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α-Amylase Inhibitors in Foodstuffs

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ABSTRACT

The incidence of dental caries in children of South Karnataka is very high. An important etiological factor influencing the development of caries is the type of food consumed. Salivary amylase exerts a protective effect over teeth by hydrolysing large polysaccharide molecules left in the oral cavity which could otherwise serve as a matrix to encompass bacteria and result in tooth decay. Inhibitors of α-amylase present in these foodstuffs can therefore reduce this protective effect. The inhibition of salivary α-amylase of a group of 10 school children by some of the most commonly consumed local foodstuffs was studied. Rice preparations (rice idli, *rice gruel and boiled rice), fish fry and banana did not have any effect on salivary amylase activity. A statistically significant effect was observed with the sweet preparations (* kadla payisa *and* kesari baath*), and the fried foods (potato chips and groundnuts). Inhibition by other foodstuffs (* dosa, idli *and* upma*) was slight and not significant. Considerable variations in basal amylase activity and susceptibility of α-amylase of individuals to inhibition by particular foodstuffs were also observed. It is possible that some component in saliva may be able to moderate the action of the inhibitor in resistant individuals.*

INTRODUCTION

Many plants are known to have naturally occurring proteinaceous inhibitors of proteases and α-amylase. α-Amylase inhibitor was first

reported from buckwheat (Chrzaszcz & Janicki, 1934) and later from wheat (Kneen & Sandstedt, 1946; Shainkin & Birk, 1970; Saunders & Lang, 1973; Silano *et al.*, 1973), rye (Kneen & Sandstedt, 1946; Marshall, 1977), cereals (Shivaraj & Pattabiraman, 1980; Mundy *et al.*, 1983), legumes (Jaffe *et al.*, 1973) and tubers (Narayan Rao *et al.*, 1970; Sharma & Pattabiraman, 1982).

Bessho & Kurosawa (1967) tested the heat inactivation of amylase inhibitors in flour using pancreatic amylase. They found that the inhibitory activity decreased with length of baking time of bread and cakes, but that more than half the activity remained in cakes and about 13% of the activity remained in bread after baking. Gertzmann *et al.* (1980), on the other hand, used a single purified inhibitor from wheat protein and assessed the susceptibility of salivary α-amylase in a large population of human subjects. They found that the α-amylase of some individuals was completely inhibited by the purified inhibitor while others had a salivary amylase type totally resistant to the effects of the inhibitor.

With these points in mind; namely, that foodstuffs contain inhibitors of α-amylase which may not be entirely destroyed on cooking and that individuals have salivary amylase with different susceptibilities to inhibition by these inhibitors, the present study was undertaken to investigate the effect of some of the most commonly consumed foodstuffs on the salivary amylase of a group of school children. The study is more meaningful since the inhibitory effect of foodstuffs in the form in which they are normally consumed, rather than in a highly purified form, was tested.

MATERIALS AND METHODS

Materials

The following food items, which comprise the most routine weekly diet of a child of average socio-economic group in and around Manipal, South Karnataka, were tested for inhibitory activity:

Fruits:	Banana
Fried foods:	Potato chips, groundnuts, fish fry
Breakfast items:	*Dosa*, *idli* (rice, *urad*)
Snacks:	*Upma*
Meals:	Boiled rice, *ganji*, yogurt

(Brief recipes of the food items are included at the end of this paper.)

Collection of saliva

Ten school children aged between 9 and 11 years were selected for this study. Their teeth were healthy and cleaned professionally by a dentist. The basal

saliva was collected in glass beakers and then transferred to chilled glass tubes. Next the children were asked to chew a weighed amount each of the foodstuffs for a constant period of time (about 30 s) and then asked to spit out the food. The saliva collected within the next few minutes represented the stimulated saliva samples. All the samples were centrifuged at 5000 × g for 5 min and kept at 0°C. Assays were usually done immediately.

Enzyme activity determination

α-Amylase activity of the basal and stimulated saliva samples were estimated as described by Bernfeld (1955) using 1% starch as substrate. The assay medium contained 30 μmoles of sodium phosphate buffer, pH 6·9, containing 10 μmoles of sodium chloride and 0·2 ml of suitably diluted enzyme (1:100) in a total volume of 1·5 ml. This was preincubated at 37°C for 10 min and the reaction started by the addition of the substrate. After 5 min incubation, the reaction was arrested by the addition of 1 ml of 3,5-dinitrosalicylate reagent. The contents were kept in a boiling water bath for 10 min, cooled under tap water and diluted to 11 ml with distilled water and the optical density read at 540 nm. Enzyme activity was expressed as OD units per milligram of protein. Inhibition was assessed by comparing amylase activity of stimulated saliva samples to that of the respective basal samples. Student's '*t*' test was done for statistical evaluation.

Protein estimation

Protein content in basal and stimulated samples was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Protein

Table 1 shows the mean protein content in saliva in mg/ml. The mean protein content in basal saliva samples varied from 1·50 ± 0·19 mg/ml (potato chips) to 2·23 ± 0·93 mg/ml (*kadla payisa*). The mean basal protein contents were comparable in all cases except *kadla payisa* and groundnut. The slightly higher values obtained here could be due to physical stimulation. The protein contents in the stimulated saliva samples were consistently higher, as expected, than the basal samples. The highest mean value was obtained with *dosa* (3·71 ± 1·31) and the lowest with yogurt (1·66 ± 0·28). Applying Student's '*t*' test, it was found that the difference in protein content between basal and stimulated saliva was not statistically significant

TABLE 1
Protein Content in Basal and Stimulated Saliva Samples[a] (mg/ml)

Food	*Basal*	*Stimulated*
Dosa	2·1 ± 0·81*	3·56 ± 1·13
Urad idli	1·81 ± 0·42*	2·58 ± 0·90
Rice *idli*	1·62 ± 0·44	2·48 ± 0·93
Ganji	1·60 ± 0·41	1·76 ± 0·46
Boiled rice	1·70 ± 0·41	2·00 ± 0·44
Upma	1·64 ± 0·54	2·36 ± 0·96
Kadla payisa	2·22 ± 0·93	2·24 ± 0·66
Kesari baath	1·69 ± 0·68*	2·73 ± 0·78
Potato chips	1·50 ± 0·19**	2·66 ± 0·60
Groundnuts	1·92 ± 0·69*	3·10 ± 1·08
Fish fry	1·54 ± 0·53	1·82 ± 0·54
Banana	1·50 ± 0·41	1·76 ± 0·40
Yogurt	1·54 ± 0·48	1·66 ± 0·28

* Significant; ** HS = Highly Significant.
[a] Number of samples = 10.

(P value < 0·1, 0·2) in the cases of boiled rice, *ganji*, *kadla payisa*, *upma*, fish fry, banana and yogurt. A significant difference (P value < 0·01, 0·05) was seen with *dosa*, *urad idli*, groundnuts and *kesari baath*. A highly significant difference was observed only with potato chips ($P < 0·001$). Burgen & Emmelin (1961) and Schneyer & Hall (1966) have also reported that total protein in saliva increases with flow rate and parasympathetic stimulation.

α-Amylase activity in basal and stimulated saliva samples

Table 2 depicts the amylase activity in basal and stimulated saliva samples expressed as OD units/mg protein. Highest mean amylase activity in basal saliva was seen in the case of *idli* (239 ± 97 units/ml) while the lowest was observed with potato chips (109 ± 22). Mean amylase activity in stimulated saliva was depressed with the consumption of *dosa*, *urad idli*, *upma*, *kadla payisa*, *kesari baath*, groundnuts and yogurt. Of these, groundnuts and the sweet dishes (*kadla payisa*, *kesari baath*) were most potent inhibitors of salivary amylase.

Applying Student's '*t*' test, inhibitions by *dosa*, *urad idli*, *upma* and yogurt were not statistically significant, while those with groundnut and the sweet dishes were statistically significant. Potato chips, fish fry, banana and rice preparations (rice *idli*, boiled rice, *ganji*) did not have any inhibitory action. In fact, in these cases, a higher amylase activity in stimulated saliva

TABLE 2
Amylase Activity in Basal and Stimulated Saliva Samples[a] (OD Units/mg Protein)

Food	*Basal amylase activity*	*Stimulated amylase activity* (in vivo)	*Basal amylase activity + food extracts* (in vitro)
Dosa	193 ± 84	132 ± 54	—
Urad idli	237 ± 97	190 + 82	—
Rice *idli*	159 ± 73	191 ± 73	161·8 ± 65
Ganji	176 ± 52	184 ± 60	178·2 ± 50
Boiled rice	180 ± 50	182 ± 57	180·3 ± 48
Upma	157 ± 51	125 ± 68	—
Kadla payisa	123 ± 50	81 ± 41*	—
Kesari baath	177 ± 93	93 ± 69*	—
Potato chips	109 ± 22	142 ± 23*	174·7 ± 20
Groundnuts	148 ± 68	88 ± 83*	—
Fish Fry	189 ± 69	204 ± 75	214 ± 41
Banana	150 ± 60	173 ± 54	163·77 ± 32
Yogurt	228 ± 74	169 ± 71	—

* Significant.
[a] Number of samples tested = 10.

than in basal was observed. In order to simulate the in-vivo finding, in-vitro extracts of the foodstuffs which has a stimulatory action on amylase were incubated with the basal saliva samples and assayed similarly. The in-vitro results were also increased and comparable to the in-vivo findings (Table 2). Kneen & Sandstedt (1946) and Granum (1979) had reported no amylase inhibitory activity in rice and potato, respectively. Hence the increased amylase activity in the respective stimulated saliva samples could be due to endogenous amylase activity in the foodstuffs.

The pH optimum for α-amylase activity being around 6·8–7·0, it was expected that upon the consumption of yogurt (pH around 4·5), a sharp fall in amylase activity would result. However, this was not observed. The decrease in the stimulated saliva sample was not statistically significant. In-vitro studies showed that yogurt possessed low endogenous amylase activity. It is possible that saliva is able to buffer the pH change adequately.

In general, the basal amylase activity showed considerable variation from child to child. Aguirre *et al.* (1987) have also reported wide individual variations in amylase concentration. A high carbohydrate diet has been correlated to increased amylase activity (Squires, 1953). In addition, the susceptibility of salivary amylase to inhibition by a particular foodstuff also varied. Gertzmann *et al.* (1980), in their study on the action of purified wheat inhibitor on the salivary amylase of a large population of people, also

observed a difference in susceptibility to the inhibitor. It is possible that, in the inhibitor-resistant types, lack of inhibition may be an expression of an indirect effect, where some component in saliva is able to moderate the action of the inhibitor. Large polysaccharide molecules, if left as such in the oral cavity, could serve as a matrix to encompass bacteria and result in plaque formation. Bacteria anaerobically metabolize carbohydrates and produce acid. Acid pH is favourable for demineralization of tooth and results in formation of caries (Guggenheim, 1968). Other workers (Krasse, 1965; Frostell *et al.*, 1967) have reported that, although sucrose is responsible for caries development, maltose and starch hydrolysates (products of salivary amylase digestion) do not favour plaque formation and subsequent caries development. In view of the fact that certain individuals' amylases are more susceptible to inhibition than others, and since salivary amylase exerts a protective effect over teeth by preventing the formation of polysaccharide matrix for bacteria, it is quite possible that there exists a definite relationship between an individual's amylase type, diet and caries susceptibility. Since the incidence of dental caries in children of this locality is very high (Shetty & Tandon, 1988), a large population study is now in progress to further substantiate this view.

Brief recipes of the food preparation

Dosa: Made up of rice and black gram (3:1 w/w), soaked for a few hours, ground to a fine paste, allowed to ferment overnight. This batter is spread out on a pan like a flat pancake.
Idli: Can be made up of rice alone or rice and black gram (3:2 w/w) soaked for a few hours, ground to a thick, coarse paste, allowed to ferment and steamed as small cakes.

Upma: Coarse wheat powder, fried with a little oil, cooked with water, with salt, seasoning.
Ganji: Boiled rice gruel.
Kesari baath: Like *upma*, but instead of salt and seasoning, sugar along with raisins and cashew nuts is added.
Kadla payisa: Sweet dish prepared by boiling bengal gram with jaggery to which fresh coconut milk is added.

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