Quantification of betel quid hygroscopicity in context to saliva: A proof of concept for future studies

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A R T I C L E   I N F O

Keywords:
Betel quid
Saliva
Oral submucous fibrosis

A B S T R A C T

The physical and chemical properties of various constitutional ingredients of betel quid (BQ) are well known in the literature. However, BQ as a whole has been rarely investigated for identifying its physical properties that can impact and modulate the disease progression of various pathologies, mainly oral submucous fibrosis (OSMF). It is quite conceivable that in the oral cavity environment, the amalgamated BQ might absorb saliva by virtue of its hygroscopic properties. As a proof of concept, we developed a methodology to quantify the hygroscopicity of the ‘whole BQ’ when it is mixed with saliva. To the best of our knowledge, this is the first study to report and quantify the hygroscopicity of BQ when mixed with saliva, and this forms a proof of concept for future implications in the pathogenesis of various pathologies.

1. Introduction

Betel quid (BQ) is defined as ‘a substance, or a mixture of substances, placed in the mouth, usually containing at least one of the two basic ingredients, tobacco or areca nut, in raw or any manufactured or processed form’. Other ingredients that can additionally be present in BQ are catechu, slaked lime, cardamom, fennel seeds, betel leaves, flavouring agents, etc. Different forms of BQ are available across the world, such as gutkha, pan masala, mawa, khaini, etc. Although ingredients are present in various permutations and combinations, areca nut, and tobacco are common across all the products consumed routinely by the users. The physical and chemical properties of each of these ingredients are well known in the literature. However, BQ as a whole has been rarely investigated for identifying its physical properties that can impact and modulate disease progression of various pathologies, mainly oral submucous fibrosis (OSMF).

When BQ is chewed in the oral cavity, it comes into contact with the saliva and stays with it for a significant period. Moreover, BQ chewing also stimulates the salivary glands to secrete more saliva in the oral cavity. Although most of the collected saliva in the oral cavity is either swallowed or spit out by the users, it is quite conceivable that the amalgamated BQ might absorb some of the saliva. This property of absorption of water is known as hygroscopicity. A careful and thorough search of the literature in various known databases such as PubMed, Scopus, and Google Scholar was carried out but we could not find any evidence for the hygroscopicity of BQ or areca nut. Hence, as a proof of concept, we developed a methodology to quantify the hygroscopicity of the ‘whole BQ’ when it is mixed with saliva.

1.1. In-vitro experiment

Gutkha is banned in most of the Indian states and hence we used the most commonly used BQ products like mawa (freshly made BQ at pan shops) and pan masala for quantification of hygroscopicity. Mawa and pan masala (widely consumed tobacco products) were procured from the local markets and were ground into a granulated powder with the help of mortar and pestle. Then, 0.5 g of ground BQ sample was weighed and transferred to the Eppendorf tube. One ml of saliva or water was added to the mixture and amalgamated with the help of vortexing for an hour. After vortexing, the visible supernatant liquid was carefully checked and measured by pipetting out and transferring to the fresh Eppendorf tube. Next, the BQ and saliva/water amalgamated tubes were subjected to centrifugation at 5000 × g for 1 h to check the saliva content that is measured as supernatant. Then, the volume of the supernatant on the top of the tube was pipetted out and measured on a volumetric basis.

In the end, hygroscopicity was calculated after centrifugation as the
The salivary mucus gel performs as a protective diffusion membrane on the surface. The superficial cells of stratified squamous epithelium contain unique ridge-like folds called microplicae, which act as a permeability barrier. This process will make the oral epithelium more susceptible to the carcinogenic stimulus and thus increase the malignant potential of the oral mucosa.

BQ has been widely investigated concerning its fibrogenic, mutagenic, and toxic potential. However, physical properties have been rarely envisaged as a cause for BQ-related pathogenesis. In a recently published paper, BQ has been proposed to support mechanotransduction for causing carcinogenesis in OSF patients. Moreover, the coarseness of BQ is also linked with the micro-trauma to the oral mucosa and subsequent fibrosis. To the best of our knowledge, this is the first study to report and quantify the hygroscopicity of BQ when mixed with saliva, and this forms a proof of concept for future implications in the pathogenesis of various oral pathologies. We propose that BQ hygroscopicity might have a role in some reported OSF events such as minor salivary gland hypofunction, diminished reactive adipogenesis, dysfunctional myoepithelial cells, and matrix stiffness. It is premature to draw any conclusion regarding the pathogenic mechanism, however BQ hygroscopicity would act as complementary to the already known mechanisms of OSMF.

We recommend future studies on the further standardisation of techniques for the quantification of hygroscopicity. Moreover, the methodology should exactly recapitulate the real-life situation of BQ chewing. Future studies should also aim at identifying any contributory effect of hygroscopicity on the pathogenesis of OSF and oral squamous cell carcinoma. An interesting query may be raised to see the effect of hygroscopicity on the quality and quantity of the saliva along with functional alterations in the salivary glands.

Funding source
The author received no specific funding for this work.

Declaration of competing interest
All the authors associated with the present manuscript declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

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Table 1

| Sample | Mixed with | Measured volume of Saliva after Centrifugation (μl) | Hygroscopicity (%) a |
|--------|------------|---------------------------------------------------|---------------------|
|        |            | After amalgamation | After centrifugation |   |
| Mawa + saliva | 1 ml saliva | Nil | 213.3 ± 11.5 | 78.6 ± 11.5 | 78.6 ± 1.1 |
| Mawa + DW | 1 ml DW | Nil | 246.6 ± 5.7 | 753.3 ± 5.7 | 75.3 ± 0.5 |
| PM 1 + saliva | 1 ml saliva | Nil | 50 ± 86.6 | 950 ± 86.6 | 95 ± 8.6 |
| PM 1 + DW | 1 ml DW | Nil | 83.3 ± 55 | 916.6 ± 55 | 91.6 ± 5.5 |
| PM 2 + saliva | 1 ml saliva | Nil | 48.3 ± 2.8 | 951.6 ± 2.8 | 95.1 ± 0.2 |
| PM 2 + DW | 1 ml DW | Nil | 73.3 ± 25.1 | 926.6 ± 25.1 | 92.6 ± 2.5 |

DW: Distilled Water; PM: Pan Masala.

Footnotes:
(a) Hygroscopicity (%) = (a-b) × 100/(a).

percentage of supernatant of saliva or water retrieved and the actual amount added before amalgamation. In this way, the amount of saliva or water that is absorbed by the BQ particles was calculated and presented as a percentage of the hygroscopicity of BQs. For each sample, the experiment is repeated three times to understand the deviation in the obtained results (Table 1).

2. Results and discussion

The husk is about 15–30% of the weight of the raw nut. The arecanut husk fibres are predominantly composed of cellulose and varying proportions of hemicellulose, lignin, pectin, and protopectin. The fibres adjoining the inner layers are irregularly lignified groups of cells called hard fibres, and the portions of the middle layer below the outermost layer are soft fibres. Moreover, the alkaloid constituents of the BQ, namely nicotine, arecoline, arecaidine guvacine, and tannins, are known as pyridine alkaloids. These pyridine alkaloids are essentially miscible with water, and hence, potentially hygroscopic. We also believe that other ingredients of BQ, such as funnel and catechu, being of plant origin, possess hygroscopic property.

It is a fact that the methodology used in the present study, such as vortexing and centrifugation, does not recapitulate the exact real-life situation of the oral cavity. Factors such as continuous masticatory activity, masticatory load, repeated grinding of amalgamated BQ with molars, squeezing of the BQ in the vestibule (effect of muscle contraction), spitting, and swallowing are complexities that were not captured in the currently proposed in-vitro model. We recommend future experiments to capture these complexities for more standardised results. However, the significantly lower standard deviation observed for hygroscopicity in each sample justifies the standardisation of the technique. In the present study, there is a great deal of hygroscopicity observed across all the samples of BQ, which we would like to propose as proof of concept.

In the present investigation, both the samples of pan masala showed a greater percentage of hygroscopicity (95 ± 8.6%, 91.6 ± 5.5%, 95.1 ± 0.2%, and 92.6 ± 2.5%) as compared to mawa (78.6 ± 1.1% and 75.3 ± 0.5%) preparation. Mawa is prepared freshly at the pan shops and is continuously rubbed on the serrated platform before it is consumed. During this process, a small amount of water and slaked lime is continuously added to the mixture of the mawa. This could be the reason for the slightly lower hygroscopicity percentage observed for mawa over pan masala. Future studies should focus on other different types of BQ products to better understand the spread of hygroscopicity percentage.

Saliva plays an imperative role in maintaining the integrity and homeostasis of the oral epithelium by forming a highly complex structure on the surface. The superficial cells of stratified squamous epithelium contain unique ridge-like folds called microplicae, which contain mucin-binding proteins. The salivary mucin binds to these proteins and forms a unique structure called the salivary mucous gel. The salivary mucus gel performs as a protective diffusion membrane against harmful substances and thus acts as a first line of defense. Due to the high hygroscopicity of the BQ, it is quite conceivable that there will be a loss of salivary mucous gel formation, thus loosing the potential of a permeability barrier. This process will make the oral epithelium more susceptible to the carcinogenic stimulus and thus increase the malignant potential of the oral mucosa.
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