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Characterization of Alpha Amylase in Maize (*Zea mays*)

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ABSTRACT

This study characterized the alpha-amylase from maize based on the amylolytic activity initiated during the germination of the maize (Zea mays) grain. Alpha amylase was partially characterized from maize and the protein concentration was determined (found to be 4.48mg/ml) using the Biuret method. The enzyme assay; effect of metal, effect of pH, effect of substrate and temperature was determined. The optimum temperature of the enzyme activity was 50^{o}C and the enzyme activity was stable at this temperature till when a sharp decrease was observed at temperature of 55^{o}C. The crude α – amylase was optimally active at pH 7.0. The apparent km value and Vmax of the enzyme from the Line weaver-Bulk plot during hydrolysis of soluble starch were 0.535mM and 0.451µmol/min/ml respectively. The activities of the α amylase were stimulated by $MgCl_2$ and $CaCl_2$ but inhibited by $HgCl_2$. Therefore, this invention could proffer an alternative to the complex nature of malt extract, with alpha amylase considered difficult to characterize. The procedure offers characterization in a simple and efficient manner and product obtained is at least 95% pure with little impurities. This study shows that the alpha amylase activity of the maize grain was discovered to be high and this could be an alternative source of the enzyme in beer and wine production, as well as industrial source of the enzyme. Thus, it can be used in various industries to degrade starch and accurate result can be generated.

Introduction

Alpha amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar such as rice and potatoes taste slightly sweet as they are chewed because amylase turn some of their starch into sugar in the mouth. The pancreas also makes amylase (α-amylase) to hydrolyze dietary starch into disaccharide and trisaccharide which are converted by other enzyme to glucose to supply the body with energy (Ammar *et al*., 2002). The spectrum of applications of the enzyme has widened; clinical, medical and analytical practices utilizes it as well its widespread applications in starch scarification, textile, foods, brewing and distilling industries (Aktinson and Movituna, 1991).

Microbial amylases are potentially useful in pharmaceutical and fine chemical industries (Augustin *et al*., 2000). Several methods have been developed for cereal amylolytic activity estimation, the level of malt α-amylase is a key quality parameter in the brewing industry. α-amylase also finds application as a silage additive, to assist in the degradation of starch and thus to provide fermentable sugars for bacterial growth. As diastase, amylase was first enzyme to be discovered and isolated (Payen, 1833). Alpha amylase (E.C.: 3.2.1.1) (Alternative names is 1, 4-α-D-glucan glucanohydrolase; glycogenase). The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long chain carbohydrates, ultimately yielding maltotriose and maltose from amylase, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster acting than β-amylase. It is a major digestive enzyme and its optimum pH is 6.7 – 7.0 In human physiology, both the salivary and pancreatic amylases are α-amylase. (Maton *et al*., 1993).

Maize (*Zea mays L.ssp. mays*, also known in some countries like Nigeria as corn), is a cereal grain with the seed coat referred to as "caryopsis", typical of the grasses, and the entire kernel is often referred to as the "seed" (Tilman and Stoskopf , 2002). When ground into flour, maize yields more flour, with much less bran, than wheat does. However, It lacks the protein, gluten of wheat and, therefore, makes baked goods with poor rising capability and coherence (Udani, 2004). Maize is a major source of starch. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, a sweetener; and also fermented and distilled to produce grain alcohol. Grain alcohol from maize is traditionally the source of *Bourbon* whiskey (Tilman and Stoskopf, 2002). Maize extract is of complex nature. It contains water, minerals, salts, dextrin's, sugars, inactive proteins, and multitude of enzymes, including alpha and beta amylase, phosphate, proteinase, oxidase, and phosphorylase (Liggett, 1948).

The aim of this work is to characterize alpha amylase from maize and determine the effect of temperature, pH concentration, and substrate concentration on the obtained enzyme that is, determines its kinetic parameters. The significant of this study is that alpha amylase that degrades starch can be characterized from maize (*Zea mays*). This alpha amylase characterized can be useful in industries such as in bread making industry and pharmaceutical industries.

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Methods

Collection and Preparation of Sample: The maize grains sample used for this study was purchased at Oja titun (New Baboko Market), Ilorin Kwara State, Nigeria. The maize grains was selected to remove damaged, infected ones before being soaked in water for an hour. It was then spread on a tray and was watered continuously for five days at regular interval to assist in germination until sprout was observed on the fifth day. 50g of the sprouted maize grains were blended with 200ml of phosphate buffer, pH 7.5 using blender. The mixture was then filtered twice using a white piece of cloth into a conical flask. The filtered sample was poured into centrifuge tubes (about 12ml in each), and centrifuged at 5000rpm for an hour. The supernatant was decanted and the extract obtained is to be referred to in this study as the crude extract and kept inside a conical flask. It was immediately stored in the refrigerator to be used for the various determination.

Protein Determination: The protein concentration of the crude extract obtained was determined using Biuret method, using BSA as standard (Ammar *et al*., 2002). Concentration of BSA = 0.5%, absorbance was read at 540nm.

Enzyme Assay: Effect of substrate was performed following the method of Ayernor *et al*, (2002). The enzyme extract was transferred into various test tubes containing varied concentration of 1% soluble starch solution (0.2 - 1.0ml) and 1.5ml of phosphate buffer (pH 7.5) each. The mixtures were incubated at 60^{o}C for 10minutes and 3ml dinitrosalicyclic acid reagent (DNS) was added to the mixture in the test tube. The tubes were placed in boiling water in the water bath for 5minutes and cooled at room temperature. The content in the test tubes were diluted to 4ml with distilled water and the absorbance was determined at 546nm. The result was expressed by plotting graph of the absorbance against substrate concentration.

Effect of metals was done according to Raimi *et al*, (2011) to know the metal ion that has positive or negative effects on α-amylase activity. The enzyme extract (1ml) was transferred into three test tubes, each containing 0.5ml of 1% soluble starch solution, 1.25ml phosphate buffer pH 7.5 and 15µL of $CaCl_2$, $MgCl_2$, $HgCl_2$. The solution was treated as above and the absorbance was determined at 546nm.

Effect of pH on α-amylase activity was determined according to Ayernor *et al*, (2002). 1ml of enzyme extract was transferred to five test tubes containing 0.5ml of 1% soluble starch solution, and 1.5ml of various phosphate buffers at pH 7, 8, 9, 10 and 11. These were treated as above and the absorbance was determined at 546nm.

The effect of temperature on the amylase was carried out according to Ayernor *et al*, (2002). The enzyme extract (1ml) was transferred into various test tubes containing 0.5ml 1% of soluble starch solution and 1.5ml of phosphate buffer (pH 7.5) each at varying temperature. The mixture was treated as above and the absorbance was determined at 546nm.

Results

Table 1: Showing Value of Protein Determination (Standard Curve)

Reagent	Absorbance at 540nm
Blank	0.022 ± 0.001
Sample	0.350 ± 0.012

Protein concentration extrapolated from the standard curve **= 4.48 ± 0.01mg/ml**

Table 2: Showing Value of Effect of Substrate on Alpha-Amylase

Test tubes	Absorbance (546nm)
1	2.010
2	2.002
3	2.018
4	2.010
5	2.105

Table 3: Showing Values for Double Reciprocal Plot of Alpha Amylase

1/[s]	1/ [v]
5.000	1.060
2.500	1.040
1.670	0.501
1.250	0.408
1.000	0.400

Table 4: Showing Value of for the Determination Effect of Metals on Alpha-Amylase

Test tubes	Soluble starch (ml)	Metals (ml)	PO_4^{2-} Buffer (ml)	α-amylase (ml)	Distilled water (ml)	Absorbance (546nm)
$MgCl_2$	0.5	1.5	2.5	1.0	4.0	1.976 ± 0.31
CCl_2	0.5	1.5	2.5	1.0	4.0	2.004 ± 0.43
$HgCl_2$	0.5	1.5	2.5	1.0	4.0	1.988 ± 0.03
Control	0.5	1.5	2.5	1.0	4.0	2.004 ± 0.01

Table 5: Effect of pH on Alpha-Amylase

PO_4^{2-} buffer (pH)	Soluble starch (ml)	PO_4^{2-} buffer (ml)	α-amylase (ml)	DNS (ml)	Distilled water (ml)	Absorbance (546nm)
7	0.5	1.5	1.0	3.0	4.0	1.877 ± 0.11
8	0.5	1.5	1.0	3.0	4.0	0.601 ± 0.01
9	0.5	1.5	1.0	3.0	4.0	0.587 ± 0.00
10	0.5	1.5	1.0	3.0	4.0	1.232 ± 0.12
11	0.5	1.5	1.0	3.0	4.0	0.917 ± 0.00

Table 6: Showing Values on Effect of Temperature on Alpha- Amylase

Temperature (^{o}C)	30	35	40	45	50
Soluble starch (ml)	0.5	0.5	0.5	0.5	0.5
PO_4^{2-} buffer (ml)	1.5	1.5	1.5	1.5	1.5
α-amylase (ml)	1.0	1.0	1.0	1.0	1.0
DNS (ml)	3.0	3.0	3.0	3.0	3.0
Distilled water (ml)	4.0	4.0	4.0	4.0	4.0
Absorbance (546nm)	0.266 ±0.01	0.367 ±0.01	0.322 ±0.00	0.322± 0.01	0.422 ±0.01

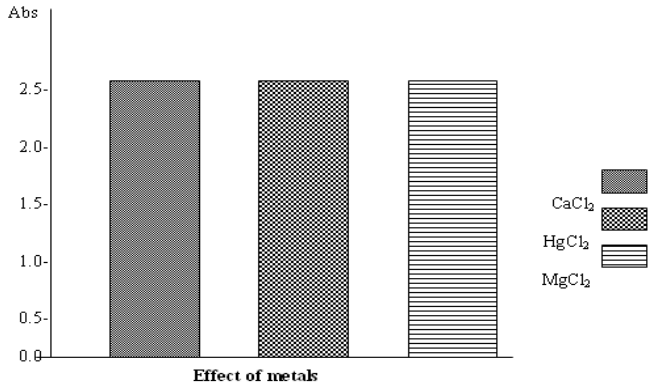

Figure 1: Effect of certain metals on the enzyme amylase

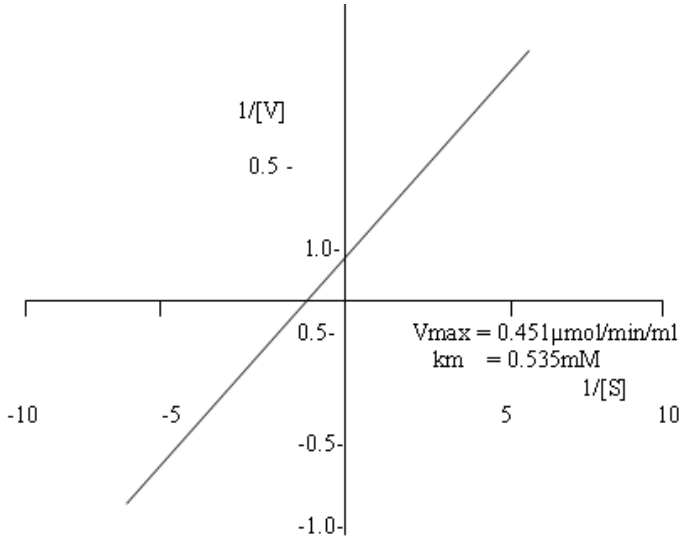

Figure 2: Lineweaver-Burk plot of the enzyme during the hydrolysis of starch from maize grains. Double Reciprocal Graph

Discussion

Alpha – amylase was partially characterized from maize grain. α – amylase was produced from maize grain (*Zea may*) and the protein concentration was determined (4.48mg/ml) using the Biuret method (BSA as standard). The enzyme assay, effect of metal, effect of pH, effect of substrate and temperature was determined using spectrophotometric method to obtain the absorbance. α-amylase has been partially characterized from maize. The protein concentration was 4.48mg/ml (Figure 1). The effect of temperature on α-amylase was more pronounce at temperature of about 50^{o}C (Figure 2). Activity increased with temperature and reached optimum at 50^{O}C after which there was a declined at 55^{O}C. This is in agreement with enzyme's reaction to temperature reported by Foster (1980).

The optimum pH was found to be at pH 7 at absorbance of 1.877 which is a neutral pH and has highest effect on α-amylase while pH 8 and 9, an alkaline pH has a lowest effect on α-amylase (Figure 3) also seem to be at optimum too, this was because the enzyme was not purified, therefore the reagent reacted with the crude enzyme. The effect declined at pH 11. Also, From the Figure 2 above, Vmax and km was obtained to be 0.451µmol/min/ml and 0.535mM respectively from Lineweaver-burk plot. Thus**,** the α-amylase has a km of 0.535mM and Vmax of 0.451µmol/min/ml respectively (Figure 2) obtained from the Lineweaver-Burk plot during the hydrolysis of starch.

All metals are having the same effect on α-amylase in the Figure 1 above, the metals ($MgCl_2$, $CaCl_2$, $HgCl_2$) are having the same effect on the enzyme because of the rate of reaction depends on the turnover rate of the enzyme. The activities of the alpha-amylase were stimulated by $MgCl_2$ and $CaCl_2$ but inhibited by $HgCl_2$.

It can therefore be concluded that α-amylase has been partially characterized and used as an enzyme source in industries such as brewing industries and pharmaceutical industries. This is very important in biotechnology especially as regard production of enzymes using simple and cost effective materials.

Conclusion

The characteristics high temperature, neutral pH of 7 observed in this study are novel qualities for application in industrial amylase production, which could be exploited in starch and other food industries.

Based on this result, it has been observed that the alpha amylase degrades starch and may be useful in textile, foods, brewing and distilling industries. According to present invention, complex nature of malt extract, and it's characterization can be overcome in a simple and efficient manner and product obtained is of high purity though with the sole impurity being certain mineral elements that are not a serious barrier to its use in various industries to degrade starch.

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