

# Influence of amylase, trypsin and pepsin on the progression of dental erosion

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## ABSTRACT

**Aim:** The gastric and pancreatic enzymes could perhaps degrade the organic matrix of eroded dentine. This study sought to investigate whether pepsin, trypsin, or the amylase have an influence on erosive mineral loss in dentine and whether they are able to degrade the organic matrix. **Materials and Methods:** The saliva samples were taken from the dental problem facing subjects and the influence of amylase, pepsin, and trypsin enzymes was checked. The enzymes were assayed using standard methods and the absorbance was recorded. **Results:** The levels of the amylase, pepsin, and trypsin present in saliva of eroded tooth subjects were found to be significantly higher ( $P < 0.001$ ) than the level of these enzymes in saliva samples from normal individuals. **Conclusion:** The combined impact of pepsin, amylase, and trypsin increases the dentine erosion. This could be one reason for the fast proceeding of dental erosion in patients with chronic vomiting and ulceritis.

**KEY WORDS:** Amylase, Dental erosion, Enzymes, Pepsin, Trypsin

## INTRODUCTION

Eating disorders are psychosomatic diseases with a prevalence in western societies in 16–35-year-old females.<sup>[1,2]</sup> Bulimia nervosa, one form of these disorders, is characterized by repeated episodes of intake of large quantities of high-calorie food and self-induced vomiting and episodes of restrictive ingestion oftentimes in combination with frequent consumption of fruit, vegetables, and acidic beverages during the restrictive period. The most common cause of erosion is by acidic foods and drinks.<sup>[3]</sup> All these intrinsic and extrinsic acids can lead to dental erosion, particularly in patients with regular vomiting.<sup>[4,5]</sup> Similar applies for patients with gastro-oesophageal reflux disease (GERD). In these patients, the gastric juice reaches the oral cavity with either high frequency or with long duration<sup>[6]</sup> resulting in higher risk for dental erosion.<sup>[7]</sup>

In enamel, erosion is a surface phenomenon where the acid impact leads to a centripetal mineral loss.<sup>[8]</sup> The rapid progress of enamel loss in patients with bulimia nervosa can be explained by the high acidity and

erosivity<sup>[9]</sup> of the hydrochloric acid that is contained in the gastric juice.<sup>[10]</sup>

However, in dentine, the erosive tissue loss is not a simple surface process, and the acid impact leads to an exposure of the organic structures.<sup>[11]</sup> As the matrix is not soluble by clinically relevant acid impacts, it remains on the surface whilst mineral loss continues. The progression of erosive tissue loss is inversely related to the exposure of the organic matrix; the thicker the matrix becomes the lower the mineral loss proceeds because it acts as a diffusion barrier.<sup>[12,13]</sup> However, in case of an enzymatic removal of the organic structures, the erosion progression rate increases.<sup>[12,14,15]</sup>

The organic matrix (mainly composed by collagens) is protected against enzymatic degradation as long as it is mineralized, but after demineralization, it can be assailed by enzymes.<sup>[12,15-17]</sup> During GERD, the gastric enzyme pepsin can reach the oral cavity.<sup>[18]</sup> Therefore, it is quite conceivable that during vomiting pepsin or even enzymes from pancreas, like trypsin, could also reach the oral cavity and impact the dental tissues. It has been previously shown that pepsin is able to degrade the isolated organic dentine structures completely after a harsh and less physiologic incubation time of more than 3 days.<sup>[16,17]</sup>

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The aim of this study is to evaluate the influence of amylase, trypsin, and pepsin on the progression of dental erosion.

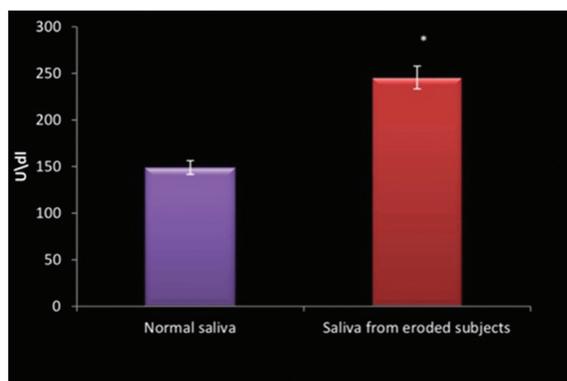
## MATERIALS AND METHODS

The saliva samples were collected from healthy controls and patients with eroded teeth. Concentration of amylase, pepsin, and trypsin was assayed. Amylase was assayed using direct substrate method with working reagent. Pepsin was assayed according to the method of Anson (1938) using hemoglobin as substrate. Trypsin was assayed using chymotrypsin inhibitor solution and trypsin inhibitor solution. The results were statistically analyzed and p value was calculated.

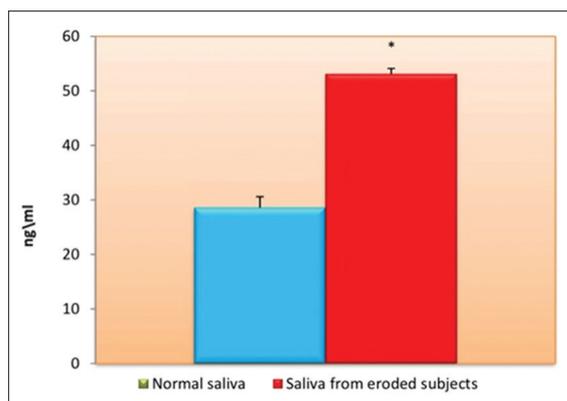
## RESULTS AND DISCUSSION

The levels of the amylase, pepsin, and trypsin present in saliva of eroded tooth subjects were found to be significantly higher ( $P < 0.001$ ) than the level of these enzymes in saliva samples from normal individuals. Figure 1 shows the level of amylase in normal saliva and saliva from eroded subjects. Similarly, Figure 2 shows the level of pepsin in normal saliva and from eroded sample. The level of trypsin in eroded subject saliva and normal saliva samples is shown in Figure 3. From all the figures, it is observed that there is a statistically significant difference in the levels of enzyme between both the groups.

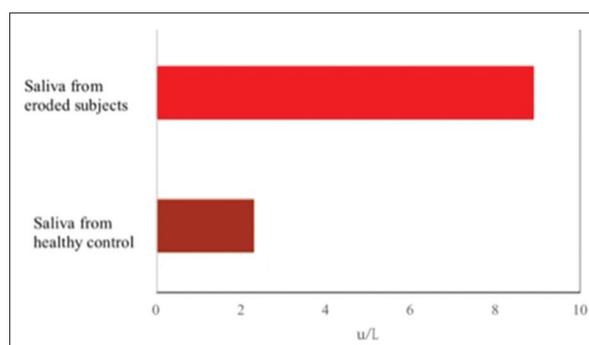
Until now, the impact of various enzymes on the eroded organic dentine matrix has been only rarely a focus of research; in particular, very little is known about the impact of different digestive enzymes on these organic structures and their influence on erosive tissue loss. The experimental design of this study should simulate the situation that could possibly occur in patients with eating disorders in combination with vomiting (bulimia nervosa). Even if nothing is published about the occurrence of digestive enzymes in the oral cavity in these patients, it is all but certain that the gastric enzyme pepsin reaches the oral cavity during vomiting. In addition, also intestinal enzymes (e.g., trypsin) can be present in the stomach due to reflux of duodenal content.<sup>[19]</sup> Therefore, it is also most likely that this enzyme is as well regurgitated. Not only in case of eating disorders but also in patients with GERD the presence of digestive enzymes in the oral cavity can be relevant. Pepsin has been found in the mouth of these patients.<sup>[18]</sup> Moreover, also a reflux of duodenal secretion, containing bile acids and trypsin, into the stomach is often a symptom of severe cases of GERD.<sup>[20]</sup> The duodenal secretion even often reaches the lower esophagus,<sup>[21]</sup> and therefore, potentially the oral cavity.



**Figure 1:** Level of amylase in normal saliva and saliva from eroded subjects



**Figure 2:** Level of pepsin in normal saliva and saliva from eroded subjects



**Figure 3:** Level of trypsin in normal saliva and saliva from eroded subjects

Pepsin has its pH optimum between pH 1 and 3. Therefore, it was added to the HCl solution at a physiological concentration (750 mg/ml);<sup>[10]</sup> the demineralization solution had a pH comparable to this of gastric juice.<sup>[22]</sup> However, trypsin has its pH-optimum at pH 7–8 and needs calcium ions for an optimal activity.<sup>[23]</sup> Trypsin can be reversibly inactivated at low pH (i.e., during vomiting) and is reactivated with an increase of pH in the presence of calcium ions, that is, during neutralizing by saliva.<sup>[24]</sup> It is known that trypsin can only degrade the native collagen matrix in eroded dentine to a limited extent.<sup>[25,26]</sup>

Amylase is one of the main enzymes present in saliva which helps in the breakdown of starch. Amylase may be measured for purposes of medical diagnosis. A higher than normal concentration may reflect one of several medical conditions, including acute inflammation of the pancreas and peptic ulcer.<sup>[27]</sup> From this study, it was observed that the level of amylase was high in the saliva taken from eroded dentine sample. In patients with reduced salivary flow rate, acid clearance is reduced and less dilution of acid will be present on attack of the tooth surface, contributing to erosion progress, especially where there is a direct contact with the acid.<sup>[28]</sup>

Regarding the results of the present study, the levels of the amylase, pepsin, and trypsin present in the saliva of eroded tooth subjects were found to be significantly higher ( $P < 0.001$ ) than the level of these enzymes in saliva samples from normal individuals. In a study done by Schlueter *et al.*,<sup>[29]</sup> it was observed that the combined impact of pepsin and trypsin leads to an increase in mineral loss (45%). It is believed that trypsin only changes the structure of the organic matrix on an ultra-structural level or destabilized the tertiary structure of the collagen molecule without splitting the amino acid chain itself. This procedure possibly opened more binding sites for pepsin and made the matrix more accessible for this enzyme, resulting in increased degradation.<sup>[29]</sup>

## CONCLUSION

The digestive enzyme pepsin, amylase, and trypsin were able to degrade the eroded organic dentine matrix. The combination of both pepsin and trypsin leads to an increase of erosive mineral loss, which was probably a result of increased matrix digestion. This mechanism could play a role in patients with eating disorders in combination with vomiting who suffer from dentine erosion because both enzymes could possibly reach the oral cavity during vomiting and affect the eroded dental hard tissue.

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