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"TO STUDY THE EFFECT OF TEMPERATURE ON ENZYME ACTIVITY"

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ABSTRACT:-
The current study was carried out to determine the effect of temperature on enzyme activity. The activity of the enzyme was measured after incubating the crude enzyme preparation under assay conditions at temperatures ranging from 10 to 60 °C, as well as at room temperature. Foods like potatoes, soybean seeds, ginger, apples as well as saliva, are sources of the enzyme beta-amylase, which is employed in industry. These sources were used to isolate, purify, and characterised beta amylase. DNS reagent, 5% Starch, 0.5% NaCl, 0.5M NaOH, and pH 4.6 Acetate buffer were used to complete this process. Using a photocolorimeter (ESICO) and UV-Visible Spectrophotometer (SHIMADZU), the effect of temperature (absorbance) was determined. To assess the impact of temperature, we used several extracts of beta amylase in the current investigation. The majority of enzyme extracts demonstrated that, up until a maximum, the speed of an enzyme reaction increases with an increase in temperature before decreasing. Temperature coefficient, or Q10, is defined as the increase in enzyme velocity when the temperature is raised by 10°C and is typically observed as a bell-shaped curve. For a Q10 is 2 for the vast majority of enzymes between 0°C and 40°C.Most enzymes work best at a temperature of 35 to 40°C ,However there are a few enzymes. The natural (tertiary) structure of the enzymes is typically destabilised when they are exposed to temperatures above 50 °C due to denaturation. At temperatures above 70°C, the majority of enzymes become inactive. We draw the conclusion that the majority of the enzyme exhibits maximum activity at the optimum temperature and then becomes denaturized as temperature is increased based on a literature review and experimental data. Therefore the activity of each of our enzyme extracts was in accordance with the mentioned catalyst for enzyme function. Temperature increases do have an impact on enzymes. The temperature at which an enzyme starts to function and the temperature at which a protein starts to break down are therefore the two factors that dictate the range of an enzyme activity.

KEY WORDS:- β-amylase, Denaturation, Enzyme activity, Optimum Temperature.

1.INTRODUCTION:-

"Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception-RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action"[3].

- Factors Affecting enzyme activity:-
- Contact between enzyme and substrate.
- Concentration of enzyme.
- Concentration of substrate.
- Order of reaction.
- Effect of temperature.
- Hydrogen ion concentration or pH.
- Effect of product concentration
- Coenzymes and activators.
- Effect of time.
- Effect of Radiation.
- Oxidation.
- Water effect[3,4,5]

- **Effect of Temperature on Enzyme Activity:-**

i. Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed.
ii. Temperature coefficient or Q10 is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q10 is 2 between 0°C and 40°C.
iii. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and Interaction for the reaction to proceed faster.
iv. The optimum temperature for most of the enzymes is between 35°C–40°C. However, a few enzymes (e.g. Taq DNA polymerase, muscle adenylate kinase) are active even at 100°C.
v. Some plant enzymes like urease have optimum activity Around 60°C. This may be due to very stable structure and conformation of these enzymes.
vi. In general, when the enzymes are exposed to a temperature above 50°C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C) [3].

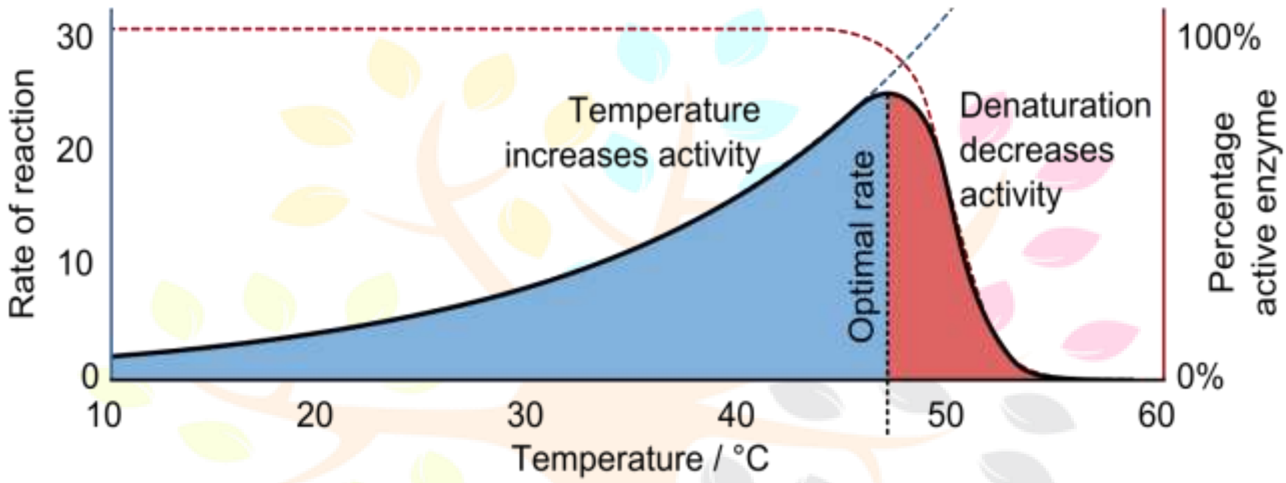


Fig 1:- Effect of temperature on enzyme activity.
(Source:- Thomas Shafee, 2016)

2.AIMS & OBJECTIVES:-

- **Aim:** To study the effect of Temperature on enzyme activity.

- **Objectives:**

i. To study and look at the review of literature on enzyme activity.
ii. To elaborate the activity of enzyme on various temperatures.
iii. To prepare different enzyme extract and to study them on different temperatures.
iv. To compute the data of temperature and absorbance.
v. To correlate the experimental and theoretical evidences of enzyme activity.

3.REVIEW OF LITERATURE:-

i. **Bernfeld et.al in 1955** studied the assay of amylase, numerous useful methods have been devised. The following phenomena seen in the enzyme digests serve as the foundation for them: (1) A rise in the reducing power of an amylopectin or soluble starch solution; (2) a modification of the iodine-staining. A decrease in the viscosity of a starch paste due to the substrate's characteristics. All three of these occurrences are indicative of how α-amylases work, but only the first one can be used to measure E-amylase. Both α- and β-amylases can be measured using the assay method , which is predicated on an increase in reducing power.
ii. **Nielsen et.al in 1997** had done work in this paper is focusing on induction of new starch degrading enzyme activity (cold induced) is β-amylase in potato tubers in sugar accumulation by storage at low temperature. The iodine staining of polyacrylamide gels containing amylopectin allowed for the separation the cold-induced activity from other amylolytic activities in zymograms. Ion exchange chromatography was used to distinguish the cold-induced enzyme from other amylolytic activities.
iii. **Gimbi et.al in 2002** worked on variations in African finger millet seed germination α- and β-amylase activity. β-amylase activity was best between 50 and 55°C at pH 6.0.

iv. **B. Kanwal et.al in 2004** have isolated, purify, and characterized alpha-amylase from Malus Pumila (apple). In this, the DNS approach, as reported by Bernfeld, was used to determine the activity of -amylase at different phases. Electrophoresis after sephadex G-150 chromatography, SDS-PAGE was used to assess the purity and homogeneity of the isolated enzyme. Characterization of α-amylase from apple was done based on Effect of temperature, pH, substrate and enzyme.

v. **Pallavi Tripathi et.al in 2004** has done research on purification and characterization of alpha from soyabean. From germination of soybean seeds, an apparent homogeneous α-amylase was isolated (Glycine max). Enzyme displayed strong starch specificity. The optimal pH for soybean alpha-amylase is 7.6, which is in the range of 4.0 to 10.6. Starch had a Km of 2.63 mg/ml at this pH, and protein had a V of 52.6 mg/ml/min. The enzyme's optimal operating conditions were determined to be 55°C, 1.85 Q10, and 12 kcal/mol of activation energy. While PMSF increased α-amylase activity, additives like EDTA decreased it.

vi. **Jenshinn et.al in 2009** had researched to purify the α-amylase by using superparamagnetic particles from human saliva. The result of this work showed that amylases from human saliva may be isolated using starch-SPIO support with 91.1% recovery and 3.5-fold purification to high specific activity. Two isoamylases with estimated molecular weights of 55 and 59 kDa were found to be present in the purified amylase, according to sodium dodecyl sulphate polyacrylamide gel electrophoresis. The activities of crude and purified amylases revealed that they functioned best at 6-7 and 7 pH levels and 40 and 30 °C, respectively. Both unpurified and purified amylases had a thermal stability range of 20–40 °C.

vii. **Olufunke et.al 2013**, in this paper, cultures of Bacillus subtilis, isolated from the kolanut weevil, Balanogastris kolae, cultured in liquid media with kolanut starch as the only carbon source, an extracellular beta amylase was generated. The enzyme was partially purified 6.4-fold by Sephadex G-150 gel filtration and 1.28-fold by acid treatment with ice-cold 1.0 N HCl. The molecular weight of the β-amylase was 39.4 kDa. The enzyme's activity peaked at 50°C and at pH 5.0, respectively.

viii. **F. Hesam a et.al in 2015** had worked on Sweet potatoes also have endogenous amylases, where α- and β-amylases are the two predominant enzymes, in addition to their high starch content. As amylase is one of the main proteins in sweet potato tubers, it is believed to be a promising source of the enzyme. The characteristics of beta amylase have been the subject of this work. It was determined how pH and temperature affected the stability and activity of the enzyme. Beta amylase was most active at 55 degrees Celsius and had a steady pH range of 3.5 to 7.5.

ix. **Flavia Villas-Boas et.al in 2016** had worked in this paper is focusing on the impact of β- and α- amylase on the structural makeup and digestibility of potato was assessed contrasted. The methods in this paper included the segments of isolation and chemical composition of starches, enzymatic treatment, distribution of molecular components of starch by gel permeation chromatography, x-ray diffraction and relative crystallinity and statistical analysis

x. **Nourbakhsh Amiri et.al 2018**, investigated the effects of temperature, retention duration, particle size, and the addition of co-solvents on extraction yield. The effects of ultrasonic and enzyme pretreatment were also investigated. Ginger with a particle size of 1 mm was used in an enzyme-assisted SWE process with 2% ethanol as a co-solvent, which was run at 130°C and 20 bars. 30 minutes is recognised as an ideal condition.

xi. **Ligtenberg et.al in 2020** researched on the objective to assess how quickly changes in external temperature affect saliva flow rate and content.20 participants (18–25 years old, 14 women, and 6 males) in a cross-over study were exposed to three temperatures (4 °C, 21 °C, and 37 °C) in random order on different days. The collection of resting saliva was commenced for 5 minutes at the same temperature after a subject had been exposed to the test temperature for 5 minutes. The following parameters were assessed in the saliva: pH, viscosity, protein concentration, mucin 5B concentration, and amylase activity. Saliva production of both proteins and amylase increases as the temperature is lowered.

xii. **Rudiana Agustini et al. in 2020** published paper, the goal of the research was to examine the reaction kinetics of hydrolyzing starch substrate. The investigation was carried out in three phases:- 1)The process of making amylase involved germination of soybean seeds, amylase isolation, purification with ammonium sulphate 35% (w/v), 2) optimization of amylase activity (germination time, pH, temperature, enzyme concentration, substrate concentration (starch), and enzyme concentration), and 3) determination of amylase reaction kinetics in starch hydrolysis.

xiii. **Duan et.al in 2021** had worked on Bacillus aryabhattai GEL-09 high-specific-activity β-amylase was expressed and characterised biochemically and structurally for use in starch hydrolysis. The recombinant enzyme purified 4.7-fold to homogeneity, with a molecular weight of ~57.6 kDa and maximal activity at pH 6.5 and 50 °C.

xiv. **Sonica Sondhi et.al in 2021** reported on several microbes, plants, fungi, and animals generate amylases, which break down starch. It has a wide range of industrial applications. Amylases are employed in the fruit business, among other places, to clarify fruit juices and lessen their viscosity. Because there is a lot of starch in fruits, the produced juices have a higher viscosity. Amylases work on the component of starch and break it down. In the current study, apple and other juices were clarified using B. licheniformis amylase. With an increase in amylase concentration, it was discovered that total suspended particles, viscosity, and total acidity all decreased.

xv. **Dalsasso et.al in 2022** had carried out in review based on the relation between ginger processing and their bioactive compounds, focusing especially on gingerols and shogaols, as well as the main processes that increase the content of 6-shogaol without compromising other phenolic compounds to produce highly functional extracts for future applications in the food packaging sector. It include broad overview of how these methods affect the composition and functionalities of ginger extracts.

xvi. **Murakami et.al in 2022** worked in this paper mainly focusing on biochemical characteristics of red algae β-amylase and immobilization to increase thermostability. Polysaccharides like starch are hydrolyzed by amylase to produce maltose. Pharmaceutical and food industry both employ it as an industrial enzyme. The unicellular eukaryotic red alga Cyanidioschyzon merolae thrives best at a pH of 2.0 to 3.0 and a temperature of 40 to 50 °C.

4.MATERIALS AND METHODS:-

- **Materials:-** Extract of soyabean seeds, apples, ginger, potatoes and saliva sample solution were prepared and used.

- **Chemicals and Reagent:-** DNS(Dinitro salicyclic acid) reagent, 5% Starch solution, 0.5% NaCl solution, 0.5M NaOH, Acetate Buffer 4.6 pH.

- **Instruments:-**

a. UV-Visible Spectrophotometer:-
Make:- SHIMADZU
Model No:- UV-1800_Shimadzu

b. PHOTOCOLORIMETER:-
Make:- ESICO
Model No:- 1313

- **Preparation of solution:-**

i. DNS Reagent:-1gm DNS, 30gm of Sodium potassium tartarate and 1.6gm of NaOH dissolve in water and making up the volume upto 100ml with distilled water.
ii. 5% Starch solution:-5gm of starch is dissolved in 100ml distilled water.
iii. 0.5% NaCl solution:-0.5gm of NaCl is dissolved in 100ml distilled water.
iv. 0.5M NaOH:-2gm of NaOH is dissolved in 100ml distilled water.
v. Acetate Buffer (pH-4.6):-Dissolve 5.4gm of sodium acetate in 50ml of distilled water, add 2.4ml of glacial acetic acid and dilute with distilled water to 100ml. Adjust the pH, if necessary.

- **Preparation of enzyme extract:-**

a) Enzyme extract from potato:-
Take 2-3 potatoes. Use cold water to rinse these off. The goal is to remove surface dirt. Peel the raw potatoes by using peeler and cut into small pieces. Grind them in a mortar and pestle with sufficient water. Collect the potatoes homogenate into a beaker and add enough water. Then filter the homogenate through a muslin cloth to remove the particles. Allow the filtration to settle. Starch rapidly settles at the bottom. Collect the starch free supernatant solution carefully. Again filter it by using buchner funnel.

b) Enzyme extract from soyabean seeds:-
Take handful of soyabean seeds, washed with distilled water and kept immersed overnight. Grind the soaked seeds in a mortar and pestle with sufficient water. Collect the homogenate into a beaker and add enough water. Then filter the homogenate through a muslin cloth to remove the particles. Uniform slurry was prepared. After preparing the slurry, filter it by using buchner funnel.

c) Enzyme extract from ginger:-
Take a ginger and use cold water to rinse these off. Peel the raw ginger by using peeler and cut into small pieces. Grind them in a mortar and pestle with sufficient water. Collect the ginger homogenate into a beaker and add enough water. Then filter the homogenate through a muslin cloth to remove the particles. Collect the slurry in a beaker and filter it by using buchner funnel.

d) Enzyme extract from apple:-
Take 2-3 apples, wash the apples with water. The goal is to remove pesticides and surface dirt. Peel the apples by using peeler and cut into small pieces. Grind them in a mortar and pestle with sufficient water and turned into apple mash. Collect the apples homogenate into a beaker and add enough water. Then filter the homogenate through a muslin cloth to remove the particles. Collect the slurry in a beaker and again filter it by using buchner funnel to obtain uniform slurry.

e) Collection and preparation of saliva solution.
Rinse your mouth with clean water. Tale a mouthful of water and hold it in your mouth for two minutes, moving it with the tongue. Collect it in a beaker[2].

- **Procedure for determining the effect of temperature on enzyme activity:-**

i. Prepare series of test tube.
ii. Incubate all test tube at different temperature for 30 mins.
iii. After incubation add 1ml DNS reagent and 0.5M NaOH(1ml) solution and 2ml distilled water.
iv. Keep all the test tubes on boiling water bath for 10 mins.
v. Allow to stand for cooling and dilute the solution 10 times with distilled water.
vi. Determine the absorbance at 540nm.
vii. Plot the graph of temperature v/s absorbance [1].

5.OBSERVATION TABLE

Table 1:-Enzyme extract from potato against different temperature

Test Tube	Starch (ml)	Buffer (ml)	NaCl (ml)	Enzyme (ml)	Temperature (°C)	Absorbance (nm)
1	1	1	1	1	10	0.015
2	1	1	1	1	20	0.054
3	1	1	1	1	30	0.020
4	1	1	1	1	40	0.067
5	1	1	1	1	50	0.800
6	1	1	1	1	60	0.108
7	1	1	1	1	R.T	0.004

Table 2:-Enzyme extract from soyabean seeds against different temperature

Test Test	Starch (ml)	Buffer (ml)	NaCl (ml)	Enzyme (ml)	Temperature (°C)	Absorbance (nm)
1	1	1	1	1	10	0.050
2	1	1	1	1	20	0.060
3	1	1	1	1	30	0.060
4	1	1	1	1	40	0.090
5	1	1	1	1	50	0.010
6	1	1	1	1	60	0.050
7	1	1	1	1	R.T	0.050

Table 3:- Enzyme extract from ginger against different temperature

Test Tube	Starch (ml)	Buffer (ml)	NaCl (ml)	Enzyme (ml)	Temperature (°C)	Absorbance (nm)
1	1	1	1	1	10	0.011
2	1	1	1	1	20	0.001
3	1	1	1	1	30	0.037
4	1	1	1	1	40	0.072
5	1	1	1	1	50	0.065
6	1	1	1	1	60	0.037
7	1	1	1	1	R.T	0.003

Table 4:- Enzyme extract from apple against different temperature

Test Tube	Starch (ml)	Buffer (ml)	NaCl (ml)	Enzyme (ml)	Temperature (°C)	Absorbance (nm)
1	1	1	1	1	10	0.018
2	1	1	1	1	20	0.015
3	1	1	1	1	30	0.008
4	1	1	1	1	40	0.027
5	1	1	1	1	50	0.004
6	1	1	1	1	60	0.019
7	1	1	1	1	R.T	0.004

Table 5:- Enzyme extract from saliva against different temperature

Test Tube	Starch (ml)	Buffer (ml)	NaCl (ml)	Saliva (ml)	Temperature (°C)	Absorbance (nm)
1	1	1	1	1	10	0.022
2	1	1	1	1	20	0.035
3	1	1	1	1	30	0.044
4	1	1	1	1	40	0.055
5	1	1	1	1	50	0.043
6	1	1	1	1	60	0.013
7	1	1	1	1	R.T	0.015

6.OBSERVATION TABLE:-

Table 6- Enzyme extract: Potato

Temperature (°C)	Absorbance (nm)
10	0.015
20	0.054
30	0.020
40	0.067
50	0.800
60	0.108
R.T	0.004

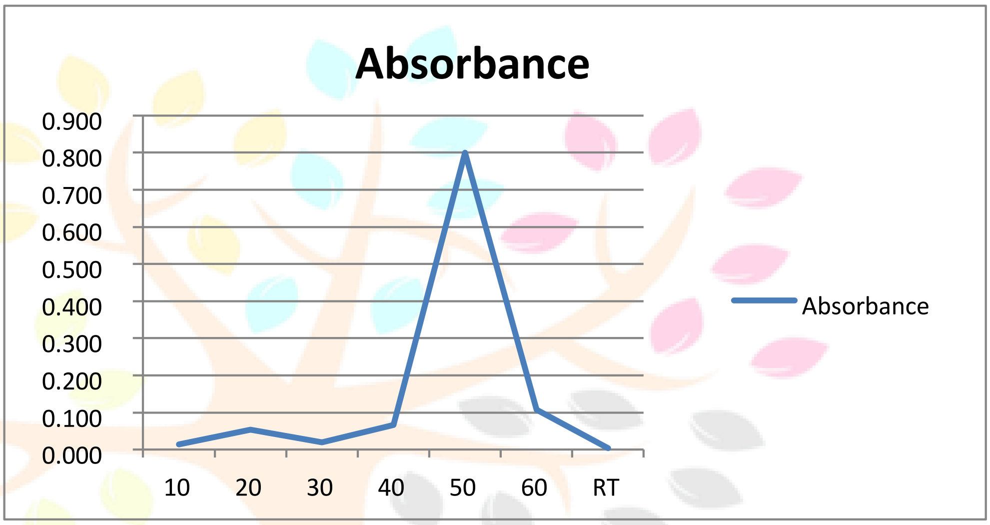


Table 7- Enzyme extract: Soyabean Seeds

Temperature (°C)	Absorbance (nm)
10	0.050
20	0.060
30	0.060
40	0.090
50	0.010
60	0.050
R.T	0.050

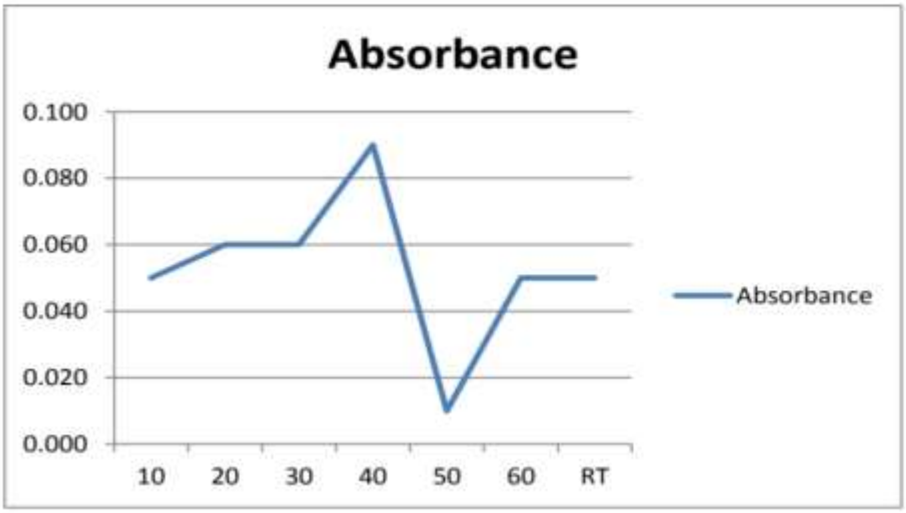


Table 8- Enzyme extract: Ginger

Temperature (°C)	Absorbance (nm)
10	0.011
20	0.001
30	0.037
40	0.072
50	0.065
60	0.037
R.T	0.003

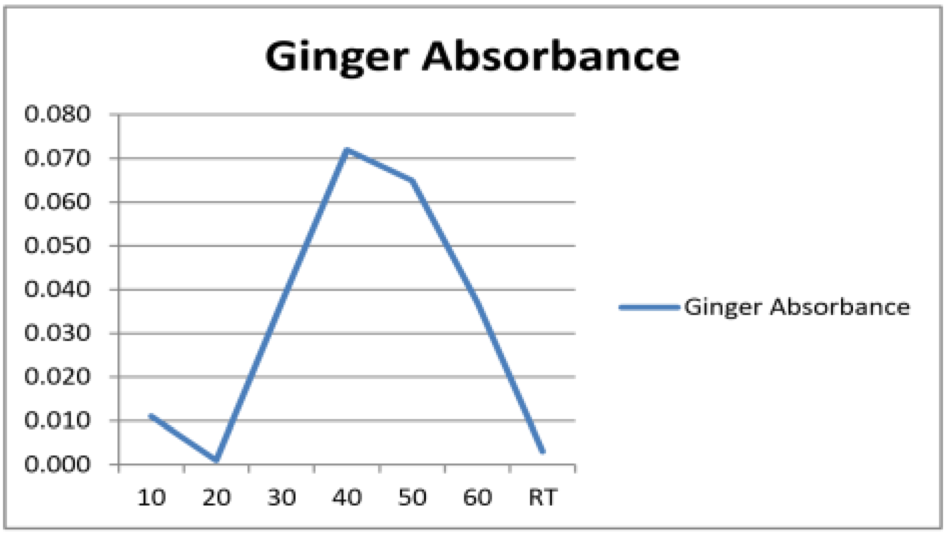


Table 9- Enzyme extract: Apple

Temperature (°C)	Absorbance (nm)
10	0.018
20	0.015
30	0.008
40	0.027
50	0.004
60	0.019
R.T	0.004

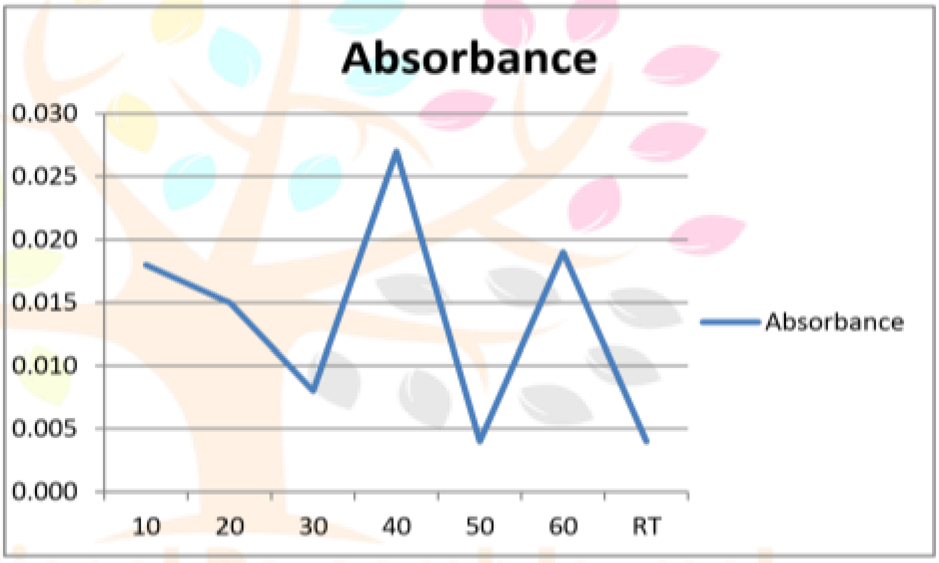
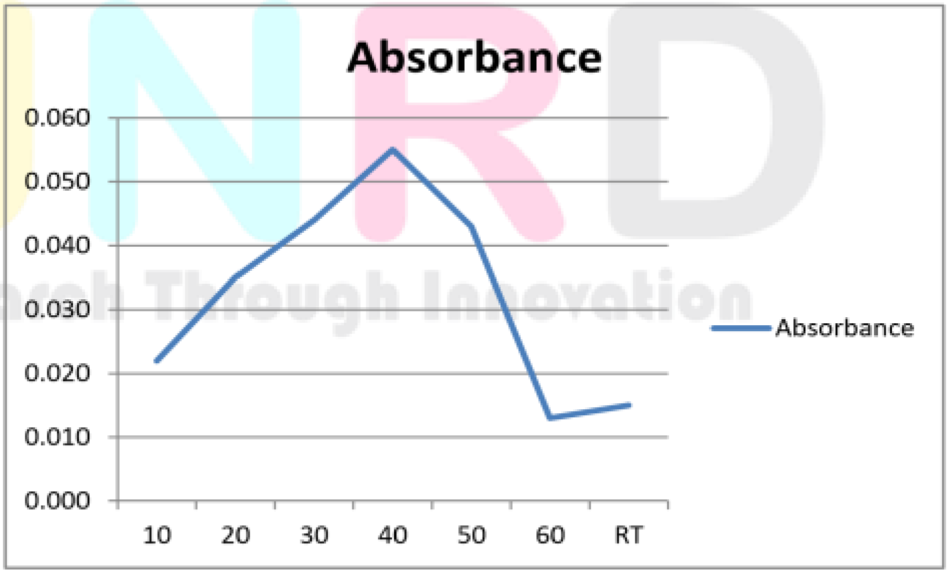


Table 10- Enzyme extract: Saliva

Temperature (°C)	Absorbance (nm)
10	0.022
20	0.035
30	0.044
40	0.055
50	0.043
60	0.013
R.T	0.015



7.DISCUSSION:-

The present investigation was carried on to check the effect of temperature on enzyme activity. The activity of enzyme was determined after incubation of the crude enzyme preparation, during assay conditions, under different temperatures ranging from 10 to 60 °C and also at room temperature. The results obtained in Graph.1 while plotting the graph of absorbance against temperature of enzyme extract of potato, showed that a gradual increase in enzyme activity from 10-50°C. The optimum temperature for maximum enzyme activity was recorded at 50°C. Beyond this range there is decline in the activity at 60°C and room temperature because of the structural unfolding transition at high temperature. The activity of crude enzyme extract from soyabean seeds at temperature variations is shown in Graph.2, it shows the optimum temperature at 40°C. Gradual increase in enzyme activity from 10-40°C, it shows least enzyme activity at 10°C and maximum activity at 40°C. As the temperature rises more than optimum temperature 40-60°C,the enzyme activity gets lowers. The protein nature of enzyme makes them extremely sensitive; so after optimal temperature the rate starts to drop that is because the protein starts to denature so it loses its shape and don't function well. The results obtained in Graph.3 while plotting the graph of absorbance against temperature of enzyme extract of ginger, it shows the optimum temperature at 40°C. Till 40°C it was in increasing order and at 40°C it has highest enzyme activity and after 40°C it is in declining phase, because the enzyme activity gradually lowers as the temperature rises more than the optimum temperature. The results obtained in Graph.4 while plotting the graph of absorbance against temperature of enzyme extract of apple, showed that a gradual increase in enzyme activity from 10-40°C. The optimal temperature for maximum enzyme activity was recorded at 40°C. Beyond this range there is decline in the activity at 60°C and room temperature because of the denaturation of protein. The results obtained in Graph.5 while plotting the graph of absorbance against temperature of saliva. The enzyme activity is slowly increased namely at temperature 10°C, 20°C and 30°C.The optimal temperature for maximum enzyme activity was recorded at 40°C.These results indicate the thermophilic nature of the enzyme. Increasing the reaction incubation temperature namely from 50-60°C led to exponential decrease in enzymatic activity. These results collectively indicated that the enzyme is a thermophilic and showed a complete thermal stability at 40-50°C. The optimal temperature for maximum enzyme activity (40-50°C) is in accordance with what was reported in β-amylase from Cyanidioschyzon merolae (red algae) (Murakami et.al 2022), Bacillus subtilis (Olufunke et.al 2013) and Bacillus aryabhattai (Duan et.al 2021) . The obtained result of temperature for optimum β-amylase activity agrees with report of Murakami et.al 2022) and Gimbi et.al (2002).

8.CONCLUSION:-

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed. Temperature coefficient or Q10 is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q10 is 2 between 0°C and 40°C. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to proceed faster. The optimum temperature for most of the enzymes is between 35°C–40°C. However, a few enzymes. In general, when the enzymes are exposed to a temperature above 50°C, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C). As the temperature of the system is increased, the internal energy of the molecules in the system will increase. The internal energy of the molecules may include the translational energy, vibrational energy and rotational energy of the molecules, the energy involved in chemical bonding of the molecules as well as the energy involved in nonbonding interactions. Some of this heat may be converted into chemical potential energy. If this chemical potential energy increase is great enough some of the weak bonds that determine the three dimensional shape of the active proteins may be broken. This could lead to thermal denaturation of the protein and thus inactivate the protein. Thus too much heat can cause the rate of an enzyme-catalyzed reaction to decrease because the enzyme or substrate becomes denatured and inactive.

In the present study we have taken different extract of β- amylase to determine the effect of temperature. Most of the enzyme extract showed that, enzyme reaction increases with increase in temperature up to a maximum and then declines. We conclude as per literature survey and experimental data that most of the enzyme shows maximum activity at optimum temperature and then gets denaturized as temperature is increased. Our all enzyme extract showed activity as per the said factor of enzyme action. Enzymes do get affected by increase in temperature. Therefore, the range of enzyme activity is determined by the temperature at which the enzyme begins to activate and the temperature at which the protein begins to decompose.

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