INDIVIDUAL VARIATION IN EFFECT OF TEMPERATURE ON ACTIVITY OF SALIVARY AMYLASE IN A SAMPLE HUMAN POPULATION

ANKITA DUNGDUNG

Admission No.- 15ZOL/16

DEPARTMENT OF ZOOLOGY

COLLEGE OF BASIC SCIENCE AND HUMANITIES

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

BHUBANESWAR-751003, ODISHA

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A

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DEPARTMENT OF ZOOLOGY
COLLEGE OF BASIC SCIENCE AND HUMANITIES, BHUBANESWAR

Dr. Netaji Upadhyaya
Assistant Professor,
Department of Zoology

CERTIFICATE-I

This is to certify that the thesis entitled, **"Individual variation in effect of temperature on activity of salivary amylase in a sample human population"** submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology to the Orissa University of Agriculture and Technology, Bhubaneswar, is a faithful record of bonafide research work carried out by **Miss Ankita Dungdung**, **Adm. No.: 15ZOL/16** under my guidance and supervision and that no part of thesis has been submitted for any other degree or diploma or published in any form.

It is further certified that the help and sources of information availed of during the course of study have been duly acknowledged.

Place: Bhubaneswar
Date: 04/06/2018

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled, **"Individual variation in effect of temperature on activity of salivary amylase in a sample human population"** submitted by Miss **Ankita Dungdung** to Orissa University of Agriculture and Technology, Bhubaneswar, in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology, has been approved by the students' advisory committee after an oral examination on the same in collaboration with an External Examiner.

Advisory Committee

1. **Dr. Netaji Upadhyaya** — Chairman _____
   Assistant Professor
   Department of Zoology
   College of Basic Science and Humanities
   O.U.A.T., Bhubaneswar

2. **Dr. C.S.K Mishra**
   Professor and Head
   Department of Zoology — Member _____
   College of Basic Science and Humanities
   O.U.A.T., Bhubaneswar

3. **Dr. Ashis Kumar Mohanty**
   Assistant Professor — Member _____
   Department of Zoology
   College of Basic Science and Humanities
   O.U.A.T., Bhubaneswar

Name: **Signature**

Designation: **(External Examiner)**

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Ankita Dungdung

Adm. No. 15ZOL/16

ABSTRACT

The salivary α amylase is a calcium dependent secreted enzyme that helps in the initiation of starch digestion in the oral cavity. There are at least three isozymes present in the human saliva AMY1A, AMY1B, AMY1C. Diet has played a huge role in the evolving of these genes. Salivary amylase like other enzymes is influenced by fluctuations in temperature. Variation was expected to occur among individuals in a population. The study was aimed to determine the behaviour of salivary enzyme with reference to temperature as a parameter and its variation in different individuals in a population of twenty-two using DNS enzyme assay.

The result implicated more than expected variation in the activity of salivary amylase. Probable reasons for these variations are discussed in a discrete manner and are yet to be verified.

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LIST OF ABBREVIATIONS

%	:	Percentage
^{0}C	:	Degree Celsius
abs-	:	Absorbance
et al.	:	et alebi (and others)
fig.	:	Figure
i.e.	:	That is
ml	:	Millilitre
nm	:	Nanometer
pH	:	Power of hydrogen
temp	:	Temperature

1. INTRODUCTION

Variation, in biology is any difference between cells, individual organisms, or groups of organisms of any species. Variation is caused either by genetic differences (genotype variation) or by the effect of environmental factors on the expression of genetic potentials (phenotypic variation). Variations may be shown in physical appearance, metabolism, fertility, mode of reproduction, behaviour, learning and mental ability, and other obvious or measurable characters.

Genotype variations or genetic variations are caused by differences in number or structure of chromosomes or by differences in number or structure of chromosomes or by differences in the genes carried by the chromosomes genetic variations arise mainly due to mutation and recombination processes. All the changes that occur in an organism when it diverged from its common ancestor is due to genetic variation within a species. Genetic variation plays a very substantial role in the process of evolution.

Environmentally caused variations results from one factor or combined effects of several factors such as climate, food supply and actions of other organisms. These variations do not involve any hereditary alteration and in general are not transmitted to future generations. Hence this factors are not significant in the process of evolution.

Genetic variations which occur in a population may either be advantageous or deleterious for the species in that population. According to Darwin the theory of evolution starts with the existence of hereditary variation. He reasoned that variations must occur in nature that favourable or useful in some way to the organism itself in the struggle for existence. Favourable variations increases the chances of survival and procreation. These advantageous variations are preserved and multiplied from generation to generation at the expense of less advantageous ones. This process is known as natural selection. The outcome of the process is an organism that is well adapted to its environment and evolution often occurs as a consequence.

Evolution can be seen as a two-step process. First hereditary variation takes place, second, selection is made of those genetic variants that will be passed on most efficiently to the following generations. The variants that arise by mutation or recombination are not transmitted equally from one generation to another. Some may

appear more frequently because they are favourable to the organism, the frequency of others may be determined by accidents of chance, called genetic drift. The necessity of hereditary variation for evolutionary change to occur can be understood in terms of the gene pool. Assume, for example, a population in which there is no variation at the gene locus that codes for the MN blood groups, only the M allele exists in all individuals. Evolution of the MN blood groups cannot take place in such a population, since the allelic frequencies have no opportunity to change from generation to generation. On the other hand, in populations in which both alleles M and N are present, different combination may occur and evolutionary change will become possible. The more genetic variation that exists in a population, the greater the opportunity for evolution to occur. Therefore, variation forms the basis of evolution (Futyama).

1.1 SALIVARY AMYLASE

Amylases are secreted proteins that take part in the hydrolysis of 1,4α-glucoside bonds present in the oligosaccharides and polysaccharides. It catalyses the first step in digestion of dietary starch and glycogen. The amylase genes are present in a cluster on the chromosome that includes salivary amylase genes (AMY1), two pancreatic amylase genes (AMY2A, AMY2B) and a related pseudogene. (AMY2P). The extensive copy number variation in AMY1 genes is directly proportional to the salivary amylase content in saliva. The amylase amount in saliva is also influenced by other factors, such as hydration status, psychosocial stress level, and short-term dietary habits.

It has been shown that the average copy number of AMY1 gene is higher in populations that have high-starch diets versus low-starch diets. Phillips, E. (2017).It was an example of intense positive selection imposed by diet on amylase copy number during evolution. The salivary amylase (ptyalin) is an endoenzyme which require calcium-binding to function. It helps in the initiation of starch digestion in the oral cavity. Starch is a polymer of glucose consisting of two primary structures: amylose and amylopectin. Amylose is a lineal polymer of glucose units joined with _ -1,4 glycosidic bonds, making up about 20–30% of most starches. Amylopectin is a highly branched polymer that consists of 1,4 and1,6 bonds. Salivary amylase helps in the hydrolysis of internal 1, 4 glycosidic bonds, yielding a mixture of maltose, isomaltose, small amounts of glucose as well as small linear and branched (containing 1,4 and 1,6 bonds) oligosaccharides called limit dextrins. The amylase enzymes are produced by

salivary glands (encoded by AMY1A, AMY1B and AMY1C genes) and exocrine pancreas (AMY2A andAMY2B genes). Additionally, a small amount of amylase is also expressed by AMY2B gene in the liver. The salivary amylase protein composes of 496 amino acids with a15 residues signal peptide. (UniProtKB-PO47(AMY1_HUMAN))

1.2 SALIVA

Saliva is a watery liquid secreted into the mouth by the salivary glands which provide lubrication for chewing and swallowing and aiding digestion. It 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 major salivary glands include the paired parotid glands which are located opposite the maxillary first molars, the submandibular and sublingual glands, which are found in the floor of the mouth while the minor glands include those that are present in the lower lip, tongue, palate, cheeks, and pharynx.

Saliva is a very dilute fluid composed of more than 99% water and a variety of electolytes such as sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. The other substances found in the saliva include immunoglobins, proteins, enzymes, mucins, and nitrogenous products such as urea and ammonia. These components are present in a very small amount and have varying flow rate. The average daily flow of whole saliva in healthy individuals varies between 1-1.5 L. Percentage of saliva secreted from the different salivary glands in an unstimulated condition are as follows: 20% from parotid, 65% from submandibular, 7% to 8% from sublingual and less than 10% from numerous minor glands. Stimulation increases the rate of secretion from each gland out of which 50% of the total secretion is contributed by the parotid gland.

The secretory unit of the salivary glands are the acinar cells. The other cells found in the salivary glands are various duct system cells and myoepithelial cells. Acinar cells determine the type of secretion produced from the different glands. Secretions can be classified as serous, mucous, or mixed. Serous secretions are mainly produced from the parotid glands, mucous secretions from the minor glands, and mixed serous and mucous secretions from submandibular and sublingual glands. The duct cells can be classified as intercalated, striated, and excretory. The first ducts are the

intercalated ducts which connect the acinar secretions to the rest of the gland. These cells are not involved in the modification of electrolyte. Striated cells are second in network, functioning as electrolyte regulation in resorbing sodium. The final duct is the excretory duct cells, the function similar as the striated cells. They are last, part of the duct network before saliva reaches the oral cavity. Myoepithelial cells are long cell processes which are wrapped around acinar cells and help in the constriction of the acinar cells on stimulation.

The normal pH of saliva is 6 to 7 that is it is slightly acidic. The Ph in salivary flow can range from 5.3(low flow) to 7.8 (peak flow).

Great variability is seen in the salivary flow rates. The accepted range of normal flow for unstimulated saliva is anything above 0.1Ml/min. For stimulated saliva, the minimum, volume is 0.2L/min. Salivary flow during sleep is zero. Temperature greatly influences the activity of salivary amylase (Schneyer, 1951).

2. REVIEW OF LITERATURE

Salivary α- amylase is a secreted enzyme found in human saliva and helps in the initial digestion of starch (Ramasubbu, 1996). Amylase is thought to be a calcium dependent enzyme which hydrolyses complex carbohydrates at α(1,4)-glycosidic linkage.

The process of digestion begins in the mouth with the chewing of food in the presence of salivary alpha amylase to convert the starch in the food into maltose (Maureen, 2000).

In humans, all amylase isoforms are present on chromosome1p21 (Mandel *et al.,* 2010) Alpha-amylase is one of the major proteins components of saliva. Among other proteins, alpha-amylase is synthesized and secreted by acinar cells the secretory units of salivary glands, after neurotransmitter stimulation (Baum, 1993). Acinar cells are innervated by both sympathetic and parasympathetic branches of the autonomic nervous system (Emmelin *et al.,* 1981).

The main purpose of salivary alpha-amylase is the enzymatic digestion of starch into maltose and dextrin. This form of amylase is also called "ptyalin". Ptyalin acts on linear α (1, 4) glyosidic linkages and, it will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, and achrodextrin) producing successively smaller starches and ultimately maltose. Salivary amylase is inactivated in the stomach by gastric acid.it was found that when pH of the gastric juice was 3.3, ptyalin was totally inactivated in 20 minutes at 37°C. In contrast, 50% of amylase activity remained after 150 minutes of exposure to gastric juice at pH 4.3 (Fried *et al.,* 1987). It is an important factor for mucosal immunity in the oral cavity, as it helps in inhibition, adherence and growth of bacteria (Bosch *et al.,* 2002).

The variation in the consumption of starch associates with the number of copies of the salivary amylase gene and amylase saliva in human populations. Increased copy number of the salivary amylase genes seems to have enhanced survival rate and favoured a shift to a starchy diet during human evolution (Perry *et al.,* 2007). Genes that are responsible for the production of salivary alpha amylase are AMY1A, AMY1B and AMY1C. The length of human salivary amylase is 10 kb. (Nishide *et al.,*1986).

The position of the amylase gene to 1p21 was assigned by Zabel *et al.*, after conducting in situ hybridisation combined with high reduction cytogenetics.

Various works have been carried out by different researchers about the effects of temperature on the activity of salivary amylase. Salivary amylase like most other enzymes is affected by fluctuations in temperature. Schneyer, 1951 showed that with a rise in temperature of 50 degree Celsius the amolytic activity of salivary amylase increases rapidly but beyond this optimum point its activity decreases rapidly.

OBJECTIVE

Variation at individual level is extremely significant and it is much higher than expected.

Therefore, to observe the above statement I adopted a very simple experiment involving the effect of temperature on salivary amylase and study its degrading pattern i.e. whether all have same optimal temperature pattern of degradation or does it differ from individual to individual in a population.

3. MATERIALS AND METHODS

3.1 CHEMICALS REQUIRED

1. Di-sodium hydrogen phosphate
2. Sodium di-hydrogen phosphate
3. Sodium chloride
4. Sodium potassium tartrate, tetra hydrate
5. 3,5-Dinitrosalicylic acid
6. D- (+) Maltose, Monohydrate
7. Soluble starch
8. Sodium hydroxide

3.2 REAGENTS PREPARATION

3.2.1 Phosphate buffer solution

20mM sodium phosphate buffer (Di- sodium hydrogen phosphate and sodium di-hydrogen phosphate) with 6.7Mm sodium chloride is made at pH6.9. pH is adjusted using pH meter with 1M sodium hydroxide solution.

3.2.2 Dinitrosalicylic acid solution (colour reagent)

8ml of 2M NaOH solution was prepared and was used for dissolving 5.3M sodium potassium tartrate, tetra hydrate (12g). This solution was mixed using a hot plate with magnetic stirrer.

96mM 3,5-Dinitrosalicylic acid was made by dissolving 0.44g DNSA in 20 ml of distilled water and was mixed using hot plate with magnetic stirrer.

Both the solutions are then mixed one by one and the volume was adjusted to 40 ml. this solution is stored in an amber bottle at room temperature for future use.

3.3 MALTOSE STANDARD SOLUTION

0.2% maltose solution was prepared by dissolving 0.2g maltose in 100 ml distilled water.

Preparation of maltose standard curve

1. 0.2% maltose solution in the range of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml was taken in 5 test tubes named as T1, T2, T3, T4, T5 respectively.
2. A control was prepared without maltose in it.

3. Distilled water was added to each test tube including control to make up the volume to 2 ml.
4. 1ml colour reagent was added to each test tube.
5. Then the test tubes were capped and placed in boiling water bath for exactly 5 minutes.
6. Test tubes were immediately transferred to a beaker containing ice to bring down the temperature of the solution to room temperature.
7. 9 ml of distilled water was then added to each test tube to make up the volume to 12ml. All were mixed thoroughly by inversion.
8. Absorbance at 540 nm was recorded against control as blank
9. A graph is plotted with amount of maltose on X-axis and absorbance on Y-axis

3.4 ENZYME ASSAY

3.4.1 Standardization of time of incubation

- 1 ml of 1% starch solution was taken in all test tubes which were marked as TI, T2, T3 and T4 including blank.
- Saliva was diluted to 5% and 1 ml of it was added to the TI test tube and was incubated for 3 minutes. Then 1 ml of colour reagent was added to it.
- Similarly, in T2 1 ml of saliva was added and incubated for 5 minutes. And then 1 ml of colour reagent was added.
- This process was repeated for incubation time of 7 minutes and 9 minutes in T3 and T4 respectively.
- Then all the 5 test tubes were heated in boiling water bath for exactly 5 minutes and then cooled on ice to room temperature. 9 ml of water is added to all test tube.
- Reading was taken at 540 nm and a graph was plotted with absorbance on Y axis and time of incubation on X axis.
- Same procedure was performed for 10%, 15% and 20% saliva.

3.4.2 Standardization of enzyme concentration

- 8 test tubes were taken and marked as B1 T1 (blank 1 and test 1), B2 T2, B3 T3, B4 T4
- 1 ml of 1% starch solution was taken in all test tubes including a control.
- In TI 1 ml of 5% saliva was added and incubated for 3 minutes. Then 1 ml of colour reagent was added to it. In B1 1 ml of 5% enzyme was added after the addition of colour reagent

- Similar process was continued with 10%, 15%, and 20% saliva in B2 T2, B3 T3 and B4 T4 respectively.
- In control no enzyme was added. Volume was adjusted with 1 ml distilled water.
- All the test tubes were heated in boiling water bath for exactly 5 minutes. Then 9 ml distilled water was added to all and reading was taken at 540 nm.

3.4.3 Standardization of range of starch solution

- 5 test tubes were taken and marked as T1, T2, T3, T4 and T5 including a blank.
- 1 ml of 0.5%, 1%, 2%, 4%, 8% starch solution were taken in each test tube from T1 to T5 respectively. In blank 1 ml of phosphate buffer was added.
- Then 1 ml of 5% saliva was added to each test tubes and was incubated for 3 minutes.
- 1 ml of colour reagent was added to all test tubes and was heated in boiling water bath for exactly 5 minutes and then cooled on ice to room temperature.
- 9 ml of distilled water was added to all and absorbance is recorded at 540 nm.
- Graph was plotted with % of starch on X-axis and absorbance on Y-axis.

3.4.4 Collection of saliva

Table 1. Table of individuals

SL. NO.	NAMES OF STUDENTS	ABBREVIATIONS	AGE	TIME OF SALIVA COLLECTION (IN A.M.)
1	Biswajit Panda	BP	22	8:40
2	Priyadarsini Mohapatra	PD	23	8:50
3	Bibekananda Meher	BM	20	8:45
4	Akash Das	AD	21	9:00
5	Manoranjan Digal	MD	22	9:15
6	Ankit Mohapatra	AM	20	8:45
7	Kumuda	KU	21	8:55
8	Sambit	SA	20	9.00
9	Bayasis M. Sharma	BS	20	9:05
10	Promod Mohapatra	PM	21	9:20
11	Manseet Swaroop	MS	20	8:30
12	Mrutunjay Behera	MR	18	9:00
13	Sushantkumar Parida	SU	22	9:10
14	Deeptanshu Tarai	DT	18	9:15
15	Prashant Kumar Sahu	PR	22	9:20
16	Ankita Dungdung	AG	23	9;15
17	Subhakanta Besoi	SB	18	9:30
18	Anupama Bara	AN	22	8:40
19	Hitakanshi	HI	22	8:50
20	Pralaya	PL	19	9:00
21	Benudhara Majhi	BM	18	9:15
22	Sradharam Swain	SS	22	9:25

Saliva was freshly collected from different students in the morning before breakfast. The saliva was voluntarily collected from different students by asking them to rub green chilly on their tongue which acts as a stimulant and allows free flow of saliva. Mouth was cleaned using distilled water to remove any debris that may come in with the saliva. The saliva was then collected in a test tube by asking the students hold out their tongue and allow the saliva to fall into the test tube(2-3ml) After collection it was immediately transferred to an ice box with ice to stop the enzyme present in it from undergoing denaturation.

3.4.5 Saliva dilution

Saliva was then diluted to 1 % using distilled water. 0.5 ml saliva was mixed with 49.5 ml distilled water and mixed using magnetic stirrer. 50 ml 1% diluted saliva was made.

3.4.6 Centrifugation

Saliva was then centrifuged at 3000 rpm for the debris to settle down. After centrifugation only the supernatant was taken while the debris which had settled down as a white pellet was discarded and it was then transferred to an ice box.

3.4.7 Starch preparation

2% starch solution was freshly prepared by dissolving 0.4g of soluble starch in 20 ml phosphate buffer (pH 6.9). The solution was boiled in a water bath with constant stirring for 5 minutes to facilitate mixing of the starch.

3.4.8 Maintenance of temperature

Water bath was used to maintain different temperatures as required. The temperature of the water bath as well as the sample was measured using a thermometer. After the temperature of the water bath was maintained as required, the sample was allowed to stand in it for five minute till the temperature of the sample remained constant.

1. 5 test tubes were taken and marked 25, 37, 47, 57, 67 and 77 respectively.
2. 1ml of freshly prepared 2% starch was taken in each test tube.
3. In another test tube an appropriate amount of diluted saliva was taken.
4. Temperature of the water bath was then maintained as needed. The temperature was checked using the thermometer.

5. The test tube containing the diluted saliva was dipped into the water bath and allowed to stand for some time. Then the temperature of the saliva was measured. Once the saliva was found to be in the required temperature and has become stable it was allowed to stand in the water bath for 4 minutes. This procedure was repeated for all the temperatures.
6. After 4 minutes 1ml of this saliva is added to the test tube containing starch and then allowed to be incubated for 3 minutes.
7. After 3 minutes DNSA (3,5-dinitrosiacylic acid) a colour reagent was added to the reaction mixture.
8. The test tube containing this mixture was then placed in boiling water for 5 minutes. Changes in the colour of the solution mixture was observed.
9. It was then immediately placed in an ice box for the solution to cool down it to room temperature. 9 ml distilled water was added to the test tube to make the volume of the solution to 10 ml.
10. Then absorbance at 540 nm wavelength was taken using UV- VIS spectrophotometer. This process was repeated for every sample that was collected.
11. The above procedure was repeated for all the individuals.

4. RESULTS

Table 2. Readings for maltose standard curve

MALTOSE CONCENTRATION (in mg)	ABSORBANCE (540nm)
0	0
0.4	0.120
0.8	0.239
1.2	0.382
1.6	0.508
2	0.645

From this data maltose standard curve is made and it shows the straight line and line equation is given as y = 0.3237x-0.008.

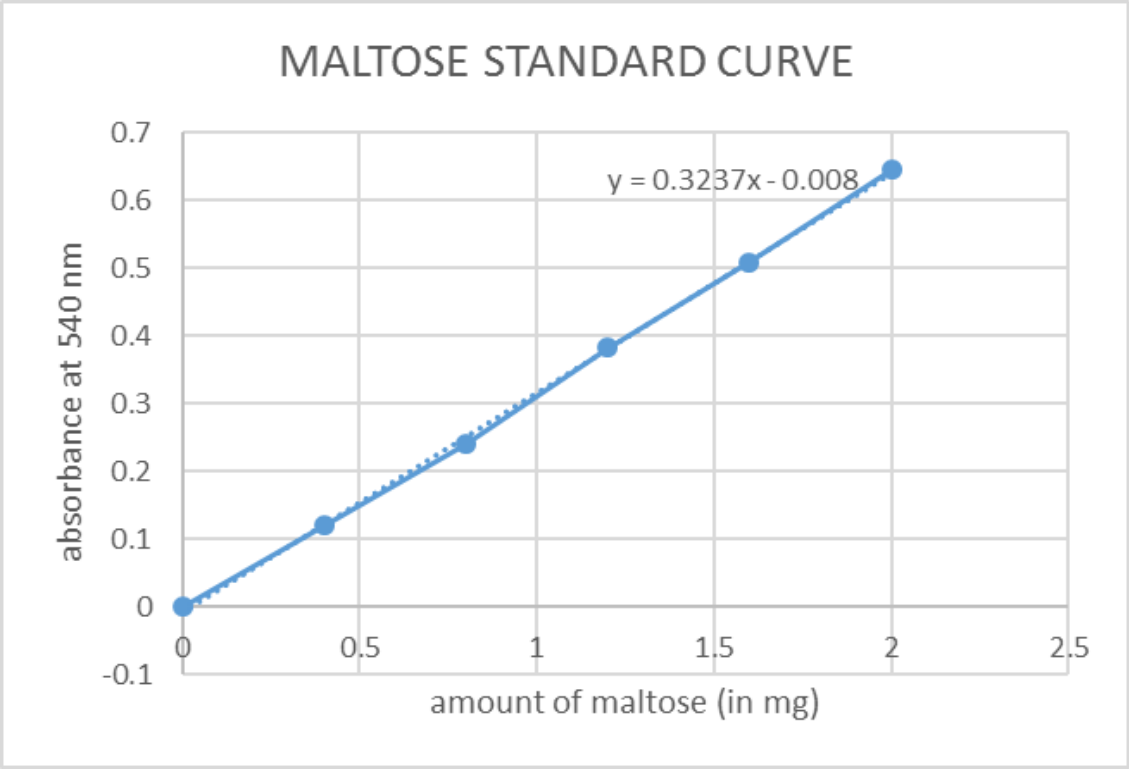

Fig. 1: Maltose standard curve

A maltose of known concentration can be used as a standard for estimating reducing sugar in unknown samples. Constructing standard curve for maltose helps us to estimate concentration of reducing sugars present in unknown sample and to determine the activity.

Table 3. Standardization of enzyme concentration

ENZYME CONCENTRATION (%)	ABSORBANCE (540 nm)
0	0
5	0.191
10	1.278
15	1.417
20	1.514

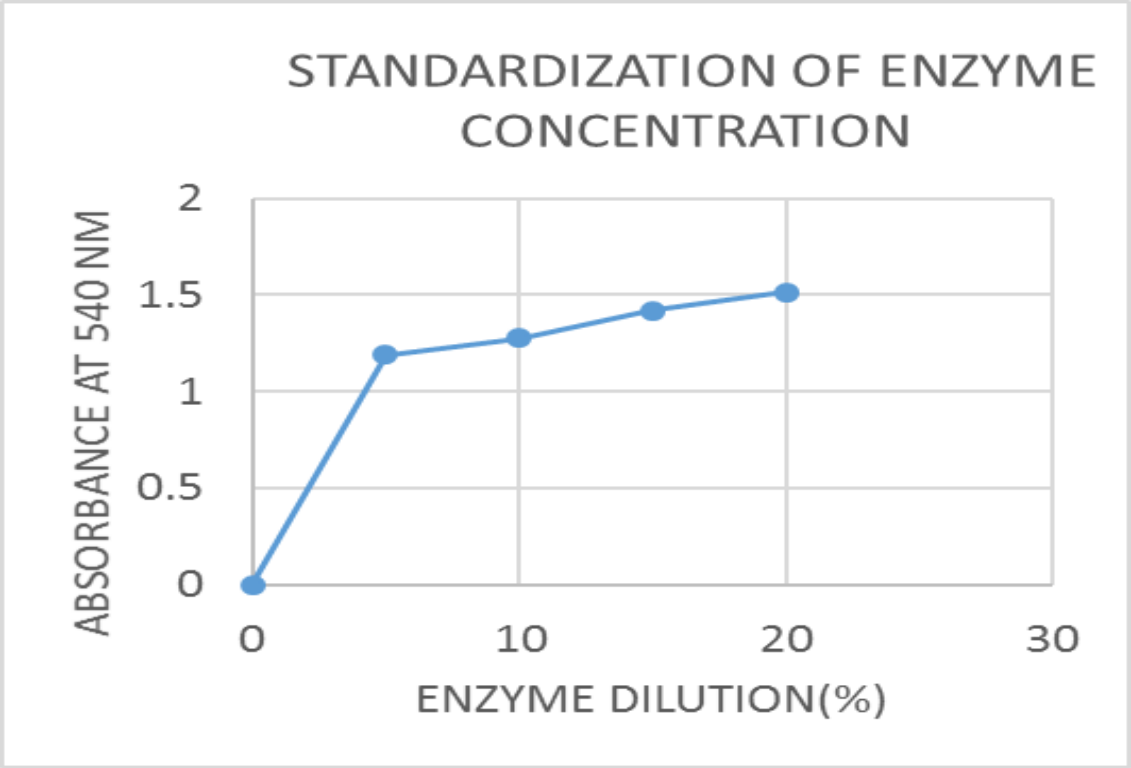

Fig. 2: Standardization of enzyme concentration

In this graph it is observed that there is linearity of enzyme dilution upto 5%. But afterward there is no such increase in values. Thus 5% dilution is considered as standard for the assay.

Table 4. Standardization of enzyme concentration

ENZYME CONCENTRATION (%)	ABSORBANCE (540 nm)
0	0
1	0.556
2	0.92
3	1.332
4	1.424
5	1.498

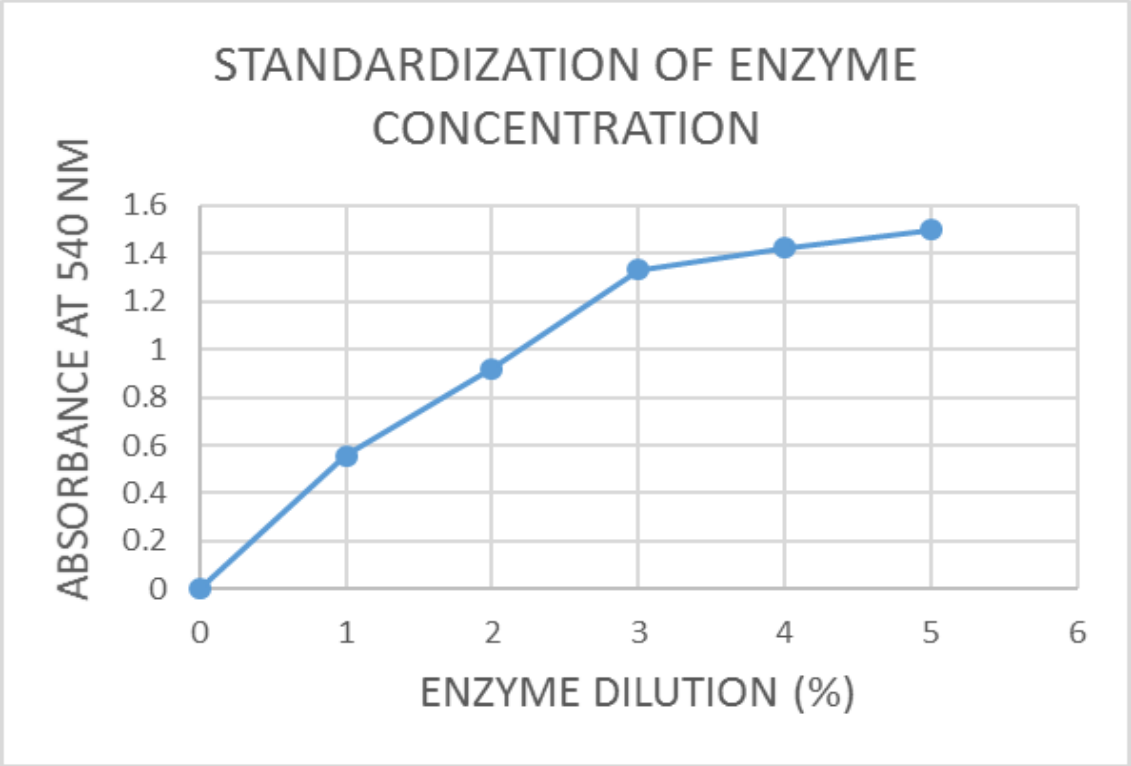

Fig. 3: Standardization of enzyme concentration

In the above graph linearity is observed from 1% to 3% dilution and after that there is no linearity seen. Thus we can consider the saliva dilution from 1% to 3%. Since in some individuals no reading is shown in 5% dilution of saliva thus least % dilution is considered so 1% dilution is considered to be standard.

Table 5. Standardization of time of incubation

TIME OF INCUBATION (in minutes)	ABSORBANCE (540nm)
0	0
3	1.16
5	1.204
7	1.287
9	1.294

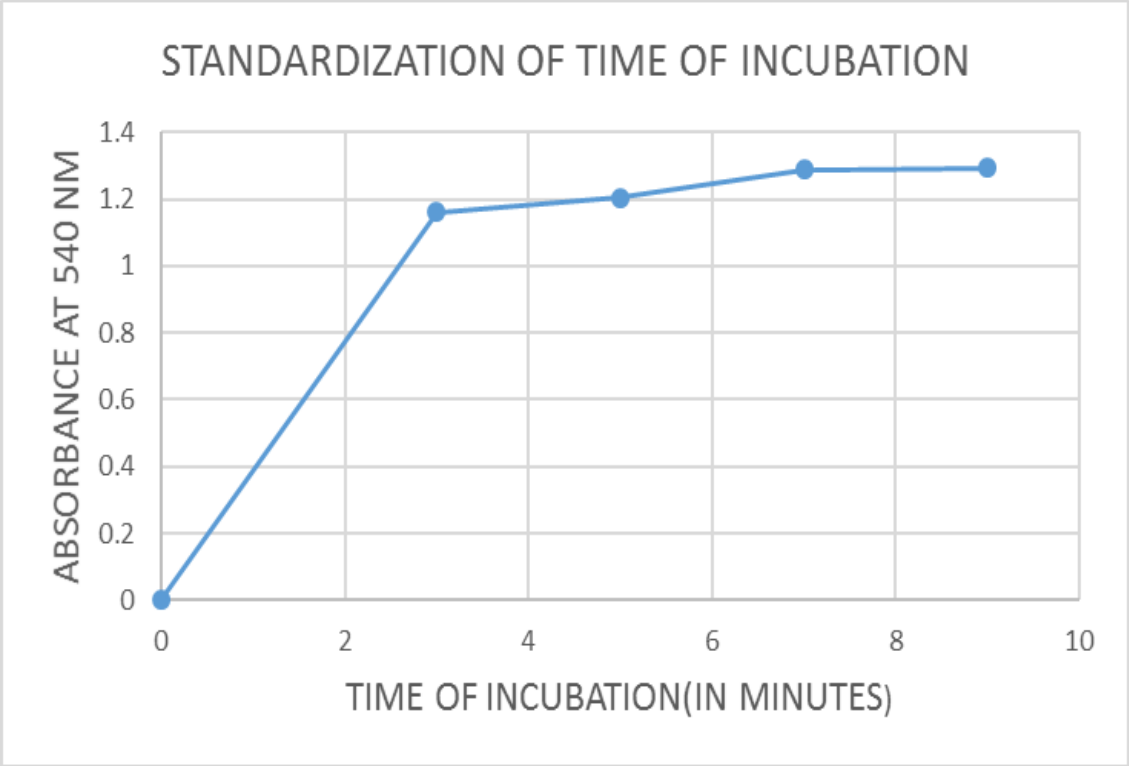

Fig. 4 Standardization of time

From the above graph straight line is obtained till 3 minutes of incubation. After that there is no much effect of incubation time and the values remain nearly equal. Thus 3-minute incubation is considered as standard for further assay.

Table 6. Standardization of starch concentration

STARCH %	ABSORBANCE (540 nm)
0	0
0.5	0.475
1	0.734
2	1.094
4	1.341
8	1.319

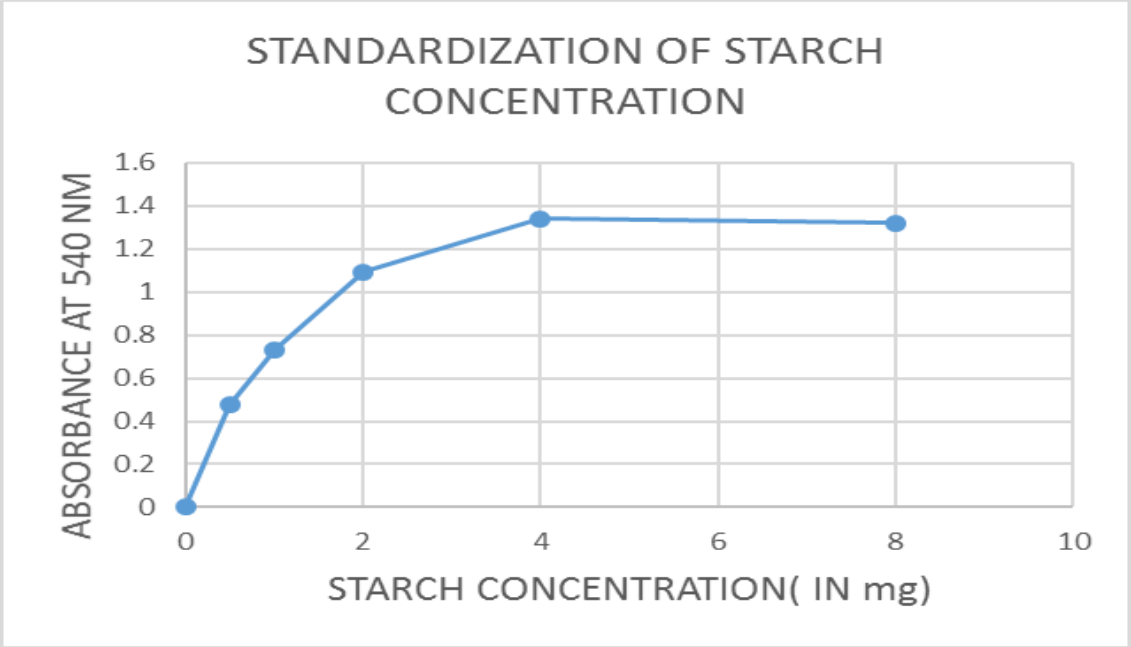

Fig. 5: Standardization of starch concentration

In the above graph these is an increase in absorbance as starch concentration increase. But after 4% starch solution slight decline is graph is observed. Thus the starch percentages from 1 to 5 is considered as standard for assay.

Considering the above standard values assay is done for each individual at different temperature (25, 37, 47, 57, 67, 77).

Table 7. Temperature readings of different individuals

Name of the individuals	Temperature in degree Celsius					
	25	**37**	**47**	**57**	**67**	**77**
BP	0.376	0.654	0.773	0.511	0.099	0.107
PD	0.214	0.383	0.334	0.568	0.095	0.046
BM	0.251	0.353	0.406	0.17	0.08	0.076
AD	0.534	1.021	1.301	0.895	0.165	0.153
MD	0.433	0.464	0.417	0.385	0.096	0.087
AM	0.291	0.403	0.415	0.401	0.054	0.065
KU	0.830	1.29	1.168	1.097	0.084	0.039
BS	0.199	0.300	0.289	0.283	0.062	0.044
PM	0.762	0.843	0.946	0.937	0.063	0.054
MS	0.717	0.716	0.597	0.238	0.122	0.109
MB	0.488	0.612	0.618	0.476	0.11	0.103
SU	0.272	0.412	0.343	0.260	0.119	0.113
DT	0.257	0.225	0.214	0.537	0.105	0.096
PR	0.14	0.200	0.214	0.202	0.118	0.115
SB	0.292	0.280	0.244	0.267	0.116	0.105
AN	0.374	0.480	0.296	0.682	0.104	0.166
HI	0.256	0.349	0.293	0.478	0.185	0.166
PL	0.278	0.273	0.311	0.413	0.502	0.179
BE	0.269	0.282	0.442	0.502	0.245	0.182
SS	0.686	0.427	0.44	0.731	0.275	0.208

From the above the given a data the following graph was plotted with temperature on the x- axis and absorbance (540nm) on the y- axis.

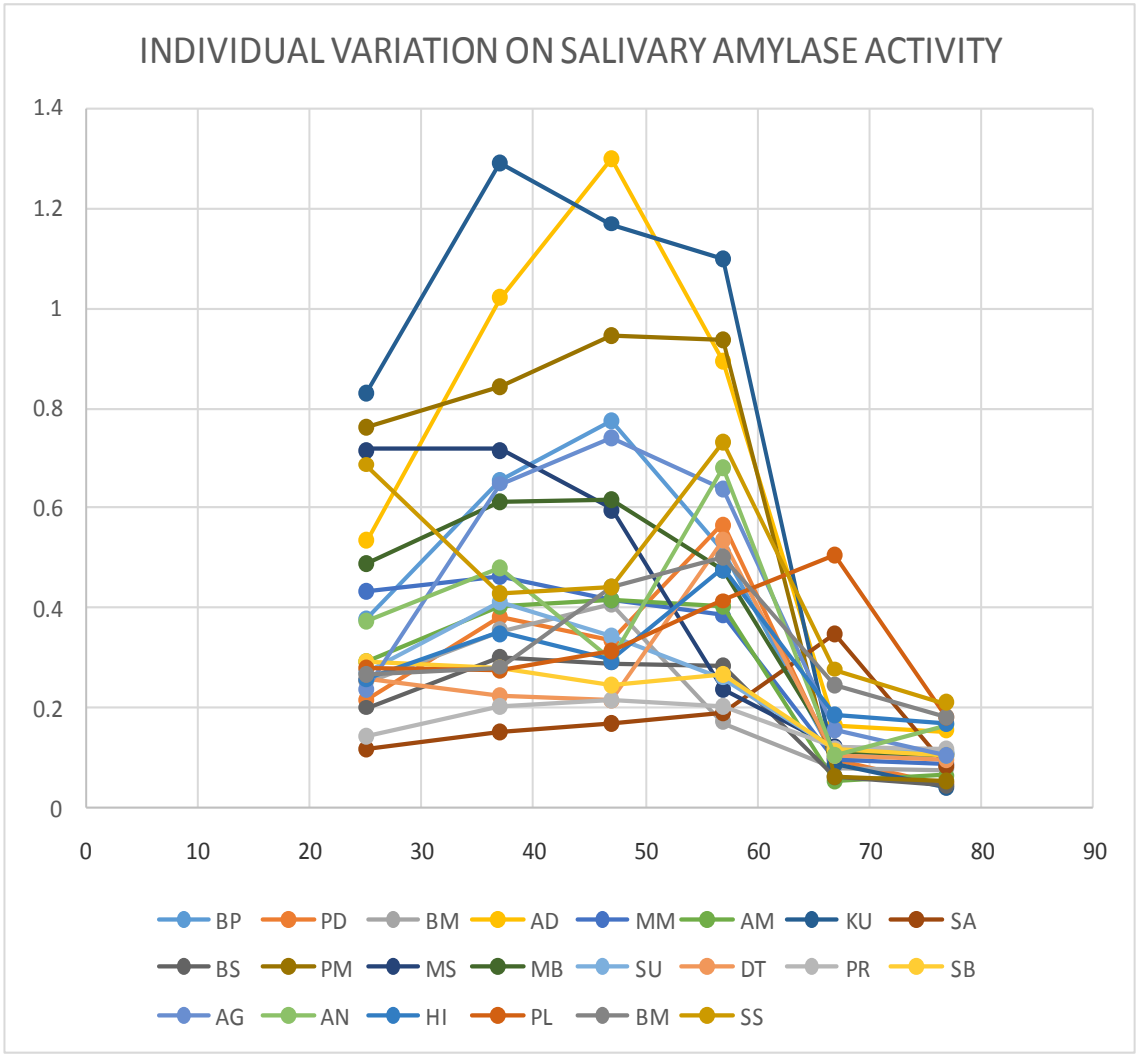

Fig. 6: Showing individual variation in amylase activity

The above graph was used to acquire various information about the variation in the effect of temperature on the activity of different individuals in a sample population. The data obtained from the graph was used to get following results.

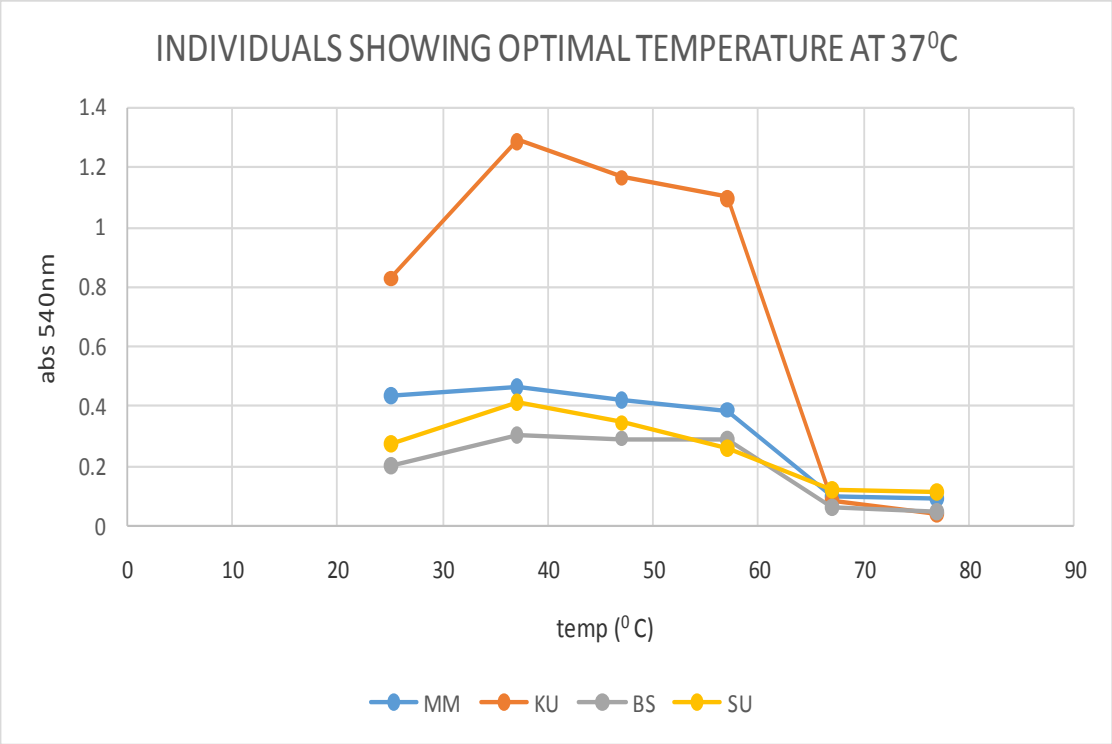

Fig. 7: Individuals showing optimal temperature at 37 ^{0}C

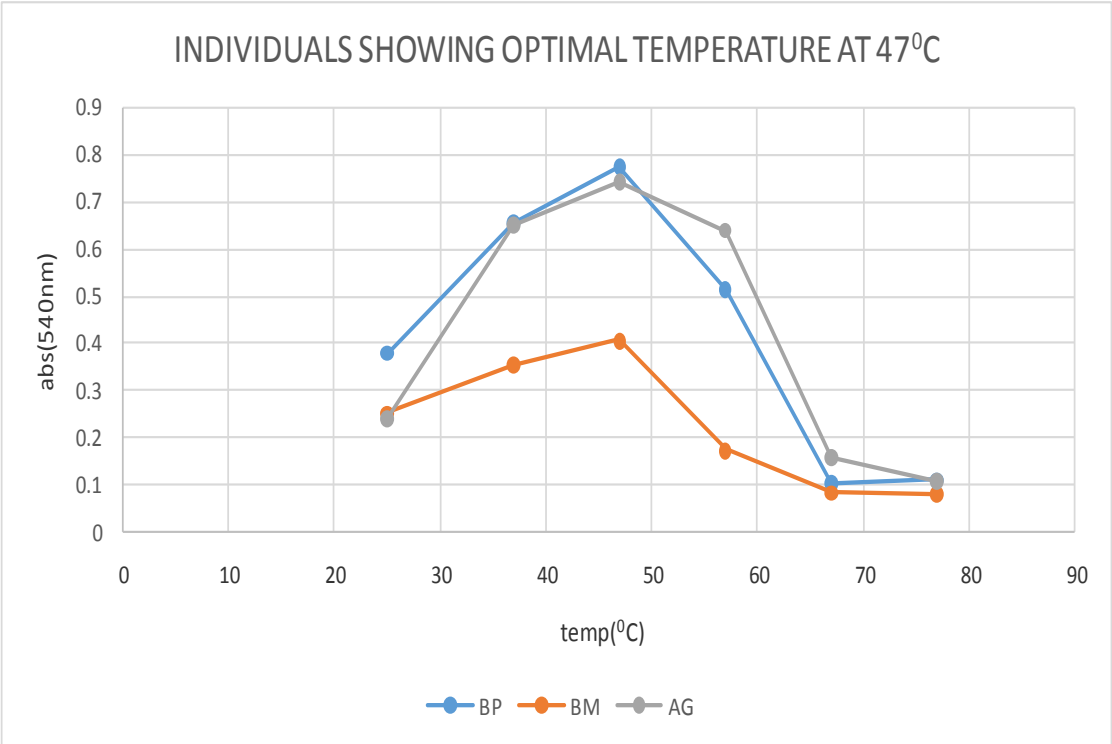

Fig. 8: Individuals showing optimal temperature at 47^{0}C

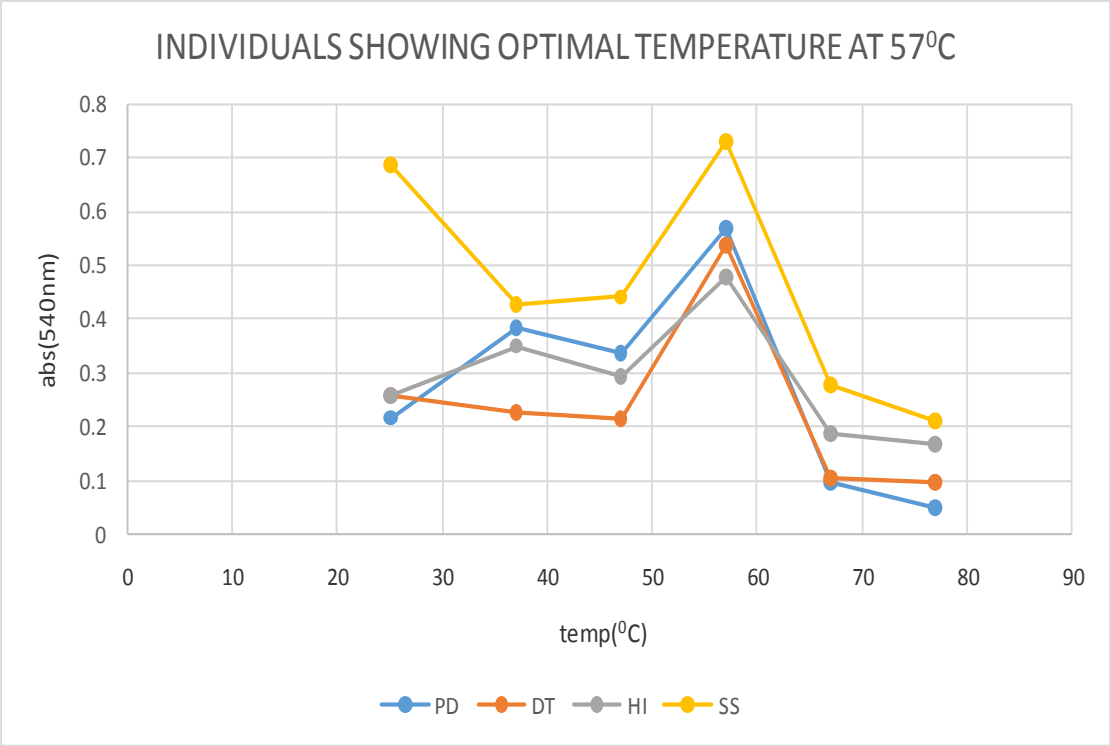

Fig. 9: Individuals showing optimal temperature at 57^0C

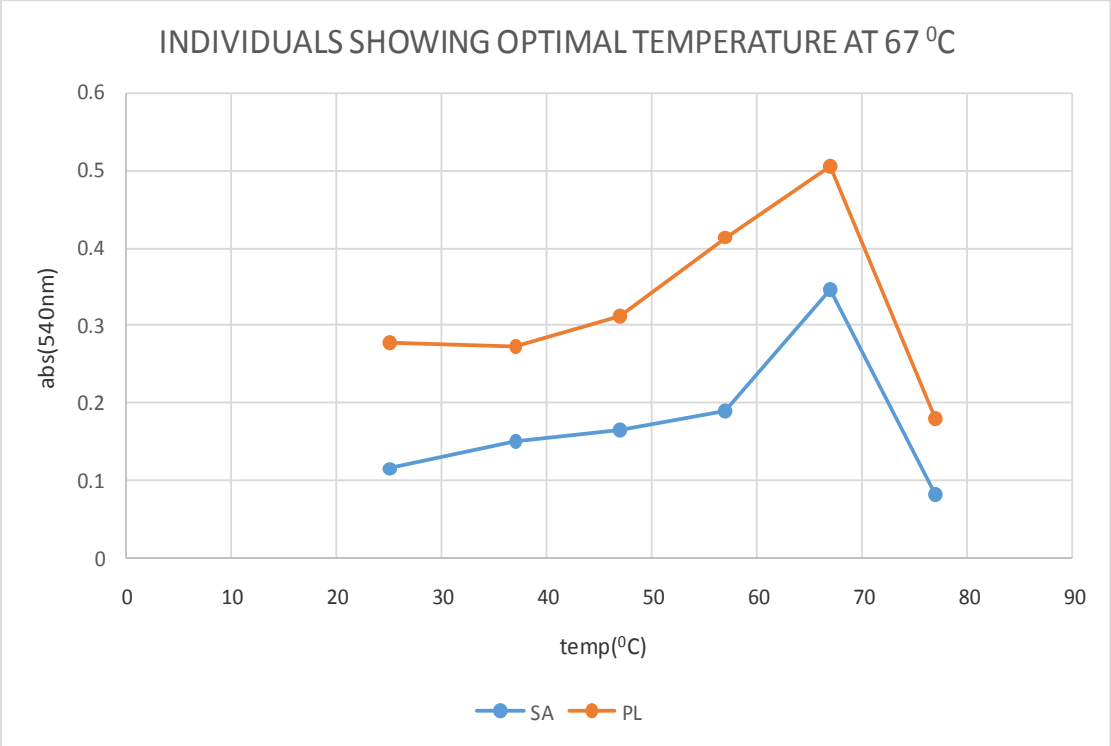

Fig.10: Individuals showing optimal temperature at 67^0C

From the above data I inferred the following results

In 4 individuals the optimal temperature was seen at 37^0 Celsius.

In 3 individuals the optimal temperature was seen at 47^0 Celsius.

In 4 individuals the optimal temperature was seen at 57^0 Celsius.

In 2 individuals the optimal temperature was seen at 67^0C Celsius.

Human saliva is mixture of 3 amylases in different proportion. Since they are different proteins, they may have different temperature response. One may have optimal temperature at 25^0C while another at 57^0C.

5. DISCUSSION

Following factors may have contributed to the observed variations in the studied population:

- We have taken 1% saliva for our experiment.
- We are ignorant of the fact that what is the exact quantity of salivary amylase in this 1% saliva in different individuals.
- We also do not know the relative proportion of isozymes (at least 3) present in each one's saliva.
- The rate of catalysis of individual's isozyme is also not known to us.

These three unknown factors definitely have significant contribution for observed variation.

Large scale copy number variation is another phenomenon which can also contribute to the quantity and proportion of particular isozyme.

Further experiments are required to find out the isozymes present in each individual's saliva.

This can also be determined by further electrophoretic study. Each one's thermal behaviour can be determined by using purified isozymes after their separation in electrophoresis.

If the amino acid sequence of this isozyme can be determined, then the variation in thermal behaviour can be predicted.

Salivary amylase has played a bigger role in human evolution. Ancestors of "homo" were not cereal eaters. Chimpanzees do have only one salivary amylase gene that means extra salivary amylase genes were introduced into the human genome by gene duplication. The rate of catalysis of salivary amylase has a positive selection value over slowly acting salivary amylase in case of individuals sub stating on starchier diets i.e. cereals. If we correlate this with human migration path and proportion of starchy and fleshy food human evolution is understood in a much better manner. Cereals are available in arid. environments. Higher AMY1 copy number and protein level probably improves digestion of starchy food and may buffer against the fitness reducing. the effects of intestinal disease.

6. CONCLUSION

This study was just an initial experiment conducted to see the individual variation in salivary amylase activity of a sample population.

More work is needed to be done to in this field to justify the above statement which includes identifying the differences in isozymes in the saliva of an individual by electrophoresis and studying their thermal behaviour individually.

REFERENCES

Bank Ruud A. *et al.* (1992). Variation in gene copy number and polymorphism of the human salivary amylase isoenzyme system in Caucasians, 89:213-222.

Baum, B. J. (1993). Principles of saliva secretion. *Annals of the New York Academy.*

Bernfeld, P. (1955). [17] Amylases, α and β. *Methods in enzymology*, *1*, 149-158.

Bosch et al. Brain Behav Immun. 2016 Feb; 52:11-17.

Butterworth, P.J. *et al.* (2011). Human α-amylase and starch digestion: An interesting marriage, 63: 395-405.

de Almeida PDV, Grégio AMT, Machado MÂN, de Lima AAS, Azevedo LR. (2008). Saliva Composition and Functions: A comprehensive review, 9(3): 72-80.

Enemchukwu *et al.* (2013). Effect of temperature, pH and substrate concentration on the kinetics of salivary alpha-amylase activity among cigarette smokers in Awka, Anambra State, Nigeria, The Bioscientist, 1(1): 108-113.

Evolution / Douglas J. Futuyma 1942.

Fried, M; Abramson, S.& Meyer, J.H. (1987). Passage of salivary amylase through the stomach in humans, 32:1097-103.

Humphrey SP, et al. J Prosthet Dent. (2001). A review of saliva: normal composition, flow, and function, 85(2): 162-69.

J Dent Res. 1987 Feb; 66(2): 509-17.

NP, Lee C, Stone AC: Diet and the evolution of human amylase gene copy number variation. Nat Genet 2007; 39: 1256–1260.

Perry, G. H., Dominy, N. J., Claw, K. G., Lee, A. S., Fiegler, H., Redon, R., & Carter N. P. (2007). Diet and the evolution of human amylase gene copy number variation, 39(10): 1256-1260.

Phillips, E. (2017). Copy number variation in salivary amylase: A participant-based study on genetic variation. The Young Researcher, 1(1), 156-166. Retrieved from http://www.theyoungresearcher.com/papers/phillips.pdf

Ramasubbu Narayana *et al.* (1996). Structure of human salivary α-amylase at 1.6Å resolution : implications for its role in the oral cavity, D52, 435-446.

Ramasubbu, N. (2005). Structure-function relationships in human salivary α-amylase: role of aromatic residues. 16: 47-56.

Santos *et al.* (2012) Copy Number Polymorphism of the Salivary Amylase Gene: Implications in human nutrition research, 5:117-131

Schneyer, 1950 the effects of temperature changes on salivary amylase activity, Volume-30.

Sidney P. Colowick and Nathan O. Kaplan. Methods in Enzymology, Volume-1.

Tucker AS, Miletich I (eds) (2010), Salivary Glands. Development, Adaptations and Disease.14:1-20.

Use of dinitrosalicylic acid reagent for determination of reducing *Analytical Chemistry*, *31*(3), 426-428.