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Thermal Stability Study of Human Salivary α-Amylase

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ABSTRACT

Saliva is a watery substance produced in the salivary glands. It contains 98 percent of water along with electrolytes, various enzymes, and antibacterial components. During the rise of forensic science, investigators used to collect blood, urine, and other biological fluids for personal identification, and over the last decade, there has been a growing interest in saliva and its use as a diagnostic tool and an alternative for blood and urine. The advantage of saliva over other fluids is its non-invasive methods for collection, even by individuals with limited training and avoidance of private functions while collection. Saliva can be a piece of evidence in crimes involving bite marks, lip prints, or in drinking cases. The saliva collected can be used to identify the species, sex, if there is any drug abuse, and also for individualization. Saliva consists of many enzymes, and the enzyme of our importance in salivary α-amylase. In this article, we have studied the stability of the salivary α-amylase enzyme at different temperatures and on different types of surfaces. It has been concluded from the study that the activity of the salivary α-amylase in human saliva decreases as the temperature and the time of deposition increases, which causes inactivation of the enzyme. Saliva being important biological evidence, it should be collected from the crime scene as soon as possible and should be preserved at low temperature immediately. The analysis of the sample should be carried out as early as possible to get accurate results.

Keywords: Salivary amylase, Thermal stability, Biological evidence, visible spectrophotometer, DNS method.

INTRODUCTION

Saliva is a watery substance, secreted by salivary glands (Delporte and Steinfeld, 2006, Varga, 2012). It contains 98 percent of water along with electrolytes, various enzymes, antibacterial components (Sánchez-López E *et al.,* 2020) and is constantly present in the mouth of humans and other vertebrates. It is composed of water, mucus, proteins, mineral salts, and amylase (van *et al.,* 2007, Davies *et al.,* 1998). Salivary analysis has become

a significant source for the evaluation of salivary conditions with physiologic and pathologic implications and is a useful tool for disease diagnosis, mainly due to its chemical composition, role, origin, and interactions with other organ systems (Lima *et al.,* 2010).

The added advantage of its non-invasive method of collection even by individuals with limited training and avoidance of private functions while collection, cost-effective nature, makes saliva a popular fluid for forensic analysis and easy for collection. Saliva is often detected in scenes of crime along with bite marks, drinking cases, or lip prints where the oral cavity may have been involved (Lester *et al.,* 2010). In cases of saliva- derived from bite marks of unknown animals, blood grouping, and species-specific profile using electrophoresis can help in the identification of the animal in question. Saliva is also an analytical tool in cases of heavy metal poisoning by reflecting the ionic imbalance and excretion of certain poisons through this route (Eisenberg *et al.,* 2016, Hostýnek *et al.,* 1993). Heavy metal poisoning can be detected by using atomic absorption spectroscopy (Willis, 1962). Saliva can also be used for personal identification, with the help of DNA fingerprinting. It can be helpful in paternity disputes and for sex determination also (Jobling, *et al.,* 1997). The presence of saliva can be confirmed by performing a starch amylase assay whose product can be detected by DNS reagent (Doane, 1969).

MATERIALS & METHODS

- Maltose
- Sodium hydroxide (NaOH)
- Dinitro Salicylic acid. (DNSA)
- Sodium potassium tartrate
- Potassium dihydrogen phosphate (KH_2PO_4)
- Potassium Hydroxide (KOH)
- Sodium chloride (NaCl)
- Starch
- Diastase

METHODOLOGY

Plotting Standard Graph

A standard maltose solution was prepared and various dilutions were prepared from it. Then a DNS solution was added to them and incubated. Then their absorbance was recorded at 540nm and a standard graph was prepared.

Preparation of Surfaces with Saliva Sample

Three different surfaces were selected namely, glass, cloth, and cigarette bud, and were disinfected with ethanol. Gargled saliva was then collected from a healthy individual and was spread on the selected surfaces. All the samples were incubated at different temperatures for different time intervals. The saliva was then collected with the help of sterile cotton swab buds. Then the collected samples were extracted with sterile saline.

Assay of Human Salivary Amylase for Enzyme Activity

The substrate (0.5%) starch was added to the extracted saliva sample and was incubated for 20 minutes at 37˚C. Then DNS solution was added to it to stop the enzyme reaction. The absorbance of the samples was measured at 540nm and the enzyme units were calculated.

Calculation of Enzyme Units

The Enzyme unit was calculated by using the following formula:

$$\text{ENZYME UNIT} = \frac{\text{Xµm x 1000}}{180} \; x \; IT$$

Whereas,

Xµm = µm of glucose produced/ml of the enzyme.

IT=Incubation Time

RESULTS & DISCUSSION

Samples collected from various surfaces may be a part of forensic evidence showing the presence of α-amylase activity indicating the presence of saliva on the surface. Sample collection was convenient on Glass, cigarette bud, cloth, indicating these are the good sources of the saliva in sampling. As the enzyme unit was variable from different samples at different temperatures indicating different concentrations of saliva. This indicates that the amount of salivary α - amylase activity is variable on different surfaces. Saliva can be observed from figure 1 that glass when compared to other surfaces, shows similar enzyme unit at 20˚C, which indicates the stability of the sample on the glass surface at low 37˚C, but does not show enzymatic activity for a long time at 45˚C. The saliva sample shows enzymatic activity on the surface for 360 hours at 20˚C, similarly, It shows up to 240 hours at 37˚C, but does not show enzymatic activity for the rest of the temperature as shown in figures 1, 2, and 3.

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Table 1: α-Amylase enzyme activity study with respect to temperature at 20˚C

S.No	Surfaces	Temperature: 20˚ Celsius																
		0 Hrs	24 Hrs	48 Hrs	96 Hrs	120 Hrs	144 Hrs	192 Hrs	240 Hrs	264 Hrs	288 Hrs	336 Hrs	384 Hrs	432 Hrs	480 Hrs	528 Hrs	552 Hrs	600 Hrs
1	Glass																	
	Absorbance	0.362	0.354	0.304	0.278	0.253	0.228	0.172	0.137	0.118	0.102	0.081	0.053	0.034	0.029	0.009	0	0
	Enzyme Unit	0.0844	0.084	0.0835	0.077222	0.070278	0.063333	0.047778	0.038056	0.032778	0.028333	0.0225	0.014722	0.009444	0.008056	0.0025	0	0
2	Cloth																	
	Absorbance	0.354	0.342	0.267	0.212	0.204	0.194	0.159	0.118	0.101	0.088	0.058	0.047	0.031	0.026	0.014	0	0
	Enzyme Unit	0.098333	0.097333	0.090333	0.080333	0.07333	0.053889	0.044167	0.041167	0.039167	0.031167	0.028167	0.024417	0.019167	0.008	0.004	0	0
3	Cigarette Bud																	
	Absorbance	0.322	0.315	0.299	0.233	0.213	0.198	0.148	0.092	0.07	0.055	0.039	0.021	0.009	0.005	0	0	0
	Enzyme Unit	0.161111	0.131111	0.101944	0.086111	0.081111	0.073056	0.060556	0.047777	0.039722	0.030278	0.019722	0.015278	0.006944	0.0025	0	0	0
4	Standard- Positive Control																	
	Absorbance	2.452	2.312	2.398	2.322	2.422	2.312	2.392	2.108	2.328	2.535	2.408	2.178	2.2	2.225	2.218	2.352	2.321
	Enzyme Unit	0.681111	0.642222	0.666111	0.645	0.672778	0.642222	0.664444	0.625	0.646667	0.65361	0.668889	0.675	0.611111	0.618056	0.616111	0.653333	0.644722
5	Negative Control																	
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Blank																	
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

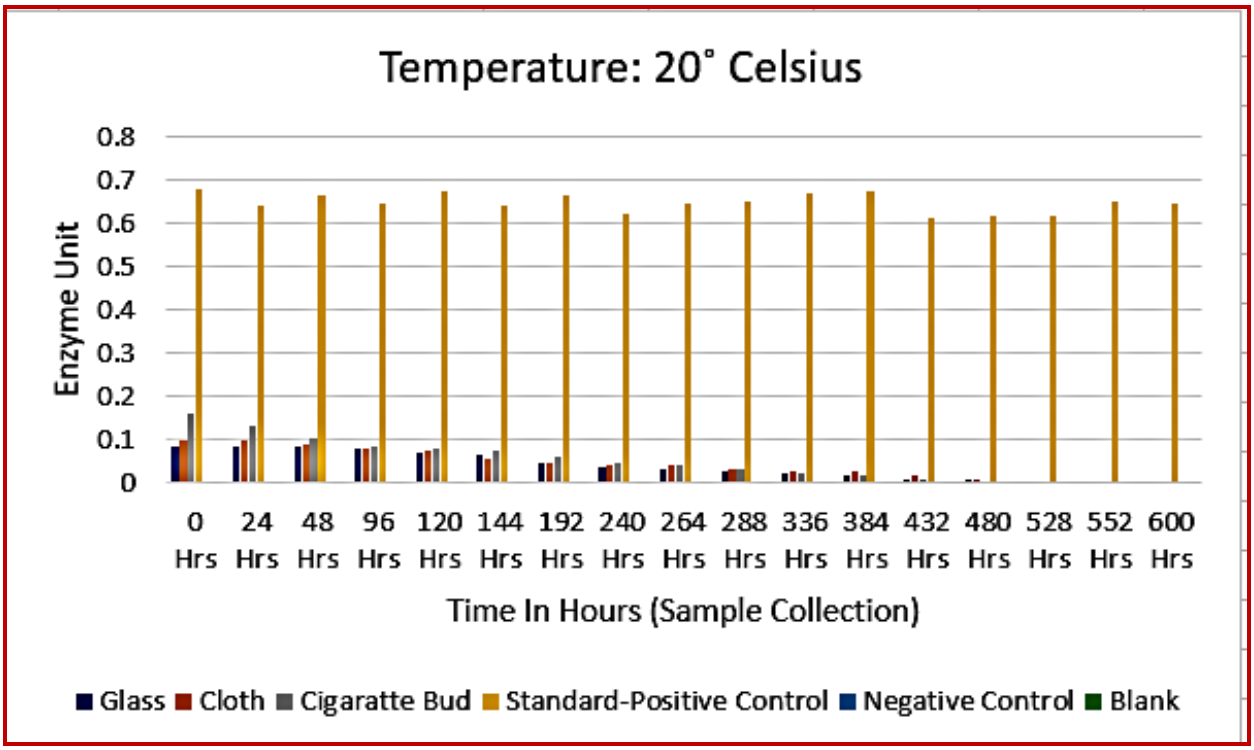

Figure 1: Graphical representation of α-enzyme activity study with respect to temperature at 20˚C

Table 2: α-Amylase enzyme activity study with respect to temperature at 37˚C

S.No	Surfaces	Temperature: 37˚ Celsius														
		0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs	168 Hrs	192 Hrs	216 Hrs	240 Hrs	264 Hrs	288 Hrs	312 Hrs	336 Hrs
1	Glass															
	Absorbance	0.343	.210.085	0.085	0.065	0.047	0.027	0.022	0.012	0.009	0	0	0	0	0	0
	Enzyme Unit	0.095278	0.058333	0.023611	0.018056	0.013056	0.0075	0.006111	0.003333	0.0025	0	0	0	0	0	0
2	Cloth															
	Absorbance	0.406	0.21	0.012	0.068	0.052	0.032	0.029	0.022	0.018	0.005	0	0	0	0	0
	Enzyme Unit	0.112778	0.080556	0.033333	0.018889	0.014444	0.008889	0.008056	0.006111	0.005	0.001389	0	0	0	0	0
3	Cigarette Bud															
	Absorbance	0.833	0.542	0.37	0.268	0.146	0.097	0.088	0.069	0.052	0.038	0.02	0	0	0	0
	Enzyme Unit	0.231389	0.150556	0.102778	0.074444	0.040556	0.026944	0.024444	0.019167	0.014444	0.010556	0.005556	0	0	0	0
4	Standard- Positive Control															
	Absorbance	2.421	2.38	2.022	2.328	2.136	2.132	2.344	2.341	2.252	2.162	2.16	0	0	0	0
	Enzyme Unit	0.6725	0.661111	0.61972	0.646667	0.64722	0.65111	0.65	0.66944	0.62556	0.66111	0.6	0	0	0	0
5	Negative Control															
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Blank															
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

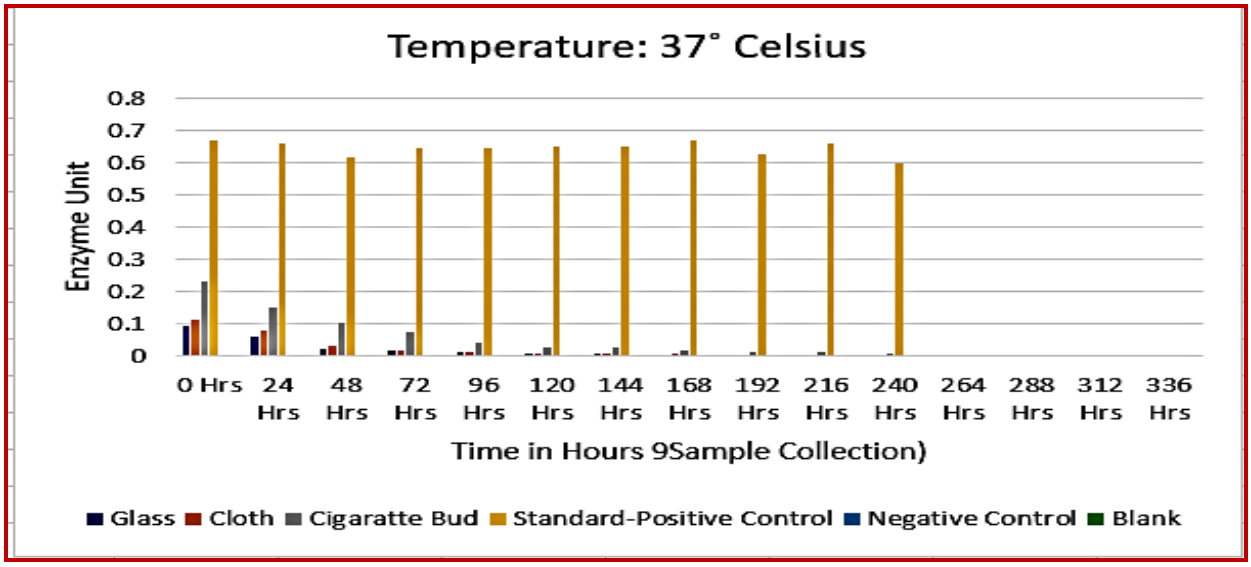

Figure 2: Graphical representation of α-enzyme activity study with respect to temperature at 37˚C

Table 3: α-Amylase enzyme activity study with respect to temperature at 45˚C

S.No	Surfaces	Temperature: 45˚ Celsius														
		0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs	168 Hrs	192 Hrs	216 Hrs	240 Hrs	264 Hrs	288 Hrs	312 Hrs	336 Hrs
1	Glass															
	Absorbance	0.343	0.225	0.093	0.081	0.03	0.022	0.009	0	0	0	0	0	0	0	0
	Enzyme Unit	0.095278	0.0625	0.02583	0.0225	0.00833	0.00611	0.0025	0	0	0	0	0	0	0	0
2	Cloth															
	Absorbance	0.406	0.26	0.17	0.144	0.115	0.087	0.072	0.04	0.012	0	0	0	0	0	0
	Enzyme Unit	0.112778	0.0722	0.04722	0.04	0.03194	0.02417	0.02	0.01111	0.00333	0	0	0	0	0	0
3	Cigarette Bud															
	Absorbance	0.833	0.575	0.498	0.255	0.204	0.13	0.111	0.065	0.024	0	0	0	0	0	0
	Enzyme Unit	0.231389	0.15972	0.13833	0.07083	0.05667	0.03611	0.03083	0.01806	0.00667	0	0	0	0	0	0
4	Standard- Positive Control															
	Absorbance	2.421	2.34	2.35	2.356	2.32	2.344	2.375	2.312	2.319	2.388	2.137	0	0	0	0
	Enzyme Unit	0.6725	0.65	0.65278	0.65444	0.644444	0.651111	0.64306	0.642222	0.644167	0.645833	0	0	0	0	0
5	Negative Control															
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Blank															
	Absorbance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Enzyme Unit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

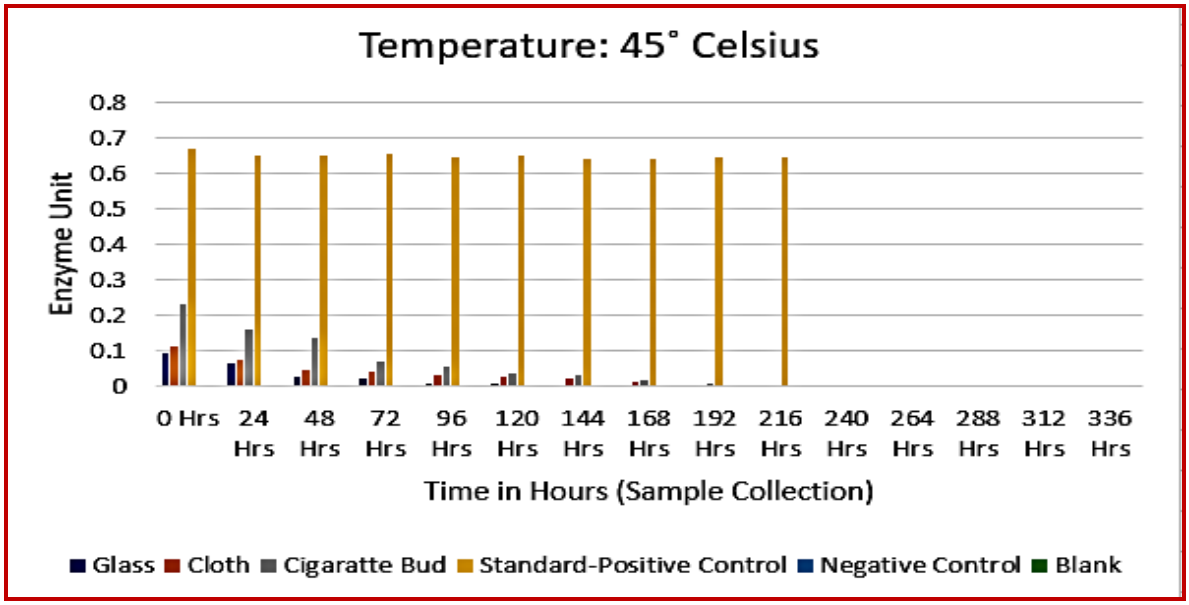

Figure 3: Graphical representation of α-enzyme activity study with respect to temperature at 45˚C

On the cigarette bud surface, the enzymatic activity is shown up to 312 hours at 20˚C. Similarly, it is shown up to 264 hours at 37˚C, but the enzymatic activity is not shown at the rest of the temperature for a long time as mentioned in Figures 1, 2, and 3.

CONCLUSION

From the study, it is concluded that the α - amylase present in the saliva sample showed its activity at temperatures 20˚C and 37˚C on various surfaces like glass, cigarette bud, cloth. However, it was found that with an increase in temperature and time, there is a reduction in α - amylase activity in human saliva, due to inactivation of the enzyme. From the forensics point of view, saliva is the most important biological sample, so it should be collected from the crime scene as soon as possible and the collected sample should be preserved at low temperature immediately. The analysis of the sample should be carried out as early as possible to get accurate results.

Declaration of Competing Interest

The authors declare no actual or potential conflict of interest related to this study.

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Ethical Approval: Approval was not required.

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