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Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method

Vardhaman Mulchand Jain, Gundabaktha Nagappa Karibasappa[1], Arun Suresh Dodamani, Gaurao Vasant Mali

Abstract:

BACKGROUND: Due to consumption of various forms of tobacco in large amounts by Indian population, it has become a cause of concern for major oral diseases. In 2008, the WHO named tobacco as the world's single greatest cause of preventable death. It is also known that certain amount of carbohydrates are incorporated in processed tobacco to make it acceptable for consumption. Thus, its role in oral diseases becomes an important question at this point of time. Through this study, it is attempted to find out the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method.

MATERIALS AND METHODS: Tobacco products selected for the study were Nandi hookah tambakhu (A), photo brand budhaa Punjabi snuff (B), Miraj (C), Gai-chhap tambakhu (D), Hanuman-chhap Pandharpuri tambakhu (E), and Hathi-chhap Bidi (F). The samples were decoded and transported to laboratory and tested at various concentrations by phenol-sulfuric acid method followed by ultraviolet spectrophotometry to determine their absorbance.

RESULTS: The present study showed Hathi-chhap bidi/sample F had a maximum absorbance (1.995) at 10 µg/ml which is a smoking form of tobacco followed by rest all smokeless forms of tobacco, i.e. sample C (0.452), sample B (0.253), sample D (0.077), sample E (−0.018), and sample A (−0.127), respectively.

CONCLUSION: As the concentration of tobacco sample increases, their absorbance increases which in turn is suggestive of increase in its carbohydrate concentration. Carbohydrates in the form of sugars, either inherently present or added in it during manufacturing can serve as a risk factor for higher incidence of dental caries.

Keywords:

Carbohydrate, caries, phenol-sulfuric acid, spectrophotometry, tobacco

Department of Public Health Dentistry, ACPM Dental College, Dhule, [1]Department of Public Health Dentistry, Ajeenkya DY Patil Dental School, Pune, Maharashtra, India

Address for correspondence:
Dr. Vardhaman Mulchand Jain, Assistant Professor, Department of Public Health Dentistry, ACPM Dental College, P. Box No. 145, Opposite Jawahar Soot Girni, Sakri Road, Dhule - 424 001, Maharashtra, India.
E-mail: dr.vardhaman.jain@gmail.com

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Introduction

India is the second largest consumer of tobacco in the world and majority of Indian population consume tobacco in various forms.[1] Tobacco is the most easily accessible, legally available addictive substance which contributes significantly to premature death and long-term suffering, and also being a major risk factor for cardiovascular diseases, chronic obstructive pulmonary diseases, cancers, reproductive outcomes, and oral diseases.[2]

In India, tobacco is consumed in myriad forms which include smoking as well as smokeless tobacco (SLT).[3] Bidi is the most popular prevalent smoking product consumed in rural areas in comparison to cigarette smoking more preferably used in urban areas. Hookah, chuttas, dhumti, chillum, cigars, cheroots, and pipes are some other smoking forms of tobacco in different parts of India.[2]

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SLT is consumed predominantly by chewing in form of pan (piper betel leaf filled with sliced areca nut, lime, catechu, and other spices chewed with or without tobacco), pan-masala or gutkha (a chewable tobacco containing arecanut), and mawa in many parts of India. Furthermore, tobacco such as mishri (a powdered tobacco rubbed on the gums as toothpaste), gul, and gudakhu are widely used as topical application on teeth and gums.[3,4]

The prevalence of various forms of tobacco usage and habit varies across different geographical areas. It has been predicted that if the current trends of tobacco use are unchecked the number of tobacco-related deaths will reach eight million by 2030, with approximately 70% of these deaths occurring in developing countries. SLT use affects nearly 300 million people worldwide and about 90% of the world's users reside in the South-East Asian Region of the WHO.[5]

The term "additive" means any substance, intended for use as a flavoring or coloring or in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding, etc.[6]

Carbohydrates are the natural tobacco components present in levels up to 20 wt% in tobacco products and used as additives during manufacturing process. They also serve as a flavoring substance and humectant in tobacco products.[7]

Due to the consumption of tobacco in such large amounts, it has become a cause of concern for major oral diseases. There is abundance literature available depicting the role of carbohydrates in causation and progression of dental caries.[8,9] Tobacco addicts in any form are prone to dental caries process.[10] It might be due to continuous consumption of these products multiple times and for chronic duration. Many studies have assessed the carbohydrate content of smoked forms of tobacco; however, there is scarcity of evidence that assesses the carbohydrate content of smokeless forms of tobacco.[7]

Phenol-sulfuric acid method is the one of the most easiest and reliable, colorimetric method, widely used among the quantitative assays available for carbohydrate estimation in aqueous solutions.[11-13] It is followed by determining light absorbance on ultraviolet (UV) spectrophotometer which is one of the oldest techniques of analysis and is the basis for number of ideal methods for the determination of micro and semi-micro quantity of analytes in a sample.[14]

The study area Khandesh being a low socioeconomic region, bidi is the most common smoked form of tobacco consumed along with numerous other smokeless forms of tobacco.[15] Hence, the present study was conducted to assess and compare the carbohydrate content of different tobacco products in smoked and smokeless forms by phenol-sulfuric acid method.

Materials and Methods

The present study is an *in vitro* study conducted at H. R. Patel Institute of Pharmaceutical Education and Research at Shirpur, Maharashtra. Ethical clearance for the study was obtained from the Institutional Ethical Review Committee.

Selection and coding of sample

The various commercially available tobacco products were taken into consideration based on their popularity, widespread use among the people, availability in the vicinity of study (Dhule) area, and of those, the following samples were randomly selected which were labeled in the following manner:

A. Nandi hookah tambakhu
B. Photo brand budhaa Punjabi snuff
C. Miraj
D. Gai-chhap tambakhu
E. Hanuman-chhap Pandharpuri tambakhu
F. Hathi-chhap Bidi.

Recently, manufactured and packed samples were purchased from the local retail outlet. The study was conducted over a period of 15 days.

Packing and transport

The samples were weighed according to 100 mg of tobacco content and were sealed in air-tight pouches with labeling of the codes (A, B, C, D, E, F). The samples were then transported in hygienic and moisture-proof conditions to the study center.

Processing

Phenol-sulfuric acid method is the most reliable method among all the quantitative assays for carbohydrate estimation.[11-13] In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow-brown-colored product with phenol and has absorption maximum at 490 nm UV spectroscopy.[12,16] This obeys Beer–Lambert's law which states that when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of absorbing solution is proportional to incident radiation as well as the concentration of solution.[17]

- $A = \log (Io/I) = EcL$
- Io = Intensity of light incident upon sample
- I = Intensity of light leaving sample cell
- c = Molar concentration of solute

- L = Length of sample cell (cm)
- E = Molar absorptivity
- A = Absorbance.

Apparatus

The apparatus used in the study were pipettes (micropipette), UV spectrophotometer (Shimadzu, Asia Pacific Pvt. Ltd., Singapore), reflux, condenser, test tube, stand, boiler, cuvette, etc.

Reagents

96% sulfuric acid, standard glucose, 2.5 N HCl, sodium carbonate, distilled H_2O, phenol solution.

Procedure for standardized sample

Take 100 mg of glucose into test tube. Add 5 ml of 2.5 N HCl and boil in water bath for 3 h to hydrolyze the sugars. Cool it to room temperature. Add sufficient quantity of solid sodium carbonate until the effervescence ceases. This indicates complete neutralization. Filter and make the volume up to 100 ml. Pipette out 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard into a series of test tubes.

The blank was set initially with all reagents without sample. Add 1 ml of phenol solution to each tube and shake well. Then, add 5 ml of sulfuric acid 96% and again shake well. Keep aside for 10 min. Shake each test tube and keep in water bath at 25°C–30°C for 20 min. Now measure the color at 490 nm. After that switch on the spectrometer, first take the absorbance (optical density [OD]) of blank and make it zero. Take the OD of all the test tubes and wash the cuvette each time after taking OD.

The values obtained of absorbance were helpful to plot a graph which is very crucial in estimating carbohydrate content. The values should be positive, above zero to plot a graph. After the plotting of a graph, the total amount of tcarbohydrate in the sample from glucose standard graph was calculated. The standard curve of absorbance was plotted at 490 nm on "Y" axis representing absorbance at 490 nm versus concentration of glucose in µg/ml on X axis [Figure 1].

From the above graph, we get an equation,

$Y = mx + c$

- Y = Absorbance of drug at 490 nm
- m = Mass
- x = Carbohydrate concentration of drug
- c = Velocity constant.

$Y = 0.195x + 0.029$

$(Y - 0.029)/0.195 = x$

- Absorbance corresponds to 0.1 ml of the test = x mg of glucose
- One hundred milliliter of the sample solution contains = x/0.1 × 100 mg of glucose = % of total carbohydrate present in it.[11,18]

Procedure for tobacco samples to be tested:

To have standard comparison of each sample, the procedure carried out for glucose sample was repeated in similar fashion with all the tobacco samples and their absorbance was determined at concentrations of 0.1, 2.5, 5, 10 µg/ml.

Results

The present study shows that Hathi-chhap bidi/ sample F has maximum absorbance (1.995) at 10 µg/ml which is a smoking form of tobacco followed by rest all smokeless forms of tobacco, i.e. sample C (0.452), sample B (0.253), sample D (0.077), sample E (−0.018), and sample A (−0.127), respectively [Table 1].

Similarly, sample F showed maximum absorbance followed by sample C, sample B, sample D, sample E, and sample A, respectively, at different concentration of 0.1, 2.5, 5 µg/ml [Table 1].

Discussion

In the present study, maximum absorption was seen with bidi followed by other smokeless forms of tobacco,

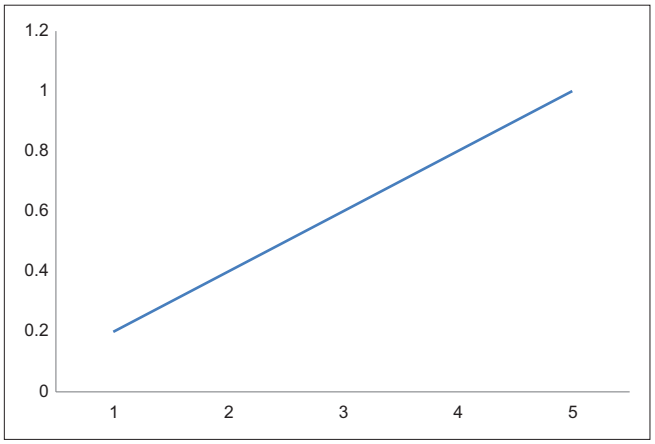

Figure 1: Standard curve of absorbance at 490 nm on "Y" axis representing absorbance at 490 nm versus concentration of glucose in µg/ml on X axis. From the above graph, we get an equation, Y = mx + c. Y = Absorbance of drug at 490 nm, m = Mass, x = Carbohydrate concentration of drug, c = Velocity constant

Table 1: Absorbance of tobacco samples at different concentrations

Concentration (µg/ml)	Absorbance					
	A	B	C	D	E	F
0.1	−1.995	−0.095	−0.058	−0.362	−0.428	−0.032
2.5	−0.427	−0.060	−0.053	−0.108	−0.236	−0.014
5	−0.192	0.005	0.146	−0.018	−0.066	0.534
10	−0.127	0.253	0.452	0.077	−0.018	1.995

i.e., sample C, B, D, E, and A, respectively. This in turn suggests that smoking form of tobacco has more carbohydrate content compared to smokeless forms of tobacco.

Phenol-sulfuric acid method was used for determining carbohydrate concentration as it is one of the most widely used colorimetric methods till date.[11] The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives, which further reacts with phenol to develop detectible color. Then, light absorption at 490 nm is recorded on a spectrophotometer.[12,16]

Carbohydrates are polyhydroxy compounds consisting of aldose or ketose sugar. Hence, placing the tobacco products for longer time in oral cavity increases the risk of dental caries, i.e. sugar and oral health are integrally related to each other.[19] Furthermore, more the frequency of tobacco consumption, more will be the risk for sugar exposure to that individual. This provided an impetus to estimate the carbohydrate in various tobacco products available in the near vicinity of study area.

We could not plot a standard absorbance curve at 490 nm of the tested samples as some of the values were negative, so only descriptive statistics for absorbance is given at different concentrations. As we could not plot the graph, Y = mx + c equation was not obtained, and hence, the carbohydrate content readings were not given by spectrophotometer. As the absorbance is directly proportional to carbohydrate content, it is predicted in the present study that as the absorbance increases, the carbohydrate content increases.

Among the samples tested, bidi which is smoking form of tobacco had more carbohydrate content compared to other smokeless forms of tobacco. It is supported by the findings of Jansen *et al*.[7] and Ramusino *et al*.[20]

Among the smokeless forms, sample C (Miraj) had maximum carbohydrate followed by sample B, D, E, and A, respectively. We could not compare the present findings with other similar studies as there were no such studies available in the previous literature. Many studies in the previous literature were conducted on smoking form of tobacco and there is paucity of literature regarding smokeless forms of tobacco.

The literature associating SLT use with either increasing or decreasing dental caries incidence is even harder to find compared to the literature associating smoking form of tobacco with dental caries. Theories have been postulated based on limited clinical findings, chemical analysis of the content of various ST products, and *in vitro* effects of ST on the growth of bacteria that has been implicated in caries development.[21]

Evidence linking ST use with increased dental caries prevalence has been reported.[22,23] In a case report by Croft, a 54-year-old patient presented "cervical caries" in the area of tobacco placement and gingivitis and recession with same tooth.[23,24] In contrast, Zitterbart *et al*. did not find any evidence of caries in the area of quid placement in their 36-year-old tobacco chewer.[23,25] Another study, which was performed among Swedish children did not report any prevalence of caries among snuff users.[26] On the other hand, higher prevalence of caries was observed in snuff dippers than in nontobacco users among teenagers in Gothenburg.[27]

There is abundance of literature which states that sugar is responsible for causing dental caries,[21,28-30] but in the present study, very less amount of sugar is present in tobacco products which might be inherently present or added in it. This shows that sugar is not responsible for directly causing caries in these individuals. Even though ST do not directly cause dental caries, placing tobacco product for longer duration in the oral cavity may lead to chronic irritation which in turn causes gingival recession, denudation of root surface of teeth that might increase the risk for occurrence of root caries.[10,31-33]

Although there is insufficient evidence, to conclude that SLT has a direct causal role in either caries formation or inhibition, literature suggests SLT do play an important role in caries activity and are harmful with respect to an individual's health.

Review of studies conducted on oral consequences of snuff and chewing tobacco use among professional baseball players in US found that ST use showed a significantly higher prevalence of root caries compared to nonsmokers.[34] Data from the multipurpose health survey conducted in USA showed that the mean number of decayed and filled root surface for those who used chewing tobacco was four times higher than for those who did not use tobacco. It is important to note that the decayed or filled surfaces tended to match the side of mouth on which the ST was used. The results showed that the mean number of decayed and filled root surface increased with increasing number of chewing tobacco packets used per week and duration of its use.[35]

The results obtained in the present study show that the absorbance was low of all the smokeless forms of tobacco compared to smoking form of tobacco which indicates very low or negligible quantity of carbohydrate present in it comparatively. This suggests less amount of inherent sugars as well as additives present in smokeless forms compared to smoking

form of tobacco. The types of sweeteners and sugars commonly found in ST are fructose, glucose, sucrose, maltose, and isomaltose.[36] This addition is presumed to be having a neutralizing effect on the bitter taste of tobacco.[37] Large variations in sugar among tobacco products can exist within form-to-form, brand-to-brand, and state-to-state. This may explain the diverse opinions of dental practitioners and investigators with respect to the concept of tobacco, increasing or decreasing incidence of dental caries.

The present study had its own limitations such as exact carbohydrate content of the samples was not estimated as some values obtained were negative due to which graph could not be plotted. The present study was done by phenol-sulfuric acid method taking financial constraint into consideration. Further research has to be conducted to determine carbohydrate content of different forms of SLT by various other techniques such as chromatography, capillary electrophoresis, infrared spectroscopy, light scattering detection, and nuclear magnetic resonance spectroscopy.

Conclusion

As the concentration of tobacco increases, the absorbance of the tobacco products (sample) increases which in turn is suggestive of increase in its carbohydrate concentration.

- Among the tested samples, maximum absorbance was seen with sample F (Bidi) which is a smoking form of tobacco
- Bidi had more absorbance that indicates more carbohydrate content compared to smokeless forms of tobacco
- Among the smokeless forms of tobacco, sample C (Miraj) had maximum absorbance that indicates maximum carbohydrate content followed by sample B, D, E, and A, respectively
- Carbohydrates in the form of sugars, either inherently present or added in it during manufacturing can serve as contributing risk factor for higher incidence of dental caries.

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Conflicts of interest

There are no conflicts of interest.

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