is an oval-shaped opening in the head. It is vital for many bodily functions outside chewing, which includes: breathing, speaking, digesting of food, talking, swallowing, talking etc. (Kamrani & Sadiq, 2023). Kamrani and Sadiq (2023) reported that the mouth allows air and food nutrients into the body. Thus, according to Willis and Gabaldon (2020), there exist several microbial flora in the mouth, among which are the bacteria and fungi groups. The oral microbiome, a community of microbes (Willis & Gabaldon, 2020) is reported to play a huge role in oral health, which when distorted, contributes to oral diseases such as dental caries, bad breath, periodontitis, gum disease, oral mucosal diseases etc. However medicated chewing gum has been made available for the control and treatment of some diseases associated with oral health (Aslani & Rostami, 2015). The importance of chewing gum as an oral

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therapy has continued to be successful following the active ingredients target against the causative agent for which health challenges emerge (Aslani & Rostami, 2015). Although chewing gum sold in the public domain is not medicated. Chewing gums such as spearmint, double bubble and chiclet with diverse active ingredients have been noted as non-medicated in action yet continue to flood the market (Aslani & Rostami, 2015). Thus, the significance of this study lies in line with the need to promote oral health, following the existence of several active ingredients in different gums sold in the market space. Sequel to this, the study aimed to evaluate the effect of the active ingredients in chewing gum on the oral microbiome of the mouth of consumers.

Materials and Methods

The chewing gum market has continued to blossom amongst the young generation (Konar et al., 2016). The purchase and production have greatly increased with high demand with vendors making daily sales (Choudhury, 2024). The study identified three commonly sold and consumed gums with their ingredients namely; (i) azonbin-based banana bubble gum with active ingredients: banana flavour, gum, gum base, glucose syrup, acidity and citric acid (ii) xanthan-based centre fruit filled chewing gum with active ingredients: strawberry flavour, gum, gum base, glucose syrup, acidity and citric acid (iii) sorbitol based centre fresh filled chewing gum, with active ingredients: peppermint flavour, gum base, glucose syrup, acidity, citric acid, glycerin and brilliant blue. All three gums are differentiated by their flavour content, stabilizer content and colour.

The gums, based on their ingredient composition namely; azonbin based banana gum, xanthan-based centre fruit gum and sorbitol-based centre fresh gums were purchased from vendors in the study area of the research (Port Harcourt, Nigeria) at a quantity size of 30 pieces each. The three units of gums were given to 90 volunteers, 30 volunteers each per gum unit for the sample collection exercise. Swab samples from the oral gravity of the volunteered participants were taken before and after chewing the gum after 10 minutes with a sterile swab stick. The swab was streaked severally, on the sides of the oral cavity for 5 seconds before being immersed into a sterile prepared diluent for 4 hours to allow viable microbial proliferation. The swabbed samples were then transferred to the Biology laboratory of Ignatius Ajuru University, Rumuolumeni, Port Harcourt for Microbial analysis.

The agar namely: (i) nutrient agar, a general purpose media, for the growth of heterotrophic bacteria (ii) sabouraud dextrose agar, for isolation and growth of fungi, (iii) mannitol salt agar; for isolation of Staphylococcal cells and (iv), macconkey agar, for growth and development of coliforms were all prepared based on manufacturer instruction as noted. The agar was first weighed, allowed to dissolve in a measured distilled water, thereafter autoclaved and allowed to cool before dispensing into a sterile petri dish for inoculation purposes as carried out by Maxwell (2018) and Aryal (2022).

Standard bacteriological techniques as adopted by Ho et al. (2024) were used to determine the microbial population in the oral cavity of participants before and after chewing the gum. Prior to plating the samples, the samples were serially diluted to a dilution factor of 10^{-2}. Thereafter one milliliter (1ml) of the 10^{-2} dilution was pipetted and plated on the prepared nutrient agar, sabouraud dextrose agar, mannitol salt agar and macconkey agar media plates. Following these, the inoculated media plates were incubated at 37ºC for bacteria growth to occur within 24 hours and for fungi growth, the media was incubated at room temperature for four days. The number of colonies/growth recovered was counted and reported as colony forming unit per mill (cfu/ml) (Ho et al.,2024).

Colonial and Morphological characterization of the isolates involved macroscopic description of the colony appearance for the bacterial and fungal isolates. Colonial features such as; the colors, shapes, sizes, elevations and opacity were observed. Morphological features involved the determination of the motility of the isolate and a gram staining procedure was necessary. Gram staining was carried out as documented by Agrawal (2019).

A motility test was carried out to determine if the isolates were motile, with flagella (a locomotory organelle). In achieving this a semi-solid nutrient agar was prepared in a test tube and a sterile straight wire was used to pick and inoculate the test bacteria by stabbing into the medium. Thereafter, the tube was incubated at 37^0C for 24 - 48 hrs. Growth in diffused form, from the line of stab into the medium indicated a positive result, whereas growth only along the line of stab indicated a negative result (Agrawal, 2019).

The isolates were screened for their response to Gram stain. The Gram stain was carried out to classify the isolates properly. The classification involved heat fixing the isolate on a glass slide followed by staining the slide with crystal violet for 60 seconds and then rinsing for two seconds. The slide was again stained with iodine as done with crystal violet. A decolourizer (ethanol) was applied on the slide for ten seconds and the slide was

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stained finally with safranin for 60 seconds; rinsed with running water and viewed under a microscope (Agrawal, 2019).

The isolates were identified using the identification scheme of Agrawal (2019), where the isolates were inoculated into freshly prepared media accordingly, as directed by the manufacturers. The mannitol fermentation test, coagulase test, indole test, capsule formation test, catalase test, lactose test and Urea test were all employed to confirm the identity of the isolates (Agrawal, 2019).

The indole test was adopted to determine the ability of the isolates to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme tryptophanase. A loop-full of the isolates was inoculated into a sterile peptone water medium and incubated at 37^0C for 48 hours. Thereafter, 0.3 – 0.5 ml of Kovac's reagent was added using a Pasteur's pipette. The appearance of a red ring layer on the medium indicated a positive indole test while the development of a yellow ring indicated a negative result (Agrawal, 2019).

The isolates were screened for capsule formation. The screening was carried out by introducing a few drops of crystal violet onto a clean slide, followed by inoculating the isolate onto it. Both components were stirred and thereafter viewed under a light microscope. A light blue appearance signified a capsulated cell while an absence of a light blue appearance did not suggest capsule formation (Agrawal, 2019).

The enzyme urease was investigated to confirm the ability of the recovered isolate to utilize urease. In carrying out the test, the urease media was prepared, dispensed into a test tube and sterilized. Thereafter the isolate was introduced into the medium. A change in colour from pink to yellow indicates the isolate could utilize the urease (Agrawal, 2019).

A coagulase test was adopted to differentiate the isolates into coagulase positive. In carrying out the coagulase test a loopful of the isolate was introduced into a clean glass slide followed by the addition of a rabbit plasma which made a complete suspension after the component had been stirred coagulating positive cell formed clumps after 20 seconds while non-coagulase isolated failed to form clumps (Agrawal, 2019).

The lactose test was used to determine the ability of the isolates to ferment lactose. In preparing the lactose broth, 1% of the lactose, peptone agar with a litmus red indicator reagent was constituted, and a Durham tube was placed in an inverted position inside a test tube in the broth. After sterilization, a loopful of the isolate was then incubated into the broth at 37^0C for 18 - 24 hours. The change in colour of the broth culture from red to yellow and a gas inside Durham's tube indicated a positive lactose (Agrawal, 2019).

The mannitol fermentation test was used to determine the ability of the isolates to ferment mannitol. The fermentation broth was prepared by adding, 1% of mannitol, peptone agar and litmus red indicator reagent to constitute the broth. The broth was then sterilized and thereafter a loop-full of the isolate was transferred into the broth. Following this, the broth was incubated at 37^0C for 18 - 24 hours. The change in colour of the broth culture from red to yellow indicated mannitol fermentation (Agrawal, 2019).

The catalase test was done to determine the ability of the isolates to break down Hydrogen Peroxide into Oxygen and Water. The test involved the transfer of a loopful of the isolate into a clean glass slide. Thereafter, Hydrogen Peroxide was introduced onto the slide and the components stirred. Rapid production of effervescence indicated the enzyme catalase while the absence of catalase was indicative of weak effervescence (Agrawal, 2019).
The data recovered were analyzed using a statistical analysis system wherein mean and standard deviations were determined. The means were separated using Duncan's multiple range test at a probability level less than or equal to 0.05 level of significance.

Results

Mean Counts of the Oral Microbiome Before and After Chewing Gums

Table 1 shows the mean counts of total heterotrophic bacteria (THB), total heterotrophic fungi (THF) total staphylococcal count (TSC) and total coliform count (TCC) recovered from the oral cavity of volunteers before and after chewing gums. Counts of THB were noted significantly different before and after chewing azonbin and sorbitol-based gums at a probability level of 0.05, while volunteers who chewed azonbin-based gum showed no significant difference in THB counts obtained. THF counts were significant before and after volunteers chewed sorbitol and xanthan-based gums at a probability level of 0.05 while azonbin-based gums showed a

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significant difference in counts. TSC showed no significant difference in all three gums before and after. No counts were reported for total coliform (TCC).

Table 1: **Mean Counts of the Oral Microbiome Before and After Chewing Gums**

	Sorbitol Gum (CFU/ml)			**Azonbin Gum (CFU/ml)**			**Xanthan Gum (CFU/ml)**		
	T_2	**T_1**	**T-test**	**T_2**	**T_1**	**T-test**	**T_2**	**T_1**	**T-test**
THB	$3.3x10^3$	$1.7x10^3$	p<0.05	$7.5x10^3$	$8.4x10^3$	p<0.05	$2.9x10^3$	$3.4x10^3$	p>0.05
THF	$1.5x10^3$	$1.4x10^3$	p>0.05	$7.x10^2$	$1.5x10^3$	p<0.05	$1.5x10^3$	$1.2x10^3$	p>0.05
TSC	$1.8x10^3$	$1.3x10^2$	p>0.05	3×10^2	7×10^2	p>0.05	4×10^2	8×10^2	p>0.05
TCC	-	-	-	-	-	-	-	-	-

Keys: **T_2**=After Chewing, **T_1**=Before Chewing, **THB=** Total Heterotrophic Bacteria**, THF=** Total Heterotrophic Fungi**, TSC=** Total Staphylococcal Counts, **TCC=**Total Coliform Count, **CFU/ml=**Coliform forming unit

Colonial Characterization of the Bacteria Isolates Recovered

Table 2a shows the colonial appearance of the isolates on the media plates. The nutrient agar plate showed a fishy smell, colorless colonies, and opaque colonies while the mannitol salt media plates showed golden yellowed colonies.

Table 2a Colonial Characterization of the Bacteria Isolates Recovered

Media Plates	**Color**	**Opacity**	**Edge**	**Elevation**	**Surface**	**Shape**	**Size**
Nutrient Media	Golden Yellow	Opaque	Entire	Convex	Smooth	Round	Small
Nutrient Media	Colorless	Opaque	Round	Convex	Smooth	Round	Small
Mannitol Salt Media	Greyish White	Opaque	Round	Low Convex	Smooth	Round	Large

Colonial Characterization of the Fungal Isolates Recovered

Table 2b shows the colonial appearance of the fungal isolates on the media plates. The sabouraud dextrose media plates showed phenotypic features such as; small size, rapid/increased colonial growth rate and a creamed textured / coloured appearance.

Table 2b Colonial Characterization of the Fungal Isolates Recovered.

Media Plates	**Color**	**Elevation**	**Surface**	**Morphology Appearance**	**Fungi**
Sabouraud dextrose Media	White/ milkish	Raised	Rough	Seed-like colonies in chains attached to hyphae	*Candida* spp.

Biochemical Characterization of Bacterial Isolates

Table 3 shows the biochemical characterization of the bacteria isolated. This revealed the presence of *Staphylococcus aureus, Proteus* spp. and *Bacillus* spp. which showed negative Gram stain, mannitol fermentation and coagulase. However, *Proteus* spp. were positive for motility, indole production and urease whereas, *Bacillus* spp. was negative for the same tests. A negative reaction was also observed with lactose and capsule for *Proteus* spp. Unlike *Bacillus* spp. which had a positive response for lactose and capsule. *Staphylococcus aureus* showed positive for Gram stain, lactose, catalase and glucose tests.

Table 3: Biochemical Characterization of Bacterial Isolates

Mann	**Moti**	**Coa**	**Indo**	**Cap**	**Cata**	**Urea**	**Lac**	**Gram Stain**	**Bacteria**
-	+	-	+	-	-	+	-	-	*Proteus* spp.
-	-	-	-	+	-	-	+	-	*Bacillus* spp.
+	-	+	-	-	+	-	+	+	*Staphylococcus aureus*

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Keys: Mann= Mannitol Fermentation, **Moti**= Motility, **Coa**= Coagulase, **Indo**=Indole, **Cap**=capsule formation, **Cata**=Catalase, **Lac**=Lactose

Frequency of Fungal and Bacterial Occurrence Recovered

Table 4 showed the occurrence of the fungal and bacteria recovered in the oral cavity of volunteers before and after chewing gums. High prevalence of the isolates were noted with *Staphylococcus aureus*. Followed by *Proteus* spp. and then *Candida* spp. *Bacillus* spp. showed the least occurrence with respect to the various gums.

Table 4: Frequency of Fungal and Bacterial Occurrence Recovered

Microbial Isolates	Sorbitol Gum	Azonbin Gum	Xanthan Gum	Frequency of Occurrence	% Frequency of Occurrence
Staphylococcus aureus	4	3	1	8	40
Proteus spp.	1	4	1	6	30
Bacillus spp.	1	1	0	2	10
Candida spp.	1	0	3	4	20

Key: %= Percentage

Discussion

The difference in the counts of heterotrophic bacteria and fungi in the various gums chewed may be due to the presence of indicator microbes as reported by Amanidaz et al. (2015). Amanidaz et al. (2015) noted that viable counts of bacteria are a result of some cells that may be viable but non-culturable. Hence, microbial counts may differ in this case, where the active ingredients present in the gum may be responsible for such changes. The flavour, the stabilizer and colour ingredients of the various gums are believed to proliferate lactic acid bacteria (Keyitesi et al., 2023) whereas, a stabilizer ingredient such as potassium sorbate does not kill yeast cells but inhibits the replication of cells (Eze et al., 2021). The insignificant difference in counts with respect to Staphylococcal counts clearly, showed that Staphylococcal were not affected by the active ingredient compositions of the gums. Coliform counts were not reported, thus the ingredients in the gums may have an effect on coliform (Wessel et al., 2015). The microbial colonial characterization obtained in this study is dissimilar from studies carried out by Kure et al. (2022). Kure et al. (2022) identified *Xanthomonas* species yellow/orange on nutrient agar as against colonies observed on nutrient agar in this study. Basically, Xanthan-based centre fruit gums were expected to develop on the nutrient agar media as reported by Kure et al. (2022), thus, this was not observed in the study. The study identified *Proteus* spp., *Staphylococcus aureus*, *Bacillus* spp. and *Candida* spp. which also is not in line with studies carried out by Kure et al. (2022). *Staphylococcus aureus* exists in the mouth as a normal flora; hence, could cause no harm but when compromised by sorbitol active ingredient present in centre of fresh gums, there could be a traces of toxic shock syndrome, bacteremia etc. (Mc Cormack et al.,2015). The presence of *Proteus* spp. in the mouth has rarely, been reported in the mouth however, *Proteus mirabilis* oral infection have been established or reported (Jamil et al., 2023). *Bacillus* spp., presence in the mouth may function as a therapeutic agent that cleanses the mouth hence, the absence of coliform as noted in this study. (Hoa et al., 2000). *Candida* spp., exists in the mouth as a normal flora but can be associated with oral candidiasis. The clinical presentation of fungal infections noted varies and is not easily recognizable. Thus, mouth odour is influenced by the action of *Candida* spp., conditions which is reported as the main cause of bad breath.

Conclusion

The study has shown that the active ingredients in the various gums had an effect on the microbiome of the oral cavity. Counts of heterotrophic bacteria and fungi were significantly, different before and after chewing the gum. However, Staphylococcal counts before and after chewing the gums were insignificant, while counts of coliforms were absent. These differences in counts are reportedly attributed to the active ingredients which invariably could initiate oral diseases following the opportunistic status of the microbes so isolated.

Recommendation

1. The study recommends that gum consumers should take into consideration the active ingredients before purchase and use, to prevent distortion of their oral flora.
2. In addition, gum producers should consider more friendly active ingredients in their productions.

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