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STUDY OF COMPOSITION OF SALIVA WITH REFERENCE TO CHARACTERIZATION OF THE ACTIVITY AND STABILITY OF SALIVARY AMYLASE WITH REFERENCE TO EFFECT OF DIFFERENT SPICES

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ABSTRACT

The current appraisal included quantitative analysis of chemical composition of saliva samples after ingestion of 10 different types of spices and molasses of Ayurvedic importance followed by analysis of variations in Lugol's test results for ptyalin activity in saliva sample in different temperature conditions and also to explore highest Amylase activity. The results were obtained with reference to the control samples. In the present study highest, pH reduction in saliva was observed after ingestion of tamarind (Tamarindus indica) frequently used in most Indian foods and confectionaries followed by increase in fluoride content, decrease in salivary protein content and increase in glucose content. The results shows that no change in pH of saliva was observed after ingestion of black pepper (Piper nigrum) followed by increase in fluoride, salivary protein and glucose content. Similar results were obtained in case of Tulsi (*Ocimum tenuiflorum)* with slight change in pH followed by increase in fluoride, protein and glucose content. After qualitative analysis of the amylase activity it was found that the starch-degradation halos were greater in the plate containing saliva with Amla and Tulsi while smaller in the plate containing saliva with tamarind and clove. The results also shows that a slight decrease in fluoride content was observed after ingestion of Amla [Phyllanthus emblica]. No change in pH followed by increase in salivary protein and glucose content as well as greater starch-degradation halos were observed in saliva with molasses which can be preferred in place of cane sugar in confectionaries.

KEYWORDS: Spices, molasses, saliva composition, ptyalin activity, Lugol's test.

INTRODUCTION

From age old tradition in India, spices have been an integral part of every food preparation and are also employed in different traditional medicinal preparations against gastric disorders. In the present scenario with busy and contemporary life schedules; maintenance of proper oral health conditions has become a challenge in a healthy individual especially among the youths Thus keeping in mind the daily routine of youths one must give due importance to the salivary composition including protein, glucose and fluoride bioavailability, salivary flow rate, salivary pH, as well as psychological factors, such as anxiety, study or job related stress, distress and strain. However, we must keep in mind that the salivary composition in human population undergoes daily and seasonal fluctuations due to the influence of various physico-chemical, biological, psychological and environmental factors. It is a known fact that the amount of ptyalin differs in people of different races but in common besides increasing our defensive mechanisms, the salivary pH as well as proteins and glucose contents of saliva sustain the biota of the oral cavity and fluoride in addition protects our teeth from caries. The optimum conditions for ptyalin activity are a pH range of 5.6–6.9 and temperature of 37^0C [1]. The main purpose of this current project was to determine the relative composition of saliva with special reference to the enzymatic activity of salivary amylase after ingestion of different spices and molasses using the Lugol test (qualitative) under different temperature conditions.

MATERIALS AND METHODS

The present study aims to analyze the chemical composition of saliva samples after ingestion of 10 different types of spices and molasses of Ayurvedic importance and also to prepare a prototype for future investigations using following parameters and as follows:

Study design-This cross-over study included 10 healthy participants (21–22 yrs old). Prior consent regarding the study design and investigation were obtained from the participants before beginning the

study. The inclusion criteria were good oral health, and the exclusion criteria were salivary amylase activity after intake of 10 different spices and molasses.

Whole saliva sampling-Whole saliva was collected from each participants into 15 ml test tubes for the control. Followed by collection of stimulated saliva which was collected after 20 minutes of ingestion of 10 different spices and molasses. Whole saliva samples were centrifuged at 6000 rpm in microcentrifuge tubes for 05 minutes to collect the supernatant saliva for further immediate analysis.

Chemical analysis of Saliva- Determination of Fluoride in supernatant saliva was done by aqua check test method Determination of Total salivary protein concentration in supernatant saliva was done by Biuret method. Determination of Total salivary Glucose concentration in supernatant saliva by Trinder's method and determination of Salivary pH in supernatant saliva was done with the help of a digital pH meter.

Lugol's Test - Qualitative Measurement of the Effect of Temperature on Intraoral Hydrolysis of Starch by Salivary Amylase after intake of spices

1. The proposed method consists of the incubation of 100µl of undiluted stimulated saliva sample in triplicates in the holes of pre-prepared starch -agar gel plate at 4^0C (refrigerator) and at 40^0C (hot air oven/ incubator) for two different incubation period of 24 hours and 48 hours.
2. To observe the amylase activity, the plates are dyed by the addition of 2-3 drops of Lugol's iodine solution and rinsed with distilled water after 10 min.

RESULTS AND DISCUSSION

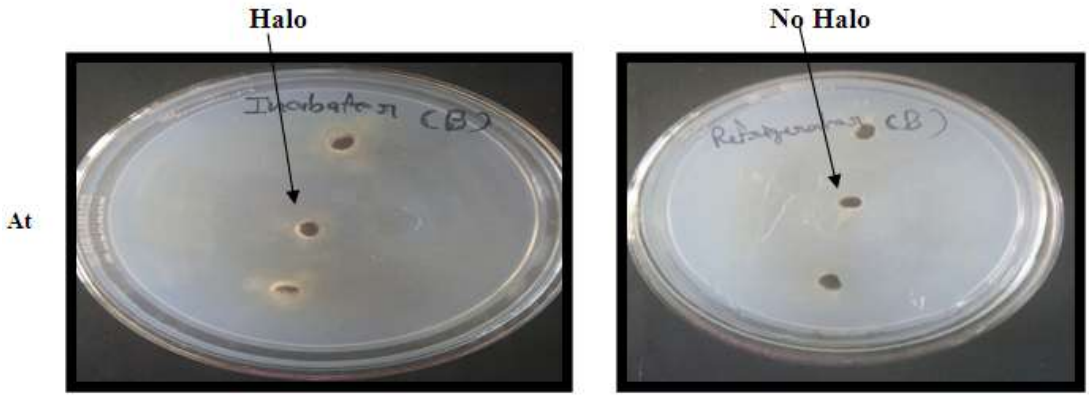
Saliva apart from being the most valuable oral fluid has nowadays become an useful tool for medical diagnosis and research for maintaining proper oral health conditions among the youths .As a result, it is extremely important to have a good knowledge of the salivary composition and function. Saliva consists of two fractions with different chemo-physical properties: supernatant saliva and salivary sediment. From physiological point of view, saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva is a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, which contains oral bacteria and food debris. The average daily flow of whole saliva varies in healthy individual between 1 and 1.5 L. On an average, unstimulated flow rate is 0.3 mL/min, with the average total for 16 hours of unstimulated flow (during waking hours) being 300 mL. Salivary flow during sleep is nearly zero and stimulated flow rate is, at maximum, 7 mL/min.[2] Stimulated saliva is reported to contribute as much as 80% to 90% of the average daily salivary production.[3]

Table 1: Composition of whole saliva before and after ingestion of different Spices and molasses [Control group and Test sample].

Food Items	pH		Fluoride in mg/l		Total Protein in mg/l		Glucose in mg/l	
	Control group	Test sample	Control group	Test sample	Control group	Test sample	Control group	Test sample
Clove *Syzygium aromaticum*	**8.4**	7.1	**2.5**	2.5	**4.4**	2.8	**26.2**	31.1
Amla *Phyllanthus emblica*	**9.9**	9.0	**2.5**	2.0	**5.5**	5.9	**35.4**	52.7
Turmeric milk *Curcuma longa*	**10.8**	8.8	**1.5**	2.0	**4.2**	5.8	**70.0**	62.8
Tamarind *Tamarindus indica*	**11.3**	7.4	**1.5**	2.5	**6.3**	6.0	**59.7**	67.2
Ginger *Zingiber officinale*	**8.3**	7.7	**1.5**	2.5	**4.2**	5.9	**64.7**	71.9
Tulsi *Ocimum tenuiflorum*	**8.0**	7.4	**1.5**	2.5	**5.0**	6.1	**50.0**	63.7
Ajwain *Trachyspermum ammi*	**8.6**	8.6	**1.5**	1.5	**14.5**	6.3	**66.1**	64.3
Black pepper *Piper nigrum*	**7.0**	7.0	**1.5**	2.0	**4.1**	6.1	**59.9**	74.8
Coriander *Coriandrum sativum*	**8.8**	7.0	**2.0**	2.5	**4.8**	6.0	**45.1**	69.0
Cane Molasses Jaggery	**7.4**	7.4	**1.5**	1.5	**4.6**	5.9	**63.4**	61.0

Table 2: Diameter (in cms) of halos representing Amylase activity of different saliva sample after ingestion of different spices and Molasses kept at 40 ^{0}C and 4 ^{0}C for 48 hours.

Incubation Temperatures in ^{0}C	Diameter (in cms) of halos representing Amylase activity of different saliva sample									
	A	B	C	D	E	F	G	H	I	J
	Clove	Amla	Turmeric milk	Tamarind	Ginger	Tulsi	Ajwain	Black pepper	Coriander	Cane Molasses
At 40^{0}C	0.0	1.5	0.43	0.26	0.4	0.7	0.1	0.2	0.6	0.66
At 4^{0}C	0.0	0.4	0.36	0.0	0.0	0.0	0.0	0.0	0.0	0.0



40^{0}C (Incubator) **At 4^{0}C (Refrigerator)**

Fig. 1: Representation of the amylase activity and results of the Lugol's qualitative test at 40 ^{0}C and 4 ^{0}C for 48 hours.

Salivary pH

The normal pH of saliva is 6 to 7, meaning that it is slightly acidic. The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow).[2] Remaining fermentable carbohydrates and the buffering capacity of saliva affect plaque pH, unless the pH of the plaque is too low for bacterial enzymes to function. The resting pH of plaque (that is, the pH of plaque 2 to 2.5 hours after the last intake of exogenous carbohydrates) is 6 to 7.3,37 The pH rises during the first 5 minutes after the intake of most foods. The pH then falls to its lowest level, to 6.1 or lower, approximately 15 minutes after food consumption. Unless there is additional ingestion of fermentable carbohydrates, the pH of plaque gradually returns to its resting pH of 6 to 7.[4] Thus, salivary buffering, clearance, and flow rate work in concert to influence intraoral pH [5] .During the present study variable changes were observed in pH value of saliva after the ingestion of different spices .The highest pH reduction was observed in tamarind (Tamarindus indica)with reduction in pH from 11.3(control) to 7.4 (test sample), no pH change was recorded in Cane Molasses [Jaggery] Ajwain [*Trachyspermum ammi]* and black pepper (Piper nigrum). However, in most cases reduction in pH was recorded. (Table -1). Naumova et.al. reported that at time intervals T0 to T3, the pH values were as follows: T0, 7.8 ± 0.60; T1, 7.5 ± 0.7 (p = 0.424); T2, 7.8 ± 0.7 (p = 1.0); T3, 8.3 ± 1.1 (p = 0.013); and T4, 8.4 ± 1.0 (p = 0.013).[6]

Salivary total protein content

In the present study ,the salivary protein content decreased sharply from 4.4 mg/lt (control group) to 2.8 mg/lt (test sample) after ingestion of clove (*Syzygium aromaticum*), while little change was observed from 6.3 mg/lt (control group) to 6.0 mg/lt (test sample) after ingestion of tamarind (Tamarindus indica). Throughout the remainder of the experiment, the protein concentration was found to increase greatly in all other cases (Table-1). Naumova et.al. reported baseline salivary protein content in the control subgroups as 0.81 ± 0.31 mg/ml and that the total protein concentration in the saliva of the stress subgroups significantly increased, immediately after stress exposure (T1).[6] Carpenter et.al. found an increase in the total protein concentration after stress exposure may be due to sympathetic activation during stress as the sympathetic innervation of the salivary glands controls protein secretion.[7]

Salivary Glucose content

In the present study, the salivary glucose content decreased from 70.0 mg/lt (control group) to 62.8 mg/ml (test sample) after ingestion of turmeric milk, slight decrease were observed from 63.4 mg/lt (control group) to 61.0 mg/lt (test sample) after ingestion of molasses (jaggery) and from 66.1 mg/lt (control group) to 64.3 mg/lt (test sample) after ingestion of Ajwain. However,in rest of the cases of spices significant increase in glucose concentration was found with highest increase from 45.1 mg/lt (control group) to 69.0 mg/lt (test sample)after ingestion of coriander, from 50.0 mg/lt (control group) to 63.7 mg/lt (test sample)after ingestion of Tulsi ,from 35.4 mg/lt (control group) to 52.7 mg/lt (test sample) after ingestion of Amla and from 59.9 mg/lt (control group) to 74.8 mg/lt (test sample) after ingestion of black pepper (Table-1). Naumova et. al. reported baseline salivary protein content in the control subgroups as 0.81 ± 0.31 mg/ml.[6] Satish Kumar et.al. reported that the salivary glucose levels showed a significant correlation with blood glucose levels, suggesting that salivary glucose levels can be used as a monitoring tool for predicting glycemic control in diabetic patients. Increased salivary glucose levels leads to increased oral candidal carriage; therefore, oral diagnosticians are advised to screen the diabetic patients for any oral fungal infections and further management.[8] Gupta et.al. reported that salivary glucose levels could serve as a potentially noninvasive adjunct to monitor glycemic control in diabetic patients.[9] Panchbhai et.al. found significant positive correlation between salivary glucose levels and the fasting blood glucose levels was seen in group with uncontrolled diabetes in the present study.[10]

Fluoride bioavailability in salivary supernatant

During the recent study period, higher fluoride concentration than that measured in control group were recorded after ingestion of tamarind, tulsi, black pepper and coriander. Slight increase in fluoride values were recorded from 1.5 mg/lt (control group) to 2.0 mg/lt (test sample) in case of turmeric milk and black pepper (1.5mg/lt to 2.0mg/lt) and for coriander (2.0mg/lt to 2.5mg/lt) while slight decrease from 2.5 mg/lt (control group) to 2.0 mg/lt (test sample) after ingestion of Amla was observed. No change in fluoride values were recorded after ingestion of clove, Ajwain and canesugar molasses (jaggery). A significant increase in values of fluoride was observed from 1.5 mg/lt (control group) to 2.5 mg/lt (test sample) after ingestion of tamarind, ginger and Tulsi (Table -1). Naumova et.al. reported at baseline (T0), the salivary sediment fluoride concentration in the control subgroups was between 0.796 and 3.15 ppm.[6] The highest fluoride concentration was reached immediately after tooth brushing (T2), but the fluoride concentration decreased within 30 min. (T4). Regarding fluoride bioavailability in supernatant saliva, a sudden decrease by T1 compared to baseline values in the control subgroups was observed. The presence of fluoride in saliva speeds up crystal precipitation, forming a fluorapatite-like coating more resistant to caries than the original tooth structure. In that sense, small amounts of demineralization have been suggested as advantageous for the tooth because enamel components of magnesium and carbonate are replaced with the stronger, more caries-resistant fluorapatite crystals.[2] Bioavailable fluoride enhances the mineralization of calcium and phosphate into hydroxyapatite, which remineralizes the crystalline structures of tooth lesions.[11]

Qualitative Measurement of the effect of Temperature on Intraoral Hydrolysis of Starch by Salivary Amylase after intake of spices

The methodological approach in the current study involves characterization of salivary amylase activity by gel diffusion method after ingestion of 10 different spices and molasses at two temperature conditions (4 ^{0}C and 40^{0}C) using two different incubation times (24 and 48 h). Using this methodology, we were able to obtain amylase activity halos only after 48 h of incubation at 40^{0}C. In regions where the starch had been degraded by amylase, clear halos appeared around the hole. The size of the halo was proportional to the amylase activity. Cristina et.al. observed different activity levels of the salivary amylase enzymes under different temperature and alkalinity conditions and it was interesting to note that ptyalin was affected to a greater extent by temperature than by pH.[1]

After qualitative analysis of the amylase activity using two different incubation times (24 and 48 h) and two different temperature conditions (at 40 ^{0}C and 4 ^{0}C) in the present study it was found that the starch-degradation halos were greater in the plate containing saliva with Amla [Phyllanthus emblica] as 1.5 cm, Tulsi(*Ocimum tenuiflorum)* as 0.7 cm and cane molasses as 0.66 cm and smaller halo of 0.1 cm in plate containing saliva with ajwain while no halo were observed in the plate containing saliva with Clove (*Syzygium aromaticum)* at 40 ^{0}C. Cristina et.al. found that the optimal temperature for the AAMY in saliva samples was 40^{0}C, which is consistent with the physiological context of the enzyme.[1] During the present investigation starch-degradation halos were not formed at 4^{0}C except in the plate containing saliva with Turmeric Milk **(***Curcuma longa) and* Amla [Phyllanthus emblica] [Figure -1]. The results of qualitative temperature assay at 40 ^{0}C and 4 ^{0}C during the current study period suggest that the enzyme activity at 40 ^{0}C seems to be higher than at 4^{0}C after 48 hours incubation period (Table -2).

CONCLUSIONS

To conclude, the observations of the current study supports the assumption that spices can be effective in reducing obesity and even carcinogenic effect among youths after long consumptions of starch-containing food items by enhancing the amylase activities in saliva and through degradation of starch. Therefore it is recommended to prepare more healthy snacks by enhancing the use of spices such as ginger, tulsi and ajwain of medicinal value. It is also recommended to increases use of molasses in dietary items. However, further investigations are necessary to appraise the role of salivary composition in maintaining in vivo homeostasis of mouth after ingestion of different food items particularly containing starch for maintenance of proper health conditions.

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