

# Effects of Cooked Starches and Sucrose or their Combination on Salivary $\alpha$ -Amylase Activity and Oral pH

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**Abstract:** *There are many questions about starch, sucrose and their combination in increasing the cariogenicity of dental plaque flora biofilm. This research aimed at evaluating the activity of salivary  $\alpha$ -amylase and oral pH, in the presence of either sucrose or cooked starches compared with when both are used together. Sixty adult males, who fulfilled the inclusion criteria according to a structured questioner interviews to assess their dental hygiene and nutritional habits, were recruited for the study, their salivary and dental plaque  $\alpha$ -amylase activity and pH measurement were assessed. Higher activity of salivary and dental plaque  $\alpha$ -amylase and lower pH were reported in the group who ate a meal containing cooked starches plus intake of sucrose in the form of soft drinks or sugared tea, compared to those subjects who only took sugars or a meal containing cooked starches. Results suggest that cooked starches when followed with sugars produce a stronger effect on salivary  $\alpha$ -amylase activity and in lowering oral pH, thus proposing an increased cariogenic activity of starch and sucrose combination.*

**Keywords:** Cooked Starches, Sucrose, Salivary  $\alpha$ -amylase Activity, Oral pH, Cariogenicity, Plaque biofilm

## 1. Introduction

Sucrose is considered the most cariogenic dietary carbohydrate due to its role in synthesis of extracellular glucans,<sup>(1)</sup> while starches were suggested to be slightly cariogenic when taken as the only source of carbohydrate in diet.<sup>(2)</sup> Recent studies have suggested that a combination of soluble starch with sucrose might be more cariogenic than sucrose alone.<sup>(3)</sup> This synergistic effect could be explained by starch fermentation by the bound dental plaque  $\alpha$ -amylase enzyme and the enhancement of formation of polysaccharides which are used as an energy source by the microbes,<sup>(4)</sup> which further secure their attachment to the pellicle-coated enamel, and also forming one of the stable components in any mature biofilm, the extracellular matrix.<sup>(5,6)</sup> The extracellular matrix retains nutrients and water and allows nutrients to pass these in between the channels to other microbes in the community,<sup>(7)</sup> which encourages acids to accumulate in the *Streptococcus mutans* biofilm.<sup>(8,9)</sup> Despite these findings, however, the role of starch in increasing the cariogenic potential of the plaque flora biofilm in the presence of sucrose has been recently questioned.<sup>(10,11)</sup> This study therefore aims at evaluating the effect either sucrose or cooked starches compared with their combination together on salivary  $\alpha$ -amylase activity and oral pH.

## 2. Subjects and Methods

### Study Design

In this retrospective observational study, sixty adult male volunteers were selected for the study on basis of structured questioner interviews to assess their oral hygiene. According to the questioner, they were to be excluded

from the study (exclusion criteria) if they had a known chronic illness, recent antibiotic medications and if they were on regular intake of caffeine (more than once daily). Their nutritional habits assessment included asking them about their recent intake, within the last 1-4 hours before conducting the interview and performing the tests, of a meal containing cooked starches and sugary sucrose component, whereby they were categorized into two groups: those who had cooked starches followed immediately by sugared tea or drinks (combination of cooked starches with sucrose), and those who had either soft sugary drinks (sucrose) or a cooked starchy meal.

### Study Procedures

**Saliva Sample Collection:** Approximately 3 ml of spitted whole mouth saliva was collected from the selected study subjects. Paraffin wax was chewed for 2 minutes as a stimulant after rinsing the mouth with water; collected saliva from each subject carried in a 15ml graduated centrifuge tubes, in an ice box to be stored at -20°C.

**Dental Plaque Sample Collection:** Immediately after saliva collection, plaque samples were carefully scaled supragingivally upon the selected teeth using sterilized dental curettes. The plaque sample was then transferred immediately to 1.5 ml Eppendorf tubes containing 0.5 ml of purified distilled water (water for injection) and transported in an ice box to be stored at -20 °C.

Salivary and dental plaque  $\alpha$ -amylase enzyme measurement:

**Salivary  $\alpha$ -amylase enzyme assay method:** On the day of the specified  $\alpha$ -amylase assay, stored saliva samples were cold centrifuged at 2000 rpm for 15 minutes. 1ml of clear

sample was transferred to 0.5 ml Eppendorf tube, for salivary  $\alpha$ -amylase enzyme assay. Salivary  $\alpha$ -amylase assay Kit (*Catalog No.1-1902*) is specially designed for the kinetic measurement of salivary  $\alpha$ - amylase activity.<sup>(12)</sup>The mean of duplicated absorbance read and the absorbance difference/minute was calculated. $\alpha$ - amylase activity was calculated according to a formula provided by the Kit (Fisher Scientific America). Levels are considered in this study according to the Kit high and low controls. Low  $\alpha$ - amylase activity was found to be <39 u/ml/minute, moderate activity =39-108 u/ml/minute and high activity =>108 u/ml/min. Absolute range in this study was 14-400u/ml/minute; Reference range<sup>(12)</sup>: Adult range, (n=75) mean = 92.4 u/ml, absolute range = 3.1 - 423.1 U/mL

**Plaque  $\alpha$ -amylase measurement:** Stored dental plaque samples in 0.5 ml purified distilled water, were weighed using a sensitive balance. Standard dental plaque mass of about 0.05 gm was chosen. Dental plaque samples were vortexed for 1 minute and then centrifuged at 2000rpm for 15 minutes. A sensitive balance was used for weighing: (*Denver instrument company*)/caloric weight variance (100-12.2mg). The clear supernatant was transferred, to 1.5ml Eppendorf tubes, ready for alpha amylase measurement as for salivary amylase measurement. The same salivary  $\alpha$ -amylase assay kit was used for the dental plaque  $\alpha$ -amylase enzyme activity assay. The dilution step (3) in the Kit was cancelled. Another step was added including preparing standard dental plaque mass around 0.05gm in 0.5 ml of purified distilled water and hence

another dilution factor and concentration had been put into consideration. Absolute range obtained for dental plaque  $\alpha$ -amylase in grams in this study was  $0.03-5 \times 10^3$  u/gm/minute (no reference for this range).

**Ethical Issues:** All participants of the study signed an informed consent form. There is no conflict of interests observed, all authors have seen and approved the manuscript being submitted.

### 3. Results

Significant higher activity of salivary amylase was noted on the eleven individuals who reported to have taken a meal composed of cooked starches followed by taking a sugary drink, compared to its activity in individuals who had taken either a meal containing cooked starches not followed with sugars or sugars alone without starchy meal (p-value=0.012 and 0.014 respectively)[Table 1]. Similarly significant higher activity of dental plaque  $\alpha$ - amylase reported after a meal composed of cooked starches-containing meal followed with sugars when compared with its activity with a starchy or a sugary intake only (p-value=0.020 and 0.004 respectively)[Table 2]. A significant lowering in salivary pH following a meal composed of cooked starches followed by taking a sugary drink is observed, when compared with the pH following a meal containing a starchy component not followed by a sugary drink (p-value= 0.000)[Table 3].

**Table 1:** Comparing the effect of a meal type on salivary  $\alpha$ - amylase activity:

Meal components	Salivary $\alpha$ - amylase (u/ml/min)			Salivary $\alpha$ - amylase (u/ml/min)		
	N	Mean $\pm$ SD	Meal components	N	Mean $\pm$ SD	p-value
A meal containing starch	43	114.23 $\pm$ 68.44	Sugars	6	86.16 $\pm$ 44.28	0.353
A meal containing starch +sugars	11	176.70 $\pm$ 80.31	Protein+ lipid+ starch	43	114.23 $\pm$ 68.44	<u>0.012</u>
Sugars	6	86.16 $\pm$ 44.28	Protein, lipid +starch +sugars	10	176.70 $\pm$ 80.31	<u>0.014</u>

**Table 2:** Comparing the effect of a meal type on plaque  $\alpha$  - amylase activity:

Meal components	plaque amylase(u/gm/min)			plaque amylase(u/gm/min)		
	N	Mean $\pm$ SD	Meal components	N	Mean $\pm$ SD	p-value
A meal containing starch	43	2.003 $\pm$ 1.475	sugars	6	0.530 $\pm$ 0.381	<u>0.020</u>
A meal containing starch + sugars	11	2.704 $\pm$ 1.451	Protein+ lipid+ starch	43	2.003 $\pm$ 1.475	0.147
Sugars	6	0.530 $\pm$ 0.381	Protein+ lipid+ starch +sugars	11	2.704 $\pm$ 1.451	<u>0.004</u>

**Table 3:** Comparing the effect of a meal type on salivary pH

Meal components	Saliva pH			Saliva pH		
	N	Mean $\pm$ SD	Meal components	N	Mean $\pm$ SD	p-value
A meal containing starch	43	7.89 $\pm$ 0.52	sugars	6	7.58 $\pm$ 0.29	0.153
A meal containing starch + sugars	11	7.23 $\pm$ 0.42	Protein+ lipid+ starch	43	7.89 $\pm$ 0.52	<u>0.000</u>
Sugars	6	7.58 $\pm$ 0.29	Protein+ lipid+ starch +sugars	10	7.23 $\pm$ 0.42	0.169

A possible relationship was detected between the activity of salivary  $\alpha$ - amylase and salivary and dental plaque pH when related to meal type Fig.[1-3]

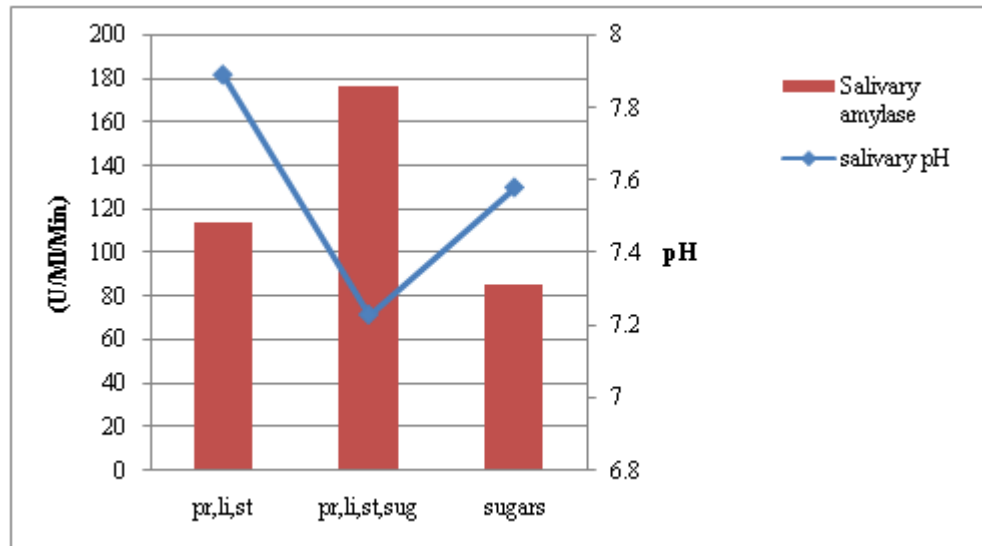


Figure 1: Relationship of salivary  $\alpha$ -amylase with salivary pH when related to meal type

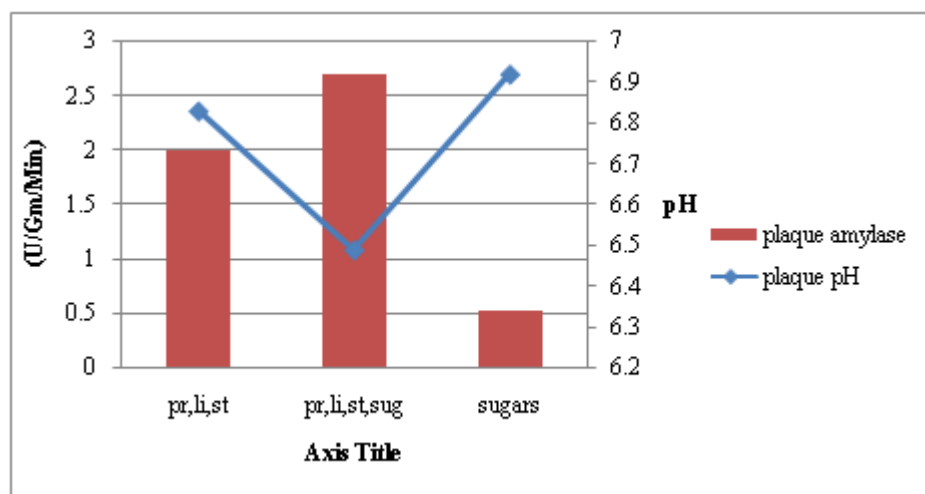


Figure 2: Relationship of plaque  $\alpha$ -amylase enzyme activity with plaque pH when related to meal type

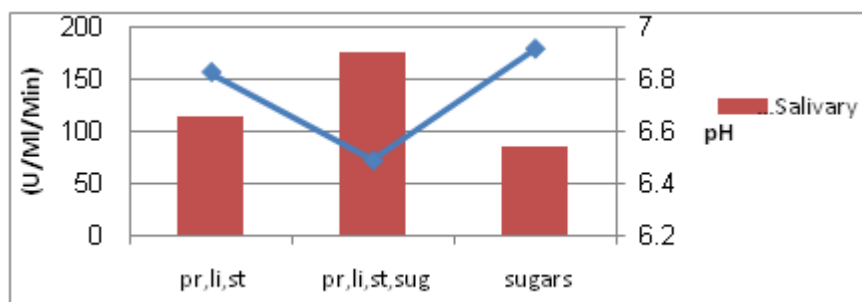


Figure 3: Relationship of salivary  $\alpha$ - amylase activity with plaque pH when related to meal type

#### 4. Discussion

The role of starch in increasing the cariogenic potential of the plaque flora biofilm in the presence of sucrose has been recently questioned.<sup>(10,11)</sup> Our findings, however, support this cariogenicity of starch, as such going in line with those studies that suggest the synergetic combination of starch and sucrose in causing dental caries by increasing the activity of salivary  $\alpha$ -amylase.<sup>(12)</sup> Dodds and Edgar have suggested that starch fermentation may be enhanced by prior plaque exposure to sucrose.<sup>(13)</sup> Bowen also suggested that sweetened starches were more cariogenic in rats than just sucrose alone.<sup>(14)</sup> Others explained these

observations by the synergistic effect between starch and sucrose or that cooked or sweetened starches are more sticky and retentive than sucrose alone.<sup>(15)</sup> In the light of these observations, a synergistic effect between sucrose and starch may be suggested to be due to the enhanced fermentation of starch by plaque-bound  $\alpha$ -amylase with a subsequent increase in caries activity. Significant lowering of pH with starch in the presence of sucrose observed in our research, is also supported by Lingstrom *et al.*,<sup>(16)</sup> who, when testing the effect of a series of processed starch and sucrose on plaque pH, found that glucose and sucrose reference solution showed the greatest pH drop in the plaque.

## 5. Conclusion

It is to be concluded that synergistic effect exists between cooked starches and sucrose on salivary and dental plaque  $\alpha$ -amylase activity, and that a drop of salivary and dental plaque pH after combination of cooked starches and sucrose is due probably to the higher salivary amylase activity, thus suggesting an increased risk of dental caries with combination of cooked starches and sucrose, an observation that might have an important nutritional implication in the preventive strategies against dental plaque and caries formation.

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