PRODUCTION AND ISOLATION OF ALPHA AMYLASE FROM RICE MALT EXTRACT

By

ASANTE, ENOCH

Bsc. (Honours) Chemical Engineering

A Thesis submitted to the Department of Chemical Engineering,

Kwame Nkrumah University of Science and Technology

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN CHEMICAL ENGINEERING

Faculty of Chemical and Material Engineering,

College of Engineering

February, 2012

CERTIFICATION

I hereby declare that this thesis is the result of my own original research work undertaken under the supervision of the undersigned and that all works consulted have duly been referenced and no part of the thesis has been presented for another degree in the University or elsewhere.

Asante Enoch 16/02/2012

(CANDIDATE) Signature Date

Certified by:

Dr. Albert Adjaottor 16/02/2012

(SUPERVISOR) Signature Date

Certified by:

Mrs. Zsuzsanna Momade 19/03/12

(HEAD OF DEPARTMENT) Signature Date

ABSTRACT

The current study investigated the production and purification of alpha amylase from malted rice (Wita 7) extract. In the course of the study, malting parameters such as germination energy, malt yield as well as malt loss of rice (Wita 7) were estimated. An appraisal of alpha amylase production during malting with rice (Wita 7) revealed that, the production level peaked on the 8th day of germination with an estimated activity of 19.436 U/ml. The crude alpha amylase from the 8th day malt extract was subsequently purified by using starch column procedures. The purified extract recorded an estimated activity of 75.549 U/ml. The project further examined the effects of varying the ratios of starch adsorbent height to column diameter (L/D) during the purification step. For the columns employed (ID = 2.45cm; 4.15cm), the 2nd elution fractions from the experiments which were conducted with L/D ratios greater than 0.33 contained the highest amount of alpha amylase. In conclusion, the current work revealed that,

- The 8th day malt was most effective (optimum malting time) for starch hydrolysis
- The purification process produced a 4-fold enzyme activity; 19:75
- The greater the L/D ratio, the higher the efficacy for purification

TABLE OF CONTENTS

CERTIFICATION i

ABSTRACT ii

TABLE OF CONTENTS iii

LIST OF TABLES vii

LIST OF FIGURES x

LIST OF PICTURES xi

ABBREVIATIONS xii

ACKNOWLEDGEMENT xiii

DEDICATION xiv

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Malting and Alpha Amylase Production 1

1.2 Problem Statement 3

1.3 Justification for Research 3

1.4 Main Objectives 5

CHAPTER TWO 6

2.0 LITERATURE REVIEW 6

2.1 Background of Rice Production in Ghana 6

2.1.1 Present State of Cereals and Paddy Rice Production in Ghana 7

2.1.2 Programs Outlined to Increase Rice Production in Ghana 9

2.1.3 Rice Demand Projection 11

2.2 Malting of Cereal Grains 12

2.2.1 Malting of Rice Grains in Ghana 15

2.2.2 Water Uptake by Rice Grains during Steeping 16

2.3 Enzyme Extraction and Diastatic Activity of Rice Malt 17

2.3.1 Malt Extract 19

2.3.2 Starch as an Affinity Adsorbent for the Purification of Alpha Amylase 19

2.3.3 Factors Influencing the Efficiency of Starch as an Adsorbent for Amylase Isolation 22

2.3.4 Starch as a Natural Substrate for Amylolytic Enzymes 23

2.3.5 Starch Hydrolysis..... 25
2.3.6 Applications of Amylolytic Enzymes 27

CHAPTER THREE..... 28
3.0 MATERIALS AND METHODS 28
3.1 MALTING OF RICE GRAINS 28
3.1.3 Drying of Germinated Rice Seeds 30
3.1.4 Milling of Dried Rice Seedlings 30
3.2 ENZYME EXTRACTION AND ALPHA AMYLASE ACTIVITY DETERMINATION 30
3.2.1 Enzyme Extraction 30
3.2.2 Preparation of Equilibrated Soluble Starch..... 31
3.2.3 Alpha Amylase Assay using the 3, 5-Dinitrosalicylic Acid (DNSA) Method... 31
3.2.4 Preparation of Standard Maltose Solution..... 32
3.3 ISOLATION OF ALPHA AMYLASE FROM RICE MALT EXTRACT..... 32
3.3.1 Extraction, Heating and Purification of Alpha Amylase 32
3.3.2 Adsorption of Alpha Amylase by Affinity Chromatography 33
3.3.3 Assessment of Alpha Amylase Concentration Trend in Elutions 34

CHAPTER FOUR 36
4.0 RESULTS AND DISCUSSIONS 36
4.1 STEEPING 36
4.1.1 Water uptake by Rice Grains 36
4.1.2 Germination Energy of Rice Grains 37
4.1.3 Malting Loss and Malt Yield Determination 40
4.2 ASSESSMENT OF REDUCING SUGARS PRODUCTION AND ALPHA AMYLASE ACTIVITIES OF CRUDE EXTRACT 41
4.2.1 Reducing Sugars Formation 41
4.2.2 Maltose Standard Curve 44
4.2.3 Alpha Amylase Activities 45
4.3 ASSESSMENT OF REDUCING SUGARS PRODUCTION AND ALPHA AMYLASE ACTIVITIES OF PURIFIED EXTRACT OBTAINED FROM VARYING ADSORBENT HEIGHT TO DIAMETER RATIO (L/D) 46
4.3.1 Reducing Sugars Production 46

4.3.2 The Influence of Adsorbent Height to Diameter ratios (L/D) on Alpha Amylase concentration in Elution Fractions. 54
4.3.3 Activities of Purified Alpha Amylase 58
4.3.4 The Optimum Number of Elutions 58
4.3.5 Estimation of Enzyme Activity likely to be exhibited by One (1) g of Rice (Wita 7) malt 60

CHAPTER FIVE 61
5.0 CONCLUSIONS AND RECOMMENDATIONS 61
5.1 CONCLUSIONS 61
5.2 RECOMMENDATIONS 62

REFERENCE 63
APPENDIX A: MALTING CHARACTERISITICS OF WITA 7 RICE VARIETY 67
APPENDIX B: ABSORBANCE AND ACTIVIITIES OF CRUDE ALPHA AMYLASE 70
APPENDIX C: STARCH ADSORBENT COLUMN 73
APPENDIX D: ABSORBANCE RESULTS OF ELUTION FRACTIONS 74
APPENDIX E: THE EFFECTS OF VARYING ADSORBENT HEIGHT TO DIAMETER RATIOS (L/D) ON ALPHA AMYLASE CONCENTRATION IN ELUTION FRACTIONS 81
APPENDIX F: ACTIVITIES OF PURIFIED ALPHA AMYLASE IN ELUTIONS 83
APPENDIX G: LIST OF PROJECT PHOTOGRAPHS 90

LIST OF TABLES

TABLE 2.1: CEREAL CROP PRODUCTION IN GHANA (1996 – 2005).....8

TABLE 2.2: RICE PRODUCTION PROGRAM 2005-2010 11

TABLE 2.3: FUTURE SUPPLY – DEMAND AND REQUIREMENT OF RICE DEVELOPMENT 12

TABLE A-1: WATER UPTAKE RATE RESULTS OF STEEPED RICE SEEDS 67

TABLE A-2: MALT LOSS AND MALT YIELD DETERMINATION RESULTS 68

TABLE A-3: GERMINATION ENERGY DETERMINATION RESULTS.....69

TABLE B-1: ABSORBANCE RESULTS OF CRUDE ALPHA AMYLASE AT 540NM.....70

TABLE B-2: ACTIVITY RESULTS OF CRUDE ALPHA AMYLASE 71

TABLE B-3: STANDARD MALTOSE CURVE DATA.....72

TABLE C-1: COLUMN ACCESSORIES DATA 73

TABLE C-2: COLUMN DATA (ID = 2.45CM).....73

TABLE C-3: COLUMN DATA (ID = 4.15CM).....73

TABLE D-1: ABSORBANCE READINGS FOR L/D = 0.08 USING COLUMN (ID = 4.15CM).74

TABLE D-2: ABSORBANCE READINGS FOR L/D = 0.16 USING COLUMN (ID = 4.15CM).74

TABLE D-3: ABSORBANCE READINGS FOR L/D = 0.33 USING COLUMN (ID = 4.15CM).75

TABLE D-4: ABSORBANCE READINGS FOR L/D = 0.41 USING COLUMN (ID = 4.15CM).75

TABLE D-5: ABSORBANCE READINGS FOR L/D = 0.49 USING COLUMN (ID = 4.15CM).76

TABLE D-6: ABSORBANCE READINGS FOR L/D = 0.08 USING COLUMN (ID = 2.45CM).76

TABLE D-7: ABSORBANCE READINGS FOR L/D = 0.16 USING COLUMN (ID = 2.45CM).77

TABLE D-8: ABSORBANCE READINGS FOR L/D = 0.33 USING COLUMN (ID = 2.45CM).77

TABLE D-9: ABSORBANCE READINGS FOR L/D = 0.41 USING COLUMN (ID = 2.45CM).78

TABLE D-10: ABSORBANCE READINGS FOR L/D = 0.49 USING COLUMN (ID = 2.45CM)
.....78

TABLE D-11: ABSORBANCE READINGS FOR L/D = 0.08 USING COLUMN (ID = 2.45CM)
.....79

TABLE D-12: ABSORBANCE READINGS FOR L/D = 0.16 USING COLUMN (ID = 2.45CM)
.....79

TABLE D-13: ABSORBANCE READINGS FOR L/D = 0.33 USING COLUMN (ID = 2.45CM)
.....79

TABLE D-14: ABSORBANCE READINGS FOR L/D = 0.41 USING COLUMN (ID = 2.45CM)
.....80

TABLE D-15: ABSORBANCE READINGS FOR L/D = 0.49 USING COLUMN (ID = 2.45CM) 80
TABLE E-1: ABSORBANCE READINGS OF ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 4.15 CM. 81
TABLE E-2: ABSORBANCE READINGS OF (3) ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 2.45 CM. 81
TABLE E-3: ABSORBANCE READINGS OF (5) ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 2.45 CM. 82
TABLE F-1: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.08 USING COLUMN ID = 4.15CM 83
TABLE F-2: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.16 USING COLUMN ID = 4.15CM 83
TABLE F-3: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.33 USING COLUMN ID = 4.15CM 84
TABLE F-4: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.41 USING COLUMN ID = 4.15CM 84
TABLE F-5: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.49 USING COLUMN ID = 4.15CM 85
TABLE F-6: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.08 USING COLUMN ID = 2.45CM 85
TABLE F-7: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.16 USING COLUMN ID = 2.45CM 86
TABLE F-8: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.33 USING COLUMN ID = 2.45CM 86
TABLE F-9: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.41 USING COLUMN ID = 2.45CM 87
TABLE F-10: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.49 WITH COLUMN ID = 2.45CM 87
TABLE F-11: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.08 USING COLUMN ID = 2.45CM 88

TABLE F-12: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.16 USING COLUMN ID = 2.45CM 88

TABLE F-13: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.33 USING COLUMN ID = 2.45CM 88

TABLE F-14: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.41 USING COLUMN ID = 2.45CM 89

TABLE F-15: ACTIVITIES OF PURIFIED ALPHA AMYLASE FOR L/D = 0.49 USING COLUMN ID = 2.45CM 89

LIST OF FIGURES

Figure 2.1: **Amylose structure of starch molecule** 25

FIGURE 4.1 WATER UPTAKE RATE BY RICE GRAINS DURING THE FIRST 12 HOURS OF STEEPING 37

FIGURE 4.2 THE GERMINATION ENERGY OF THE RICE GRAINS DURING THE MALTING PERIOD.....39

FIGURE 4.3 MALTING LOSS AND MALTING YIELD OF WITA 7 RICE VARIETY.....41

FIGURE 4.4 TREND OF PRODUCTION OF REDUCING SUGARS IN WITA 7 RICE VARIETY42

FIGURE 4.5 MALTOSE STANDARD CURVE.....45

FIGURE 4.6: TREND OF ALPHA AMYLASE PRODUCTION. 46

FIGURE 4.7: ABSORBANCE READINGS OF ELUTION FRACTIONS FROM L/D RATIO OF 0.08 (ID = 2.45 CM; 1G STARCH ADSORBENT USED, ID = 4.15 CM; 3G STARCH ADSORBENT USED).....47

FIGURE 4.8: ABSORBANCE READINGS OF ELUTION FRACTIONS FROM L/D RATIO OF 0.16 (ID = 2.45 CM; 2G STARCH ADSORBENT USED, ID = 4.15 CM; 5.8 G STARCH ADSORBENT USED).....47

FIGURE 4.9: ABSORBANCE READINGS OF ELUTION FRACTIONS FROM L/D RATIO OF 0.33 (ID = 2.45 CM; 3G STARCH ADSORBENT USED, ID = 4.15 CM; 11.9G STARCH ADSORBENT USED).....48

FIGURE 4.10: ABSORBANCE READINGS OF ELUTION FRACTIONS FROM L/D RATIO OF 0.41(ID = 2.45 CM; 4G STARCH ADSORBENT USED, ID = 4.15 CM; 14.7G STARCH ADSORBENT USED).....48

FIGURE 4.11:..... ABSORBANCE READINGS OF ELUTION FRACTIONS FROM L/D RATIO OF 0.49 (ID = 2.45 CM; 5G STARCH ADSORBENT USED, ID = 4.15 CM; 17.6 G STARCH ADSORBENT USED).....49

FIGURE 4.12: ABSORBANCE READINGS OF ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 4.15 CM 55

FIGURE 4.13:..... ABSORBANCE READINGS OF 3 ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 2.45 CM. 56

FIGURE 4.14:..... ABSORBANCE READINGS OF 5 ELUTION FRACTIONS OBTAINED FROM VARYING ADSORBENT HEIGHT (L) IN A COLUMN HAVING A CONSTANT INTERNAL DIAMETER (D) OF 2.45 CM. 57

LIST OF PICTURES

PICTURE 1.0: 4TH DAY OF GERMINATION; JPEG IMAGE FORMAT 38

PICTURE 2.0: STEEPING 90

PICTURE 3.0: 1ST DAY OF GERMINATION 90

PICTURE 4.0: 2ND DAY OF GERMINATION 90

PICTURE 5.0: 3RD DAY OF GERMINATION 90

PICTURE 6.0: 8TH DAY OF GERMINATION 90

PICTURE 7.0: DRYING 90

PICTURE 8.0: SIZE REDUCTION (BLENDING) 90

PICTURE 9.0: RICE MALT FLOUR 90

PICTURE 10.0: MALT FLOUR IN CENTRIFUGETUBES 91

PICTURE 11.0: CENTRIFUGE MACHINE 91

PICTURE 13.0: COOLED HEATED EXTRACTCONTAINING AMMONIUM SALT 91

PICTURE 12.0: HEATING OF MALTEXTRACT IN WATER BATH 91

PICTURE 14.0: AMYLASE PRECIPITATEFORMED FROM EXTRACT 91

PICTURE 15.0: STARCH SUPPORT PLATES (DIAMETER = 2.40CM) 91

PICTURE 16.0: SUPPORT PLATES IN COLUMN (ID = 2.45CM) 91

PICTURE 17.0: STARCH SUPPORT PLATES (DIAMETER = 4.00CM) 91

PICTURE 19.0: SUPPORT PLATES IN COLUMN (ID = 4.15CM) 91

PICTURE 18.0: TREATED STARCH IN COLUMN(ID = 2.45CM) 91

PICTURE 21.0: ELUTION AND COLLECTIONOF ENZYME FROM STARCH ADSORBENT 91

PICTURE 20.0: COLUMN WITH ENZYME SOLUTION PASSING THROUGH STARCH ADSORBENT 91

ABBREVIATIONS

AFD	French Development Agency
CMD	Carbohydrate Binding Module
CHA	Cycloheptaamylose
CSIR	Council for Scientific and Industrial Research
DF	Dilution Factor
DNSA	Dinitrosalicylic Acid
EU	European Union
FRI	Food Research Institute
IVRDP	Inland Valley Rice Development Project
ID	Internal Diameter
JICA	Japan International Cooperation Agency
KNUST	Kwame Nkrumah University of Science and Technology
L/D	Length to Column Diameter ratio
LRDP	Lowland Rice Development Program
MoFA	Ministry of Food and Agriculture
M_w	Molecular Weight
OD	Optical Density
PPMED	Policy, Planning, Monitoring and Evaluation Division
RME	Rice Malt Extract
S_1	Initial salt saturation
S_2	Final salt saturation
SRID	Statistics Research and Information Directorate
SRI	Soil Research Institute
U	Activity

ACKNOWLEDGEMENT

I am thankful to God for His Grace that has helped me to attain this height in life. My special appreciation goes to my supervisors; Dr Albert Adjoattor and Dr Woode whose suggestions and criticisms have contributed to the success of the project. It is also worth to note the contributions of all the Lecturers at the Chemical Engineering Department; to Mrs. Zsuzsana Momade (HoD), Dr. George Ankudey, Dr. Lawrence Darkwah, Mr. Kwaku Baah Ennum and Dr. David Kuwornu. I am also thankful to Dr. E. Annan-Afful of Crop Research Institute, Fumesua, who personally delivered to me data on rice production in Ghana.

A reserved expression of special gratitude goes to my siblings namely; Victor, Harriet, Seth, Eugene and most of all to Rev. Dennis Mudd Asante. No amount of words can suffice for the advice and encouragement from this wonderful set of people.

I also take a special pleasure in expressing my appreciation to Prof. Mrs. I. Oduro and Mr. T.D. Blutse of the Biochemistry Department, KNUST for allowing me to use their laboratory facilities. Again, the contributions of Mr. Omari Agyapong and Mr. Enos Adepah are kindly accredited. I also extend my appreciation to my fellow post graduate colleagues at the Chemical Engineering department namely; Abel, Julius, Nafiuw, Maclean and Andrew who shared in my moments of countless frustration.

Finally, I wish to say thank you to Miss Regina Boateng Ofori (Nova Scotia Agric College, Canada) for her love, support and affection.

DEDICATION

To my Lovely Mum and Daddy

Maame Afua Tiwaa

And

Opanin Kofi Agyei Frempong

CHAPTER ONE

1.0 INTRODUCTION

1.1 Malting and Alpha Amylase Production

Hagenimana *et al.* (1992, cited by Saboury, 2002) reported that amylases are widely distributed in plants, animals and microorganisms and show varying action patterns depending on their source. Rao *et al.* (2005), citing Bajpai and Bajpai (1989), referred to plants as one of the abundant sources of enzymes. Further, Boyer (1970, cited by Awoyinka and Adebawo, 2007) reported that, plants in their natural states contain enzymes that are made for their own use. These enzymes are often separated from the plant product and are used to accelerate chemical reactions in industry. Subsequently, Yaldagard *et al.* (2008) reported that, amylases specifically cleave the glycosidic bonds in starch which is the principal storage of polysaccharide present in seeds of various plants.

The potential for commercial applications of alpha amylases is enormous as they are extensively used in beverages, baby foods, medicinal and pharmaceutical manufacturing industries. Commercially, alpha amylases are produced mostly from fungal sources, but they are also extracted from other plant sources such as barley, millet, wheat, sorghum and maize (Rao *et al.*, 2005).

Alpha amylase enzymes from cereal sources are obtained in a controlled germination process called malting. Malting is a process involving the germination and drying of cereal seeds with the prime objective being to promote the development of hydrolytic enzymes that are not active in raw seeds (Dewar *et al.*, 1997). During malting, the seeds undergo various changes such as increase in the quantities of alpha and beta amylases and partial degradation of reserve substances (cell wall, gums, protein, and

starch) in the endosperm (Dewar *et al.*, 1997). The malting process involves the steeping of the grains in water, germinating and drying.

Barley holds a prominent position with regards to malting as it is known to produce relatively large amounts of alpha and beta amylases during the process. However, barley cultivation in the tropical areas, including Ghana, has not been successful, hence there has been a call for the exploration of other locally grown cereals, especially rice, for the production of malt for local sugar and brewing industries (Ayernor and Ocloo, 2007). Malting of cereals other than barley has been in the research domain in recent years (Dewar *et al.*, 1997; Ayernor and Ocloo, 2007). These developments have been attributed mainly to local availabilities and economic considerations (Hammond and Ayernor 2000).

In Ghana, various cereals such as maize, sorghum, rice and millet have been malted for use as sources of enzymes in sugar and brewing industries. Research has established that malted rice has a potential for achieving high starch conversion rate to sugar (Hammond and Ayernor, 2000). In addition, Ayernor and Ocloo (2007) cited Egwim and Oleyede who reported that, whiles barley, wheat and rye produce substantial amounts of alpha and beta amylases, rice produces entirely alpha amylase during malting. On the basis of the works done by early researchers, the current study is carried to investigate the possibility of producing and purifying alpha amylase enzyme from a local rice variety (Wita 7).

1.2 Problem Statement

In Ghana, small and medium scale industrial users of amylolytic enzymes such as alpha amylase purchase and import these enzymes at a high cost which eventually increases the total cost of production. Consequently, the current study is intended to produce alpha amylase locally from malted rice and purify it by using starch (cassava) column procedures.

1.3 Justification for Research

Hydrolysis of starch is the breaking down of the complex starch molecule into simple constituting units (glucose). This process is vital as it enhances easy handling of material in processing equipment and enables manufacturers to come up with vital semi finished-products for further processing. In industry, the process can be accomplished either by using acids or enzymes.

The use of enzymes for hydrolyzing starch has gained widespread application in recent years. Enzymes have the advantage over acid catalysts in that they exhibit specificity for certain linkages in starch. As a result, fewer byproducts are formed and actual yields are high. They also require low concentrations to accomplish their purposes and they operate under relatively mild conditions (Aiyer, 2005). The inherent specificity of enzymes in hydrolysis has crowned their dominance in their usage in the industry to the extent that they enable the users to exercise maximum control over the extent of hydrolysis. Again, the inherent specificity of enzymes allows specific linkages to be attacked in order to achieve the expected products.

Alpha amylases randomly break the bonds between glucose molecules, producing oligosaccharides (a glucose polymer containing a relatively smaller number of monosaccharide units). Beta amylases cut off pairs of glucose molecules to produce maltose until they reach a branch in the starch chain molecule where they stop working.

In contrast to the availability of many different and "sophisticated" enzyme systems used in the developed countries, most developing countries do have access to a limited range of enzymes (Ayernor and Hammond, 2001). Again, the high cost of cane sugar and its limited quantity makes it difficult to meet production requirements of sweeteners by industries (Cecil, 1995). Therefore, the use of enzymes from cereal sources for the production of reducing sugars from starch has the potential to make sugar-usage industries less dependent on conventional sugar in areas where starches are more cheaply accessible than cane sugar (Ayernor and Hammond, 2000). What's more, starch bioconversion offers alternative uses and provides an expanded market for starchy materials such as cassava which is highly perishable and lacks proper preservation techniques (Hammond and Ayernor, 2000). The use of local rice malt for the bioconversion of starch in industries would enhance the marketing of harvested rice from farmers who cultivate such crops.

In Ghana, Hammond and Ayernor (2000) screened cereals including, maize, millet, sorghum and rice for their hydrolytic power on starch and found that rice has the highest enzyme activity. Again, Kneen and Dickson, (1967, cited by Ayernor and Hammond, 2001) reported that, whereas barley, wheat and rye produce alpha and beta amylase during malting, rice essentially produces mostly alpha amylase during

germination. Egwin and Oloyede, (2006, cited by Ayernor and Ocloo 2007) have also reported that rice produces a high amount of alpha amylase during malting.

The current study therefore attempts to produce and purify alpha amylase from malted rice (Wita 7) variety.

1.4 Main Objectives

The main interest of the project is focused on the production and purification of alpha amylase from malted rice.

The specific goals include the following:

- Estimate the trend of alpha amylase and reducing sugars formation in rice seeds malted for 12 days following available literature.
- Estimate the malting characteristics of the Wita 7 rice variety such as germination energy, malting yield and malting loss
- Purify the alpha amylase from the malted rice that exhibited the highest enzyme activity.
- Assess the concentration trend of alpha amylase enzyme in the elutions from starch adsorbent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background of Rice Production in Ghana

Mobil and Okran, (1985), cited by Kranjac-Berisavljevic 2001, reported that, rice has been cultivated in Ghana for a long time. It was one of the major commercial food crops during the 17th and 18th centuries. Most of the people involved in the cultivation of rice at that time were small scale farmers, who cultivated rice under bush/fallow system and shifting cultivation practices. According to the practice, rice was cultivated 2-4 years until the fertility of soil was diminished. A fresh piece of land was acquired and the pattern repeated. Although wasteful in its use of land and labor, the system in the past was capable of sustaining the population without noticeable adverse effects on the land. Again, Kranjac-Berisavljevic, (2001) cited Benneh, *et al.*, (1990) who reported that, with the increasing population, the system has almost broken down, and the fallow periods have reduced with corresponding reduction in production yields. The two production seasons in Ghana are the main season and off seasons. Cultivation for the main season occurs between May and June with harvesting taking place between October and November. For the off-season, planting is done between January and February with harvesting occurring between May and June.

Until 1920, most of the rice in Ghana was cultivated in the Volta and Western Regions (Kranjac-Berisavljevic, 2001). Most of the rice varieties were grown without any improvement as they were inherited from ancestors and some of these varieties are still in cultivation. In the inland valley (Ashanti, Eastern, Western, Central and Brong Ahafo regions) which constitutes one of the main ecologies in the country, rice is grown on flooded fields. Since the attainment of Ghana's

independence in 1957, various agricultural policies have been developed towards self-sufficiency in rice production. An entirely mechanized system has been put in place to take over from poorly resourced small-scale farmers. These and other national policies relating to the self-sufficiency efforts have resulted in gradual shift of bulk rice production from Volta and Western regions towards flood plains of the Volta River in Northern Ghana. According to Dekuku, (1997) as cited by Kranjac-Berisavljevic, (2001), it is estimated that, the interior Savanna Agro-Ecological Zone has about 800,000 hectares of land suitable for rice production.

2.1.1 Present State of Cereals and Paddy Rice Production in Ghana

The main cereals cultivated in Ghana include maize, rice, millet and sorghum. Cereals have constituted 11% of total food production in Ghana between the periods of 1996 and 2005. Paddy rice alone contributed to 14.53% of cereals produced between these years. Table 2.1 gives the average production of the various cereals between the periods of 1996 and 2005.

Cereals including rice have become valuable to the Ghanaian due to the fact that they contain high carbohydrates and nutritional values. Rice production has increased from 162,000 to 237,000 tons between 1994 and 2004 in parallel with expansion in the cultivation area from 80,000 to 120,000 hectares. However, rice production capacity has not been able to meet the national consumption rate which at present is still supplemented with rice importation (JICA study team report, 2007).

Table 2.1: Cereal Crop Production in Ghana (1996 – 2005)

Cereal	Production (1000 ton)
Maize	1000 (58.14%)
Rice (Paddy)	250 (14.53%)
Millet	160 (9.30%)
Sorghum	310 (18.02%)
Total	**1720**

Source: (JICA study team report, 2007).

Between the periods of 1996 and 2005, paddy rice production has been in the range of 200,000 and 280,000 tons with annual fluctuations. In a quest to determine the main causes of annual fluctuations in paddy rice production, correlations for paddy rice production and yield (production in tons per hectare) as well as paddy rice production and planted area from the ten (10) regions were analyzed. Though, no significant relationship was identified between paddy rice production and yield, a clear correlation was recognized between paddy rice production and the planted area. According to the correlation, paddy rice production increased with increasing area for rice cultivation (JICA study team report, 2007). In another report, Braimoh (2009), citing Langyintuo and Dogbe (2005) attributed the increase in the average annual rice production in Ghana (80,000 tons) in the 1990s which was about 2.5 times the average production amounts in the 1960s to land area expansion. Following an assessment by the Ghana Soil Research Institute (SRI), it has been found that, 2.86 million hectares, representing 12% of the national land is suitable for paddy rice cultivation. However, the existing paddy rice field for cultivation only accounts for 123, 000 hectares or 4% of the potential area for paddy cultivation (JICA study team report, 2007).

Currently, there are three main rice cultivation fields in Ghana namely: the lowland rain-fed, upland rain-fed and the irrigated lands. As a result of fluctuations in the planted area which has been identified as the main cause of variation in annual rice production, programs have been instituted to expand the available rice cultivation fields (JICA study team report, 2007).

2.1.2 Programs Outlined to Increase Rice Production in Ghana

Notable among the programs aimed at increasing rice production in Ghana is the Lowland Rice Development Program (LRDP). According to the program, agencies such as French Development Agency (AFD) and the Ministry of Food and Agriculture (MoFA) have in terms of technical and financial assistance embarked on lowland rice development in the Northern region. Following the completion of the first phase in 2003, the lowland paddy fields in the region increased to cover a total area of 1,151 hectares with an average yield improved to 2.6 ton/ha. In the second phase of the project which was scheduled to commence in 2008, it was estimated that an additional 6000 hectares of land would be cultivated for rice production in the Northern, Upper East, Upper West and Volta regions by 2012 (JICA study team report, 2007).

Another project intended to increase rice production in Ghana is the Inland Valley Rice Development Project (IVRDP). The program is targeted at developing the inland valleys for rice cultivation in five regions namely: Brong Ahafo, Ashanti, Eastern, Western and the Central regions. The program promotes the group formation of rice farmers and assists in paddy rice production. The program has plans of water resource development such as the construction of irrigation, drainage canals as well as technical and managerial support. A hindrance to the smooth

implementation of the IVRDP is presented by the traditional land tenure system especially in the southern parts of Ghana where the owners are often reluctant to sign land lease contracts (JICA study team report, 2007

Also in the bid to expand rice cultivation fields, a release from cabinet dated 15th April, 2005 stated the program for the promotion of domestic rice production with targets to be achieved by 2010 (Table 2.2). According to the program, new development of lowland and inland valley rice was to be established in Hohoe, Jasikan, and Kpandu with a total coverage area of 7,000 hectares under the IVRDP and 10,000 hectares in the northern part of the country under EU/AFD. Once more, the paper had stated an extension of the existing irrigation schemes in both Aveyime in the North Tongu district of the Volta region and Kpong in the Greater Accra region to a total area of 6000 hectares (JICA study team report, 2007). However, data on the outcome of these projected developments were not available as at the time of submitting the thesis in 2012.

Table 2.2: Rice Production Program 2005-2010

Category	Development Area	Paddy Rice Yield	Paddy Rice Production
Lowland and Inland Valley Rice	19,000 ha	4.0 ton/ha	76,000 ton
Irrigated Rice	6,000 ha	5.0 ton/ha	30,000 ton
Total	25,000 ha		**106,000 ton**

Source: (JICA study team report, 2007).

2.1.3 Rice Demand Projection

The annual per capita rice consumption according to the JICA study team during 1999-2000 was 17.5 kg on the average and this increased to 22.6 kg during 2002-2004. Future rice demand projection as reported by the Study team was made on the basis of indicators such as per capita consumption growth and population projection by the Ghana Statistical Service. Considering the population growth only, the Study team reported that, the current demand of 470, 000 tons will increase to around 600,000 tons in 2015 (Table 2.3). Under both growths of population and per capital consumption, rice demand will increase to 1,680,000 tons which is 3 times the demands between 2002 -2004. (JICA study report, 2007).

Table 2.3: Future Supply – Demand and Requirement of Rice Development

Item	Annual Average Production between 2002-2004	Projection in 2015 Population Growth Only
Rice demand Rice • Paddy grain equivalent	470,000 ton 770,000 ton	600,000 ton 1,000,000 ton
Import (Paddy grain) Import ratio	480,000 62%	480, 000 46%
Domestic Production Self- sufficiency • Paddy production • Average yield • Production Area Area increased	38% 290,000 2.5 ton/ha 118, 000 ha	54% 520,000 3.5 ton/ha 149,000 31,000

Source: (JICA study team report, 2007).

Estimating rice production from the supply side which is largely dependent on the area of cultivation, the government seeks to increase rice production fields by 25,000 ha by 2012 and by 31,000 (Table 2.3) in 2015 to boost the domestic rice production to meet demand for consumption and other purposes (JICA study report, 2007).

2.2 Malting of Cereal Grains

Much attention has been paid to cereal alpha amylases because of their important role in the process of degrading starch (Zawistowska *et al.*, 1988). Malts are cereals that have been steeped, germinated, and dried under controlled conditions (Beleia and Varriano-Marston, 1981). Malting is, therefore, a process involving steeping,

germination and drying of cereal seeds with the prime objective of promoting the development of hydrolytic enzymes that are not active in the raw seeds (Dewar *et al.*, 1997). Alternatively, malting has been defined as the germination of cereal grains in moist air under controlled conditions with the objective being to promote the development of hydrolytic enzymes which are not present in ungerminated grains (Dewar *et al.*, 1997).

The malting process is divided into three physical conditions namely: steeping, germination and drying/kilning. Dewar *et al.* (1997, citing Briggs *et al.*, 1981) referred to steeping (the soaking of the grains in water) as the most critical stage of the malting process. During steeping, the grains absorb some amount of water necessary to initiate physiological and biochemical processes such as the synthesis of hydrolytic enzymes. In general, malt quality increases with steeping time (16-40 hrs) for sorghum (Dewar *et al.*, 1997). However, steeping time of 24 hours for rice has also been reported (Hammond and Ayernor, 2001). The soaking water enters the grain resulting in an increase of the moisture content in the grains. The increase in the water content of the grains stimulates the respiration of the embryo (germ) and at the same time hydrates the stored starches in the endosperm. As the embryo activity increases, hormones called gibberellins are produced. These natural plant hormones diffuse into the aleurone layer (which is part of the endosperm but located under the bran) where they stimulate the production of hydrolytic enzymes including alpha and beta amylase. Steeping can either be done totally during the stipulated time or can be interspersed with "air rest" periods where the steeping water is drained off from the grains with the aim of exposing the soaked grains to atmospheric oxygen.

Germination is the step after steeping which is basically the development of the embryo. It is the stage where the grain's food reserves are hydrolyzed with the aid of

enzymes into simple sugars and amino acids needed for embryonic development. Agbale *et al.,* (2007), cited Dufour (1994) who reported that, since malting is a time-limited process, grains are considered suitable for malting only if more than 90% attain maximum germination within 3 days. Germination is carried in the dark and during the process, the moist grains are spread evenly on a sterilized jute sack and are watered about 2-3 times daily to keep the grains moist. At this stage, the gibberellins from the embryo (germ) diffuse into the endosperm and they stimulate the aleurone cells to produce more amylolytic enzymes which break down the stored starches into simple sugars to provide energy for growth. Other enzymes produced include proteolytic enzymes which attack the protein content present and cellulytic enzymes which break down cell walls. In addition, the scutellum (a secreting organ located between the embryo and the endosperm) has also been reported to produce alpha amylase but in smaller quantities (Ranki and Sopanen, 1984). The amylolytic activity of rice malt increases with germination time. Different peaking periods of enzyme production in germinating rice seedlings have been reported. Azakawa *et al.,* (1969), report that enzyme production peaks on the 10^{th} day of malting. This observation was realized during the study of enzyme mechanisms of starch breakdown in germinating rice seeds. Shaw and Chuang (1982 cited by Ayernor and Hammond 2001) also reported that rice amylase reaches the maximum activity after the 8^{th} day of germination. Further, Hammond and Ayernor, (2000) also conclude that diastatic power of malted rice is highest on the 10^{th} day of malting.

The different peaking times of enzyme activities during rice-malting as reported by these researchers have been attributed to the use of different varieties and pre-

treatment method such as the addition of gibberellic acid and air-rest treatments during the steeping period (Cecil, 1995; Mensah, 2008).

Kilning is the process of exposing the germinating seeds to a gentle heat to remove moisture with the intent of stopping germination without destroying the amylolytic activity of the rice seedlings. This is achieved by either exposing the seedlings to solar heat for 20-25hrs at 38°C (Ayernor and Ocloo, 2007) or using an air-oven drier for 18hrs at 45±5°C as reported by Ayernor and Hammond (2001). Kilning of cereal grains at 50°C for 20 hours during malting has also been reported (Agbale *et al.*, 2007). Eskin (1990, cited by Ayernor and Hammond 2001) reported that, drying at extreme temperatures can deactivate the malt enzyme, hence to preserve enzyme activity the temperature for drying should be between 50 and 60°C.

2.2.1 Malting of Rice Grains in Ghana

Malting of rice grains started in some parts of the world including Ghana when there was a search for alternative sources of imported malted barley (Ayernor and Ocloo, 2007). It has been reported that enzymes suitable for starch hydrolysis occur in a number of plants and the most suitable alternative must be used for malting (Hammond and Ayernor, 2000). In this regard, studies have been conducted on the malting quality of local cereals to find the most appropriate substitute for imported barley (Ayernor and Hammond, 2001). In Ghana, several investigations have been carried out on some local cereals including millet, sorghum, maize and rice in an attempt to find the most suitable alternative. Nonetheless, the brewing qualities of these cereals for beer production did not compare favorably with those of barley malt (Ayernor and Hammond, 2002). Fortunately, rice grains among the selected local

cereals have been reported to possess the highest hydrolytic potential to convert starch into sugar.

Previous attempts to utilize rice malt in enzyme development yielded less satisfactory products (Agbale *et al.*, 2007). Relative to barley malt products, the products from rice malt had a low dextrose equivalent. The results were attributed to the fact that parameters which contribute to high enzyme yield and activity had not been defined. However, after conducting several experiments, Hammond and Ayernor (2000) reported that, the highest diastatic power in rice malt occurs around the 10th day of malting. Further, Shaw and Chuang (1982, cited by Ayernor and Hammond 2001) reported that, rice amylase production reaches the maximum after the 8th day. Accordingly, the current work intends to assess the characteristics of rice malts from Wita 7 variety harvested before and after the 10th day in order to identify its peaking time of amylase production.

2.2.2 Water Uptake by Rice Grains during Steeping

The movement of water into rice grains during steeping is influenced by the initial moisture content of the grain, grain size, temperature and nitrogenous content. Dewar (1997, citing the works of French *et al.*, 1990 and Briggs *et al.*, 1981) referred to steeping as the most widely acknowledged critical stage of the malting process.

The steeping period and air-rest treatment are known to enhance water uptake in sorghum, buckwheat, millet and barley (Dewar *et al.*, 1997; Wijngaard *et al.*, 2005). Occasionally, the steeping process may be incorporated with air-rest period where the steep water is drained off and the grain is allowed to take atmospheric oxygen over a period of time. The air-rest treatment reduces the incidence of uneven and

slow germination and most importantly, promotes the production of hydrolytic enzymes. In general, it has been concluded that, the uptake of water increases rapidly during the early hours of steeping until the initial saturation point is reached (Wijngaard, *et al.*, 2005).

In Ghana, rice grains are usually soaked continually for a maximum of 24 hours without air-rest treatment. Hammond and Ayernor (2001) soaked rice grains for 24 hours during the preparation of rice malt extract for sugar syrup production without air-rest treatment. Air-rest treatment during steeping is not a common practice in Ghana but following the

outcome of literature, the current study will employ the practice of air-rest during malting to enhance the development of the hydrolytic enzymes.

2.3 Enzyme Extraction and Diastatic Activity of Rice Malt

Malt enzymes are extra cellular in nature and hence possess less difficulty during extraction. They are usually extracted from the malt when their production reaches the maximum level during malting. Hammond and Ayernor (2001) reported that the diastatic activity of rice malt reaches its peak on the 10th day of germination whiles Agbale *et al.*, (2007) have observed a constant increase when the rice grains are malted for 11 days. The different peaking times as reported have been attributed to the use of different varieties and pre-treatment method as stated in section 2.2.

Sodium acetate buffer, water, 0.5% NaCl, L-Cysteine and Sodium-phosphate buffer of pH 8.0 are the common media for extracting enzymes from malt (Osman, 2002). Extraction is usually performed by adding the selected extraction medium to a

known quantity of finely ground malt and allowing the mixture to stand for about 30-120 minutes with intermittent stirring. The mixture is then centrifuged for 15 minutes at 2000rpm. The supernatant which is the rice malt extract is filtered and used as the enzyme source. In a similar experiment, alpha amylase was extracted from millet with 0.05M Sodium acetate buffer of pH 4.8 containing 0.01M calcium chloride solution and extraction was allowed for 2 hours (Beleia and Varriano-Marston, 1981). This was followed by centrifugation for 15 minutes at 2000 rpm and the precipitate discarded (Beleia and Varriano-Marston, 1981). Usually, the supernatant is used as rice malt extract (RME) for the hydrolysis of the cassava flour (Ayernor *et al.*, 2002).

In another report, Osman, (2002), added 4ml of an extraction medium to 0.75g ground malt only to yield an average of 3.188ml malt extract with the difference being retained by the grist. Using the same approach as Osman, Yaldagard *et al.*, (2008) recorded an extract yield of 2.95ml when 4 ml of sodium phosphate buffer was used as the extracting solvent from 0.75 g malt flour.

Although, all the four media extract reasonably well, phosphate buffer of pH 8.0 gives the maximum enzyme yield and activity (Osman, 2002). The efficiency of the phosphate buffer may be attributed to the high pH coupled with the added phosphate ions which enhance the release of more enzymes (Osman, 2002). Although, the phosphate buffer with a pH of 8.0 seems to be the best medium for extraction of starch-degrading enzymes, it is far displaced from the optimal pH (4.0-5.5) of all the enzymes, rendering it unsuitable for mashing and starch degradation. Therefore industrial mashing is preferably performed in water in which the enzymes and other soluble materials are fairly extracted (Osman, 2002)

2.3.1 Malt Extract

According to Schwimmer and Balls (1949), malt extract is of a complex nature. It contains water, mineral salts, dextrin, sugars, inactive proteins and a multitude of starch-degrading enzymes including alpha and beta amylases. Starch-degrading enzymes in malt extracts are very important for the brewing and distilling industries. Alpha and beta amylases have been identified as the most important starch degrading enzymes for brewing. They produce different products when acting alone and when they are combined. However, more fermentable sugars are produced from their combined action (Osman, 2002). As a result, food technologists and biotechnologists especially in developing countries have found it to be a useful alternative enzyme source for hydrolyzing starch. The bold step has largely been embraced due to the availability of several cereal sources and most of all, their relatively low cost of production as compared to the imported ones.

In Ghana, Ayernor and Hammond (2000), combined cassava starch and malted cereal to produce sugar syrup. Furthermore, the Food Research Institute of the Council for Scientific and Industrial Research (FRI/CSIR) also conducts research on cereal malting and use of its extract for glucose syrup production from starch (Mensah, 2008).

2.3.2 Starch as an Affinity Adsorbent for the Purification of Alpha Amylase

Chen et al. (1991, cited by Lin et al., 2009) referred to raw starch as a suitable material for the affinity separation of proteins due to its abundance, stability, non toxicity, and easy recovery. Affinity chromatography exploits biological affinities for the purification of proteins. Baird et al. (1976) cited Lowe at al. (1974) who revealed

that, in affinity chromatography, the ligand is bound to an insoluble support and depending on the specificity of the ligand-enzyme interaction, a single enzyme or group of enzymes can be bound to an affinity polymer. One partner of the interaction pair, the ligand, is usually bound to a solid matrix. The resulting affinity resin is packed into a chromatographic column which is used to adsorb the protein of interest from the crude extract that is passing the chromatographic column. With an appropriate elution buffer the purified protein can then be recovered from the affinity adsorbent.

Current works have reported that, some amylases contain Carbohydrate-Binding Modules (CMD) with discrete fold that possess carbohydrate binding activity but not catalytic activity (Rodriguez-Sanoja, 2005). It has also been reported that the adsorption of enzyme onto the granule surface does not necessarily cause the occurrence of subsequent catalytic activity (Sujka and Jamroz, 2006). The CMD allows interaction between insoluble substrate and the enzyme bringing the substrate to the active site in the catalytic domain and consequently improving hydrolysis. It has been proposed that any of the carbohydrate binding modules may bind to starch with equal affinity by both intra-chain and inter-chain interactions. As a result, the number and flexibility of the neighbor carbohydrate binding modules in the molecule should determine the affinity of the starch binding domain (Santiago *et al.*, 2007).

Evidence of interaction of alpha amylase with starch granule through a non catalytic site on the enzyme has existed for some time. Schwimmer and Balls (1949) studied the affinity of malt alpha amylase for wheat starch granules at a low temperature. Malt alpha amylase appeared to interact through a site different from the catalytic site. This was inferred because maltose noncompetitively (situation where an

inhibitor binds at sites other than the catalytic sites of the enzyme resulting in a decrease of the enzyme affinity to the substrate) inhibited the action of malt alpha amylase on starch in solution and desorbed alpha amylase from starch granules. In addition, Schwimmer and Balls (1949) demonstrated that, the efficiency of adsorption was proportional to the surface area of the granules. Using alpha amylase from various sources, Walker and Hope (1963) reached a similar conclusion. However, there is little evidence to support suggestions on the functional role for a non catalytic site on the enzyme.

Owing to the complex nature of malt extract, the adsorption of alpha amylases on granular starch in the presence of cold ethanol has long been known (Schwimmer and Balls, 1949). Walker and Hope (1963) cited the work of Holmberg *et al.*, (1933) who earlier reported that, malt alpha amylase can be separated from beta amylase in a mixture by the adsorption of the former on starch from cold 50% ethanol in the presence of maltose. Not long after the discovery, it was revealed that the adsorption of alpha amylase from malt extract on a column of wheat starch at 5°C in the presence of 40% ethanol is an essential step in the purification of the enzyme (Sigmund and Balls, 1949). Amritkar *et al.*, (2004, citing Somers *et al.*, 1989) reported that most enzymes are purified by chromatographic techniques after crude isolation by precipitation and membrane separations. Again, alpha amylases from various species have been purified to homogeneity by binding to cross-linked starch (Somers *et al.*, 1995).

The process of binding the enzyme to the starch column and its subsequent elution constitutes an important step in its purification. Amritkar *et al.*, (2004) cited Lali

(2002) who reported that, affinity interactions has been claimed to increase the selectivity of the adsorptive separation process where the bio-molecule of interest is captured and eluted in a single pass of a crude extract through a column. Usually, the choice of a purification process is strongly dependent on the potential market, the processing cost, the final quality required and the available technology (Amritkar *et al.*, 2004). As a result, the choice of cassava starch as an adsorbent for the purification process in the current experimental work stems from its local availability and relatively low cost as compared to other adsorbents such glycogen amylopectin, amylase and ion exchange resins. Again, the choice of the starch adsorbent derives from the point that, its surface area possesses a robust affinity ligand for alpha amylase enzymes. Reports also indicate the use of other polymeric substrates like cellulose, and sodium alginate for the purification of polymer degrading enzymes like amylase and cellulase (Beleia and Varriano-Marston, 1981; Somers *et al.*, 1995). However, the disadvantage of cross linked starch as an adsorbent is its slight biodegradation by the amylolyitc enzyme and desorption of bound enzyme at high temperatures (Damodara *et al.*, 2005).

2.3.3 Factors Influencing the Efficiency of Starch as an Adsorbent for Amylase Isolation

Schwimmer and Balls (1949) found that the efficiency of adsorption of malt alpha amylase on starch at 0°C in the presence of 40 % ethanol was inversely proportional to the granule size. This conclusion was revealed from their experiment when cassava starch which had an average granular size of 15µm exhibited an adsorption efficiency of 0.8 whiles banana starch with a recorded average starch size of 50µm obtained an adsorbing efficiency of 0.3. In a similar work, potato starch granules

which exhibited the largest size among all starches investigated for adsorption capacity for alpha amylase showed the lowest (Walker and Hope 1963). The adsorption efficiency of starches increases by the various treatments which decrease the granular size and apparently increase the surface area of the starch granule. Such treatments include the subjection of the starch to ball milling and mild acid treatments. However, Schwimmer and Balls (1949) reported that, the most effective adsorbents are starches freshly precipitated from solution with alcohol. In addition to starch granule size, conditions such as low temperatures (20°C) and high pH values (above 7.8) which lead to a decrease in the rate of starch hydrolysis have been reported to increase the rate of amylase adsorption on starch granules (Walker and Hope, 1963).

Moreover, recent works have increased the extent of alpha amylase enzyme adsorption on starch by incorporating the adsorbent with other ligands such as Cycloheptaamylose (CHA) which promotes adsorption (Weselake and Hill, 1983).

2.3.4 Starch as a Natural Substrate for Amylolytic Enzymes

Starch is a major reserve carbohydrate of all higher plants. Chemically, it is a high-molecular weight polysaccharide with a chain of many glucose molecules. It has $(C_6H_{10}O_5)_n$ as the empirical formula where n is a large number which is not accurately known. Starches are produced commercially from the seeds of plants, such as corn, wheat, sorghum or rice; from the tubers and roots of plants such as cassava and potato.

Starch consists of two polysaccharide units namely; amylose and amylopectin whose ratios vary in a wide range according to botanical origin (Sujka and Jamroz, 2007).

There are two types of linkages, the alpha-1, 4 and the alpha-1, 6, in starch which enable them to assume different structures. Comparing molecules of starch to cellulose, starches are glucose polymers linked together by the alpha-1, 4 and alpha-1, 6 glucosidic bonds, as opposed to the beta-1, 4 glucosidic bonds for cellulose (Shuler and Kargi, 2001). It occurs in the form of water insoluble granules. When heated in water the hydrogen bonds holding the granules together begin to weaken and this permits them to swell and gelatinize. Ultimately they form a paste, depending on the concentration of polysaccharide (Aiyer, 2005).

Amylose is composed of linear chains of alpha 1, 4 linked glucose residues (Figure 2.1) and as a result, it is extensively degraded by alpha amylase. However, some amylose is not totally degraded to maltose by this enzyme (Aiyer, 2005). Again, Aiyer (2005) cited Banks and Greenwood (1975) who reported that amylose has a degree of polymerization of several thousands of glucose units. The molecular shape and structure of amylose causes it to be unstable in aqueous solution and retrogrades (precipitates spontaneously). This is because the linear chains align themselves by hydrogen bonding and thus form aggregates. Amylose has considerable viscosity in alkaline solutions due to its molecular shape. Amylose forms a complex with iodine to form intense blue color and this forms the basis of a method for quantitative determination of amylose (Aiyer, 2005).

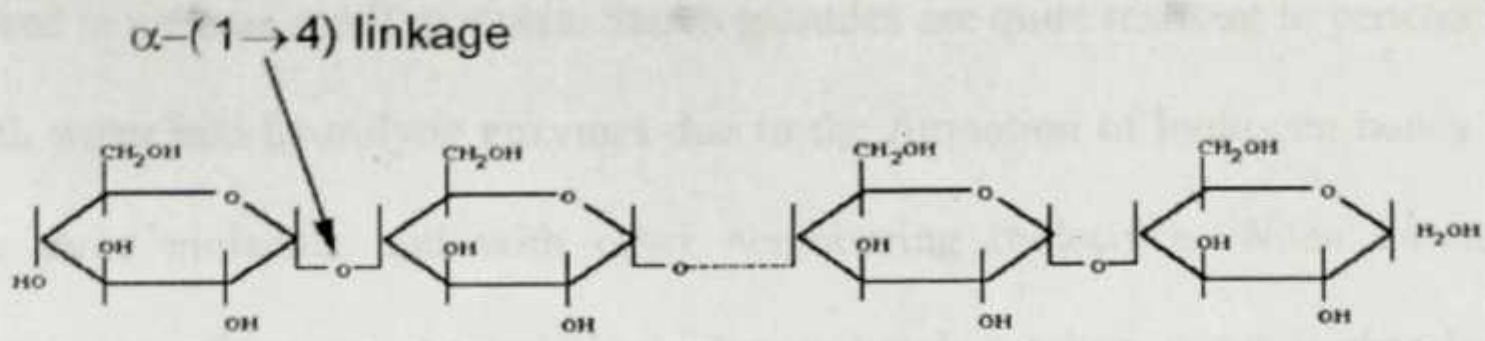


Figure 2.1: Amylose structure of starch molecule

According to Aiyer (2005), amylopectin may account for 75 to 80% of most starches. Aiyer citing the work of Fogarty (1983) referred to the branched component of starch as being amylopectin. The amylopectin is made up of several millions of glucose units in a compact structure. It is formed from the presence of many alpha-1, 6 glucosidic linkages in the starch granule. In aqueous solutions, amylopectins are relatively stable due to branched molecules that are not able to form compact aggregates. Amylopectin also presents a great challenge to hydrolytic enzyme systems. Because, most of the alpha amylase enzymes are specific for alpha 1, 4-glucosidic links, the specificity poses a problem for complete hydrolysis of amylopectin to glucose.

2.3.5 Starch Hydrolysis

Hydrolysis of starch is the depolymerization of the starch molecule into the individual glucose monomers. The process of hydrolysis by enzymes involves three (3) main stages namely: gelatinization, liquefaction and saccharification.

Gelatinization

It is the dissolution of the starch granules to form a viscous suspension. Gelatinization requires heat and free water and must precede liquefaction. Starch is generally insoluble in water at room temperature. Because of this, starch in nature is

stored in cells as small granules. Starch granules are quite resistant to penetration by both water and hydrolytic enzymes due to the formation of hydrogen bonds within the same molecule and with other neighboring molecules. When an aqueous suspension of starch is heated, the hydrogen bonds weaken, water is absorbed, and the starch granules swell. This process is commonly called gelatinization because the solution formed has a gelatinous and highly viscous consistency (Norman *et al.*, 2005).

Liquefaction

The breakdown of large molecules of starch by alpha amylases as they randomly cleave the alpha 1, 4 glucose bonds drastically reduces the viscosity of the gelatinized starch solution. The thinning of the solution is identified as liquefaction. It is the partial hydrolysis of the starch, with loss in viscosity. The principal aim for liquefaction is to reduce the viscosity of the gelatinized starch to ease subsequent processing. At this stage, the starch slurry is liquefied; that is the starch granules are pasted, hydrated and dispersed to such an extent that the hydrolytic cleavage of the starch molecules are accomplished (Norman *et al.*, 2005).

Saccharification

This is the final stage of the depolymerization of starch molecules. This process is called saccharification, due to the formation of saccharides by synergistic activities of both the alpha and the beta amylases. Beta amylase specifically splits off two glucose units at a time. The product is a disaccharide called maltose. The bond breakage is more extensive in saccharifying enzymes than in liquefying enzymes. The starch chains are literally chopped into small bits and pieces (Norman *et al.*, 2005).

2.3.6 Applications of Amylolytic Enzymes

Amylases have wide scale application ranging from textile to effluent treatment (Aiyer, 2005). The conversions of starch into sugar, syrups and dextrins which form the major part of the starch processing industry have been achieved by using amylases. The reducing sugars which result from alpha amylase hydrolysis of starch are used as carbon sources in fermentation as well as sources of sweetness in a range of manufactured food products and beverages.

CHAPTER THREE

3.0 Materials and Methods

Viable paddy rice seeds (Wita 7) were obtained from the Crop Research Institute (CRI), Fumesua, Kumasi and were stored dry at an average room temperature of 28°C. Common reagents like ammonium sulphate and other materials were also obtained from the Departments of Chemistry, Chemical and Materials Engineering, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. On the other hand, reagents such as malic acid and dinitrosalicylic acid (DNSA) which were not available were supplied by Pakus chemical ventures; dealers in laboratory chemicals, Amakom, Kumasi.

3.1 Malting of rice grains

3.1.1 Steeping of Rice Grains

400g of viable Wita 7 rice grains was cleaned by washing thoroughly in pipe – borne water and soaked using 1000ml of fresh pipe-borne water for 24 hours. However, one hour periods of air-rest was applied after the first 12 hours of steeping. Steeping was carried out in a plastic container at a temperature of 28±1°C. After steeping, the weight and average percentage weight gained were determined. The weights of fifty dry (50) viable rice grains as well as steeped grains were measured using an electronic balance. The weight gained by the steeped grains was obtained by taking the difference in weights between the fifty (50) steeped grains and dry viable rice grains. The weight gained by the steeped grains was expressed as a percentage increase in weight.

3.1.2 Germination of Steeped Rice Grains

Germination was allowed to proceed for twelve days in a cardboard box lined with a sterilized jute sack where a fairly constant temperature of 26±3°C was provided. The grains were also watered 2-3 times daily. Samples were taken out from the germination chamber for each day during the 12 day period and their germination process was halted by drying at a temperature range of 40 - 50°C in an oven. (Gallenhamp Hotbox oven; size 1).

In the course of germination, the germination energy of the rice was determined. Hough *et al.* (1975, cited by Ayernor and Hammond 2001) defined germination energy as the percentage of grains that germinate under the conditions of test. This was accomplished by selecting a specific number of rice seeds. From this number, the number of germinated rice seeds was determined at the end of each germination day. The ratio of germinated grains to the total number of grains selected was evaluated as the germination energy.

Malt loss and malt yield at different germination intervals was also determined. The dry weight of a known number of rice grains was recorded before steeping and also after drying the germinated seedlings. The malting loss was evaluated by noting the difference in dry weights divided by the initial dry weight and multiplied by 100.

$$\text{Malt loss (\% on dry basis)} = \frac{Ricegrain(wt.) - Malt(dry.wt)}{Ricegrain(wt)} \times 100$$

$$\text{Malt yield (\% on dry basis)} = \frac{Malt(dry.wt)}{Ricegrain(wt)} \times 100$$

3.1.3 Drying of Germinated Rice Seeds

The drying process took place in an electric drying oven where hot air was blown over the rice seedlings. The temperature of the oven was maintained between 40 - 50˚C for a period of 20-24 hours.

3.1.4 Milling of Dried Rice Seedlings

The dried seedlings were milled into finer particles using a Double-M-Cucina made blender Model No: DM-1731. The milled rice malt samples were packaged in a small size transparent polyethylene bags and stored at low temperatures between 0-4°C in a refrigerator.

3.2 Enzyme Extraction and Alpha Amylase Activity Determination

3.2.1 Enzyme Extraction

3g of the milled malt sample was weighed into centrifuge tubes and 12 ml of phosphate buffer solution of 0.05M with a pH of 8.0 was added. The suspension was left to stand for 30 minutes with intermittent stirring at 5 minute intervals. The enzymes were extracted into the solution. The extraction process was finally terminated after centrifuging for 15 minutes at 4500 rpm. The supernatant (malt extract) was filtered through a filter paper into a measuring cylinder. The volume of the malt extract was noted and diluted using a dilution factor of 10 with malate buffer solution of 0.1M having a pH of 5.5. The procedure was repeated for each of the 12 milled malt samples to obtain their respective malt extracts.

3.2.2 Preparation of Equilibrated Soluble Starch

An equilibrated starch is starch slurry obtained from dissolving a known amount of starch powder in a buffer solution of a known pH. The mixture obtained is usually heated and later cooled to the desired temperature. In the current experiment, the equilibrated starch was prepared by mixing 1g of starch with 20ml of 0.1M malate buffer solution of pH =5.5. In addition, 0.1g of calcium chloride was added to aid the hydrolytic activity of alpha amylase on starch. The solution formed was heated for 10 minutes at 75°C to form the equilibrated starch. It was then cooled to a temperature of 55°C before it was employed in the determination of the alpha amylase activities in the various rice malt extracts.

3.2.3 Alpha Amylase Assay using the 3, 5-Dinitrosalicylic Acid (DNSA) Method

The alpha amylase activity `was determined by measuring the amount of reducing sugars produced as a result of its action on soluble starch. To accomplish the task, the beta amylase present in the diluted malt extract was deactivated by heating to a temperature of 70°C for 15 minutes in the presence of 0.3g calcium chloride salt to prevent the deactivation of the alpha amylase during the heating process. 1ml of the heated diluted malt extract (having a dilution factor =10) was added to 5ml of the equilibrated starch and was thoroughly mixed together. The reaction was allowed to proceed for 10 minutes and was terminated by adding 2ml of 0.1M sodium hydroxide solution. The reducing sugars were measured by adding 1ml of 3, 5-dinitrosalicylic acid reagent to the mixture and boiling for 5-8minutes. The control sample (extract from unmalted rice grains) was treated in the same way except that the sodium hydroxide solution was added to the equilibrated starch before the addition of the malt extract. The optical densities of the test and the control samples

were then determined using a spectrophotometer at 540nm after cooling to room temperature.

One unit of alpha amylase activity is expressed as micromoles of apparent maltose produced per milliliter of alpha amylase solution per minute under the conditions of test (Beleia and Varriano-Marston, 1981). The procedure was repeated for all the malt extracts obtained from each day of the malting period. Accordingly, the alpha amylase activity in the malt extract was calculated using the modified form of the relation used by Beleia and Varriano-Marston, (1981). The alpha amylase from the malt extract which exhibited the highest alpha amylase activity during the test was then purified.

$$\text{Activities (U/ml)} = \left(\frac{mg/ml(maltose) \times 10^3}{Mw.maltose \times time(\text{min})} \right) \times 2$$

M_w = Molecular weight of maltose (342g/mol)

3.2.4 Preparation of Standard Maltose Solution

0.1g of maltose was weighed into a beaker and 100ml of distilled water was added resulting in a concentration of 1mg/ml standard solution. Since concentrations of 0.2, 0.4, 0.6 and 0.8mg/ml of maltose solutions were needed to plot the standard maltose curve, the 1mg/ml standard solution was subsequently diluted to obtain solutions of such concentrations.

3.3 Isolation of Alpha Amylase from Rice Malt Extract

3.3.1 Extraction, Heating and Purification of Alpha Amylase

To obtain the pure alpha amylase, 3g of milled rice malt was weighed into 8 separate centrifuge tubes and 12ml of 0.05M acetate buffer having a pH of 5.4 was added.

0.2g of calcium chloride salt was added to each of the centrifuge tubes. The mixture was allowed to stay for 2 hours to allow for the extraction process to take place. Finally, the extraction process was finished by centrifuging at a speed of 2000rmp for 15 minutes. The supernatant from each of the centrifuge tubes was collected and poured together into a conical flask. The pH of the supernatant was adjusted to 6.0 with a strong acid or base such as hydrochloric acid or sodium hydroxide respectively. The beta amylase present was denatured by heating the extract in a water bath at an average temperature of 70°C for 15 minutes and the heated extract was allowed to cool. To precipitate out the alpha amylase from the cooled extract, the amount of ammonium sulphate crystals to be added to 1 liter of cooled extract was determined according to the formula below. The amount determined was added in minute quantities to the extract until a saturation of 0.45 with respect to the salt was established. The solution obtained was allowed to stay overnight at a low temperature of 4°C in a refrigerator.

$$\text{Ammonium sulphate per liter of cooled extract} = \frac{533(S_2 - S_1)}{100 - 0.3S_2}$$

Where S_2 = Final salt saturation

S_1= Initial salt saturation

Source: (www.protein-chem.com/Resource/Ammonium.pdf)

3.3.2 Adsorption of Alpha Amylase by Affinity Chromatography

The partially purified alpha amylase in the precipitate form was again centrifuged at a speed of 4500 rpm for 15 minutes to obtain the precipitate in a form of a cake and the supernatant obtained was discarded. About 0.5ml of acetate buffer with a pH of 5.6 was first added to each of the caked alpha amylase precipitate located in the eight

centrifuge tubes and the total volume obtained was collected together in a measuring cylinder. An alcohol solution was added to establish a volume ratio of 5:1 with respect to amylase solution and alcohol (80% v/v). Finally, 0.2g of calcium chloride was also added to prevent the deactivation of the enzymes as Calcium is required to maintain the structural integrity of alpha amylase (Vallee *et al.*, 1959). The mixture was poured through a column containing cassava starch precipitated from alcohol solution. The alcohol-treated cassava starch substantially adsorbs only alpha amylase and this was kept in place on a metallic support clogged with cotton wool. The column was eluted with 5ml of 0.6% w/v calcium acetate solution. The washing process was repeated five times and each fraction was collected separately. The various elution fractions were tested for alpha amylase activities and those with higher potentials were combined to give a pure alpha amylase enzyme solution. The entire process was carried out at a temperature of 26±3°C.

3.3.3 Assessment of Alpha Amylase Concentration Trend in Elutions

In order to study the concentration trend of alpha amylase in the various elutions, varying quantities of starch (1, 2, 3, 4 and 5g of cassava starch) was used as the adsorbent in a glass column having an internal diameter of 2.45cm to establish length to diameter (L/D) ratios of 0.08, 0.16, 0.33, 0.41 and 0.49. Next, a total volume mixture of 4.0-4.5ml containing the partially purified alpha amylase precipitate, ethanol (80%v/v) and calcium chloride was passed through each of the various quantities of starch contained in the column. The adsorbents were as usual eluted with 5ml of 0.6% w/v calcium acetate solution and the eluents from each elution fraction were tested for alpha amylase activity.

To increase the scope of the research work, the process of adsorption and elution of alpha amylase using the starch adsorbent was carried out with a relatively bigger column having an internal diameter of 4.15cm. The quantity of starch used was determined from the density of starch, and the volume of column employed to again establish the same L/D ratios (0.08, 0.16, 0.33, 0.41 and 0.49). From the outcome of the calculations, 2.97, 5.75, 11.86, 14.74 and 17.61g of cassava starch was weighed respectively and used for the adsorption process. Using the same volume of elution solution (5ml of 0.6 % w/v the calcium acetate), the elution process was repeated 5 times.

It is important to note that, using the 2.45cm internal diameter column, only 3 elutions were initially carried out whiles 5 elutions were carried out when the 4.15cm internal diameter was used. As a result, the entire experiment was again repeated using the 2.45cm internal diameter column and 5 elutions were consequently carried out for each amount of starch used as the adsorbent. This was necessary to enable effective comparisons and conclusions to be made on the concentration trend of the alpha amylase enzymes in the various elutions when different columns were used.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Steeping

4.1.1 Water uptake by Rice Grains

Following 24 hours of steeping in water with an hour of air-rest after the first 12 twelve hours, the Wita 7 rice variety records a weight of 1.8g from an initial dry weight of 1.4g. Evidently, an increase of 0.4g representing 28.57% of the initial dry weight is established. The increase in weight is mainly due to the physical absorption of water by the endosperm and embryo of the rice seed as explained below.

Owing to the concentration difference established between the surrounding water and internal structures of the rice seeds such as the endosperm and embryo, the surrounding water moves by osmosis through the bran (husks) which acts as a semi permeable membrane. In effect, water diffuses into the endosperm slowly until it reaches its saturation point (Wijngaard *et al.*, 2005). Depending on the extent of concentration difference established the rate of water uptake can either be slow or fast. The rate of water uptake by the rice seeds is rapid during the first hour of steeping reaching an uptake rate of 0.2g/hr as indicated in Figure 4.1. Thereafter there is a progressive decline in the rate of water uptake to about 0.04g/hr at the fifth hour after which there is a gradual rise in uptake rate to 0.05g/hr at the sixth hour and again the decline in water uptake rate continues gradually until saturation point of the seed is established. Wijngaard *et al.*, (2005) made a similar observation while studying the effect of steeping time on the final quality of buckwheat. They concluded that the rate of water uptake increases rapidly during the initial stages but progressively slows down until the saturation point of the seed is established. The

unexpected rise during the 6th hour could be attributed to the point that, rice seeds samples having different trends of water absorption were analyzed. This might have led to incoherency of absorption rate around the 6th hour of steeping. However, instrumental errors may have accounted for the recorded unexpected rise during the 6th hour of steeping.

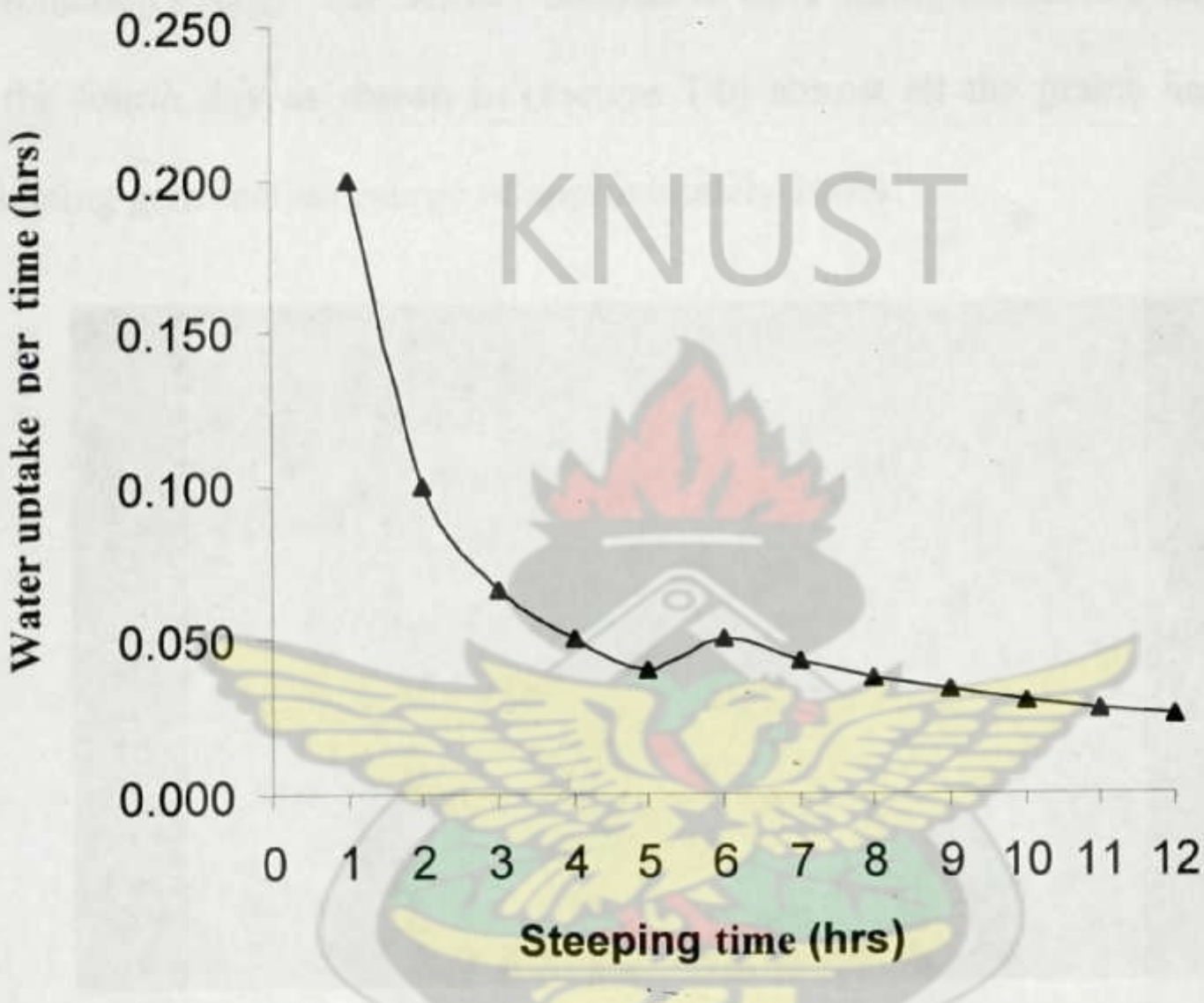
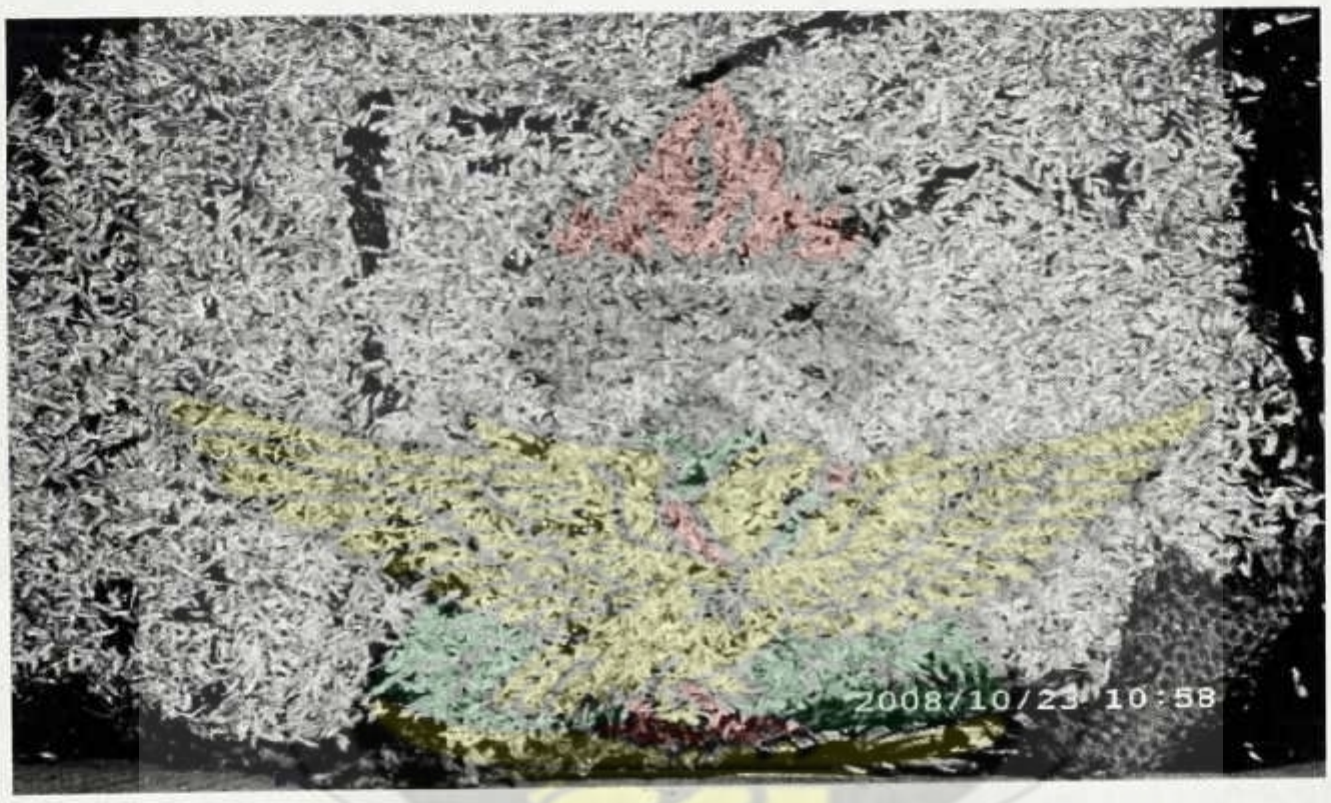


Figure 4.1 Water uptake rate by rice grains during the first 12 hours of steeping

4.1.2 Germination Energy of Rice Grains

Germination energy is the ratio of the germinated grains to the total number of grains within a specific time of germination. It is also an indication of the availability of reducing sugars which is the main source of energy for growth by the embryo. The germination energy is greatly dependent on the method of estimation with respect to the availability of water, air, temperature and the germination time allowed.

In the current study, the germination energy is determined by randomly selecting 50 rice grains from the germination chamber into a Petri dish. From the collection, the ratio of the number of germinated grains to the total number of grains at each malting period is evaluated as the germination energy or germination power. On the first day of germination, 17 out of 50 grains had sprouted representing 34% as the germination energy. The number doubles to 68% during the second day. By the end of the fourth day as shown in (Picture 1.0) almost all the grains had germinated reflecting germination energy of approximately 100%.



Picture 1.0: 4th day of Germination; JPEG image format

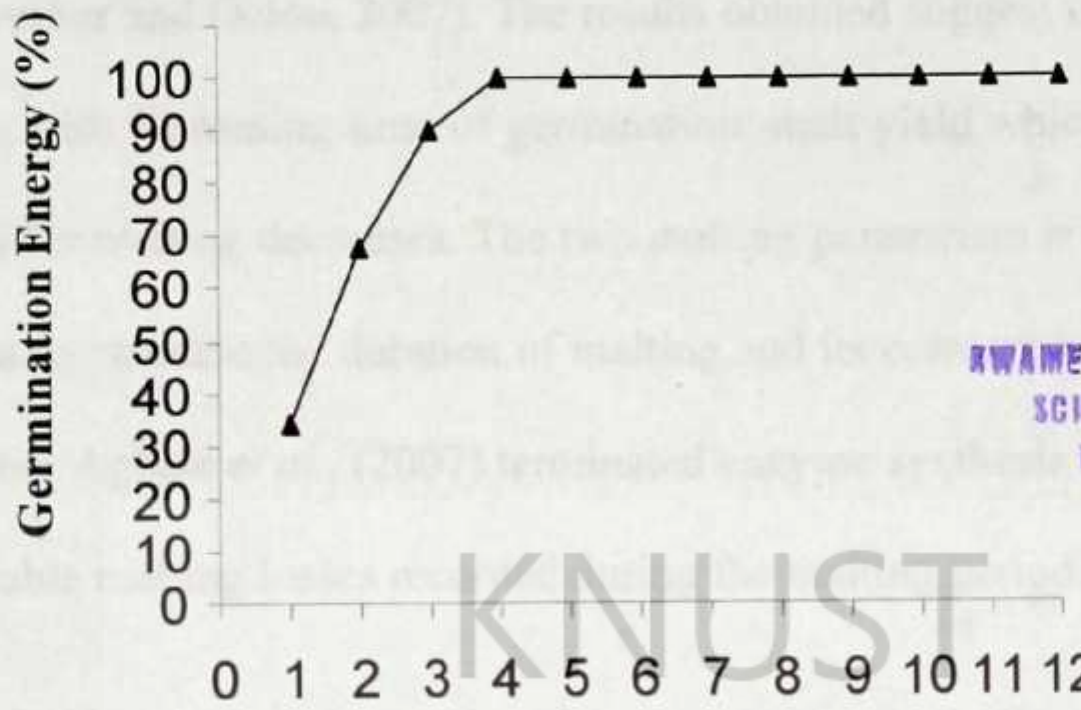


Figure 4.2 The Germination energy of the rice grains during the malting period.

Following the trend of germination energy results in Figure 4.2, it is obvious that the Wita 7 variety of rice exhibits high germination energy. Hough *et al*., (1975) cited by Ayernor and Hammond (2001) reported that, during germination, the proportion of the grains germinating varies with the quantity of water supplied and with the degree of maturity of the grain. Moreover, Agbale et al., (2007), cited Dufour (1994) who reported that, since malting is a time-limited process, grains are considered suitable for malting only if more than 90% attain maximum germination within 3 days. As shown in Figure 4.2, about 90% of the rice (Wita 7) variety grains attained the maximum germination by the third day which makes it suitable for malting.

4.1.3 Malting Loss and Malt Yield Determination

Malting loss is the material lost, as a per cent dry weight in converting the grain into malt (Ayernor and Ocloo, 2007). The results obtained suggest that as the malting loss increases with increasing time of germination, malt yield which is the residual grain content after malting decreases. The two malting parameters are important since they enable us to estimate the duration of malting and its corresponding malt yield. As an illustration, Agbale *et al*., (2007) terminated enzyme synthesis after 4 days due to the considerable malting losses recorded during the malting period.

The rapid breakdown of the starch content in the rice seeds between the 2nd and 9th day of germination as observed in Figure 4.3 contributes to malting losses. These observations can be attributed to the chemical changes that occur during the malting process. Suhasini and Mellashi (1995, cited by Ayernor and Ocloo 2007) reported that, malting loss is caused by metabolic activity. It increases with increasing duration of germination. Another report also suggested that, the partial degradation of high molecular weight materials in the starchy endosperm of the paddy rice during malting resulted in the observed increases in malting loss and decrease in malt yields (Ayernor and Ocloo, 2007).

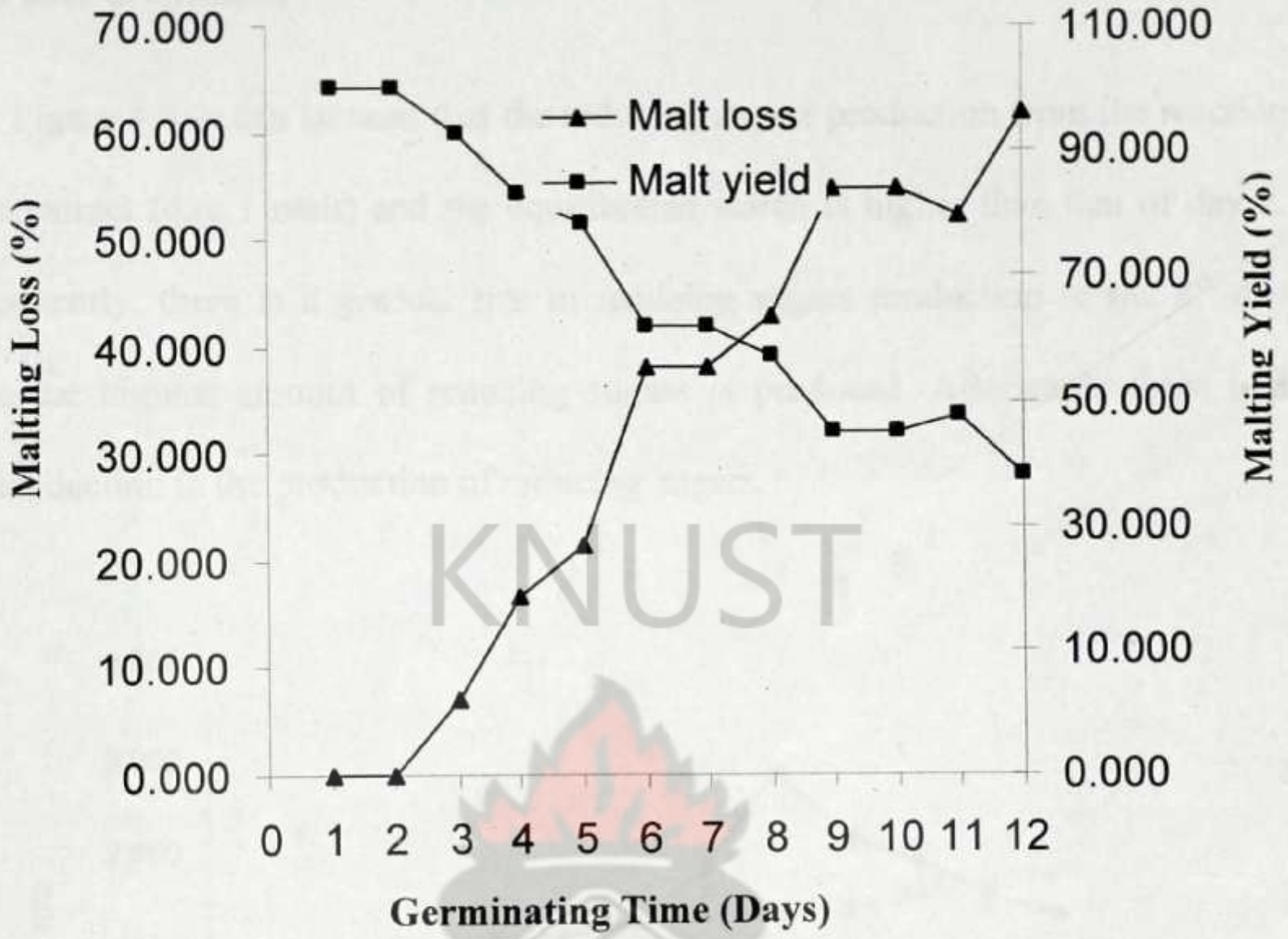


Figure 4.3 Malting loss and Malting yield of Wita 7 rice variety

4.2 Assessment of Reducing Sugars Production and Alpha Amylase Activities of Crude Extract

4.2.1 Reducing Sugars Formation

The determination of the amount of reducing sugars formed by reacting alpha amylase with starch was carried out using the 3, 5-dinitrosalicylic acid (DNSA) method. The reducing sugars formed as a result of the reaction are capable of reducing the color of DNSA reagent from yellow to red and then dark red color on boiling for 5-8 minutes at a temperature between 70-80°C. The intensity of the color change indicates the amount of reducing sugars present after a particular reaction. Following the procedures outlined in section 3.2.3, the reducing sugars formed by the

alpha amylase in each extract from the 12-day malting period and the equilibrated starch were determined.

From Figure 4.4, it can be seen that the reducing sugars production from the reaction of the extract (day 1 malt) and the equilibrated starch is higher than that of day 2. Subsequently, there is a gradual rise in reducing sugars production to the 8th day where the highest amount of reducing sugars is produced. Afterwards there is a gradual decline in the production of reducing sugars.

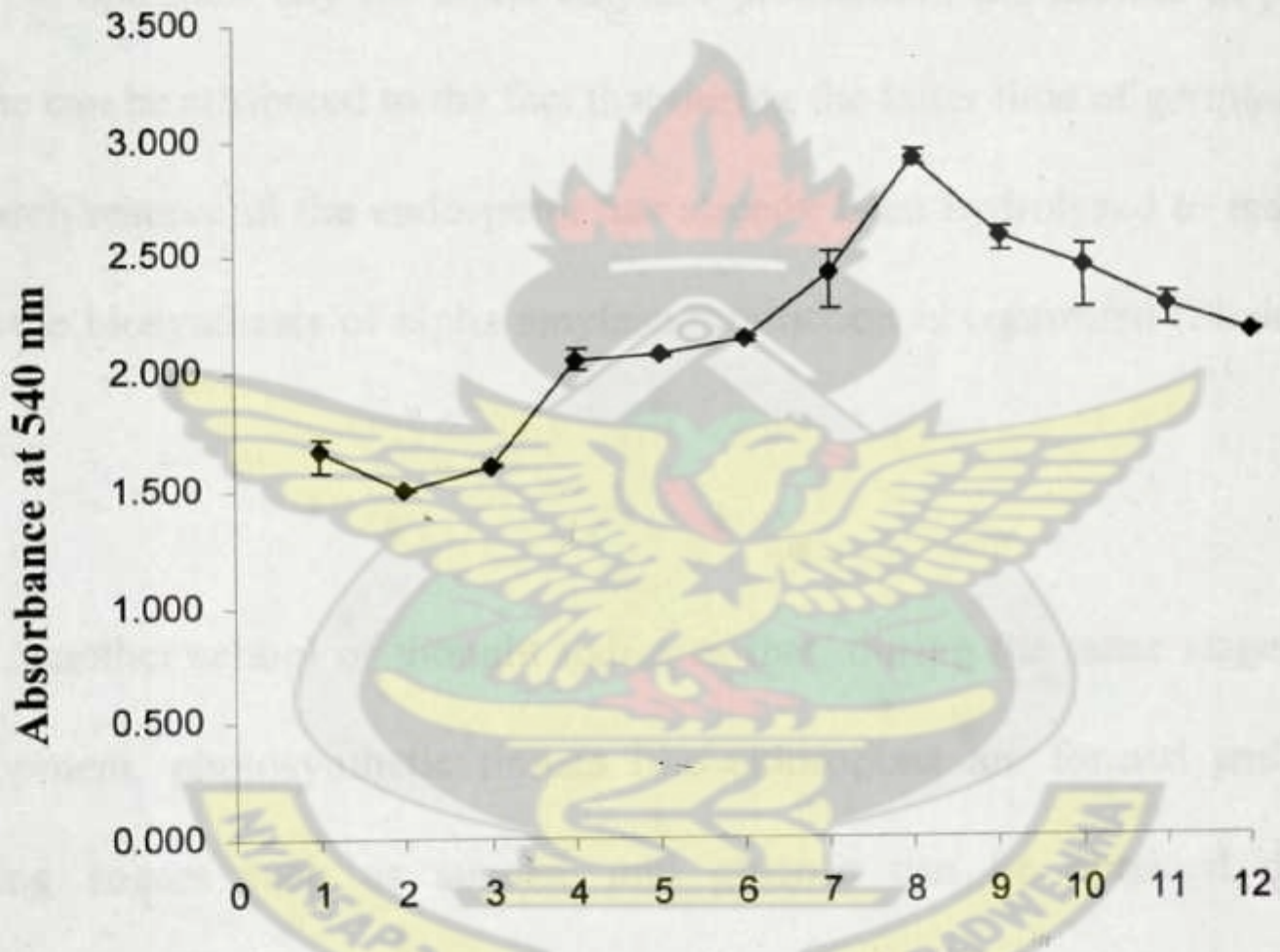


Figure 4.4 Trend of production of reducing sugars in Wita 7 rice variety

Paralleling the amount of reducing sugars formed to the level of alpha amylase present in the extract from the malt collected on day 1, it is established according to Figure 4.4 that, the level of alpha amylase present is relatively higher than the amount present in the extract from the second day malt. A similar trend was observed by Murata and T. Azakawa (Azakawa *et al*., 1968). They postulated that the high

amount of alpha amylase produced initially is necessary in order to produce enough reducing sugars (sucrose; a major respirable carbohydrate) from the reserved starch in the endosperm to meet its rapid utilization for embryonic development. In the subsequent days after the second day, the level of alpha amylase increases for the resynthesis of sucrose to keep pace with its great demand for tissue (shoots and roots) development. This trend continues until the 8^{th} day where, as a result of the high demand for reducing sugars (sucrose) for tissue development, the highest amount of alpha amylase was produced to meet the purpose of starch breakdown. After the optimum day for alpha amylase production, the decline in producing the enzyme can be attributed to the fact that during the latter time of germination most of the starch reserve in the endosperm has already been hydrolyzed to reducing sugars hence the biosynthesis of alpha amylase production is controlled (Shuler and Kargi, 2001)

Again, another school of thought indicates that, during the latter stages of the seed development, photosynthetic tissues like chloroplast are formed and as a result, reducing sugars such as sucrose and glucose can be obtained directly from photosynthesis as a source of energy for growth without necessarily sourcing them from the seed, leading to the decline of alpha amylase production in the germinating seeds.

Further, the trend of alpha amylase production in cereals can be attributed to the relative contributions of the production sites. Ranki and Sopanen, (1984), identified that alpha amylase is clearly secreted by the scutellum during the first and second days of germination in a depreciating manner before the subsequent activation of the

aleurone layers. Although it was concluded that, the scutellum secreted alpha amylase during the initial stages of germination, its contribution to the total activity in the starchy endosperm was only 5 to 10%. It was again established that, the contribution of the aleurone layer is sufficient to account for all the alpha amylase activities in the starchy endosperm that is representing the site of bulk enzyme production (Ranki and Saponen, 1984)

From the results of Ranki and Saponen (1984), the initial decline in the trend of alpha amylase production in the present study can be explained from the view of the enzyme production site. Accordingly, the decline in the amount of reducing sugars obtained between the first and second days malt extracts are as a result of the reduction in the production of alpha amylase from the scutellum site. Following the decline in enzyme production from the scutellum site, the aleurone layers become activated and take over the production of alpha amylase for the hydrolysis of the reserved starch in the endosperm during the subsequent days of germination.

4.2.2 Maltose Standard Curve

A calibration curve of absorbance versus concentration of maltose was plotted with known amounts of maltose (Figure 4.5). The activities of the alpha amylase from the rice malt extract were determined by converting the absorbance readings from the spectrophotometer to amounts of maltose formed (mg/ml) per minute from the standard maltose curve. The amounts of maltose formed as read from the standard curve was converted into activities using a modified form of the relation used by Beleia and Varriano-Marston, (1981) as stated in section 3.2.3. According to the

relation, one unit of alpha amylase activity is expressed as micromoles of apparent maltose produced per milliliter of alpha amylase solution per minute.

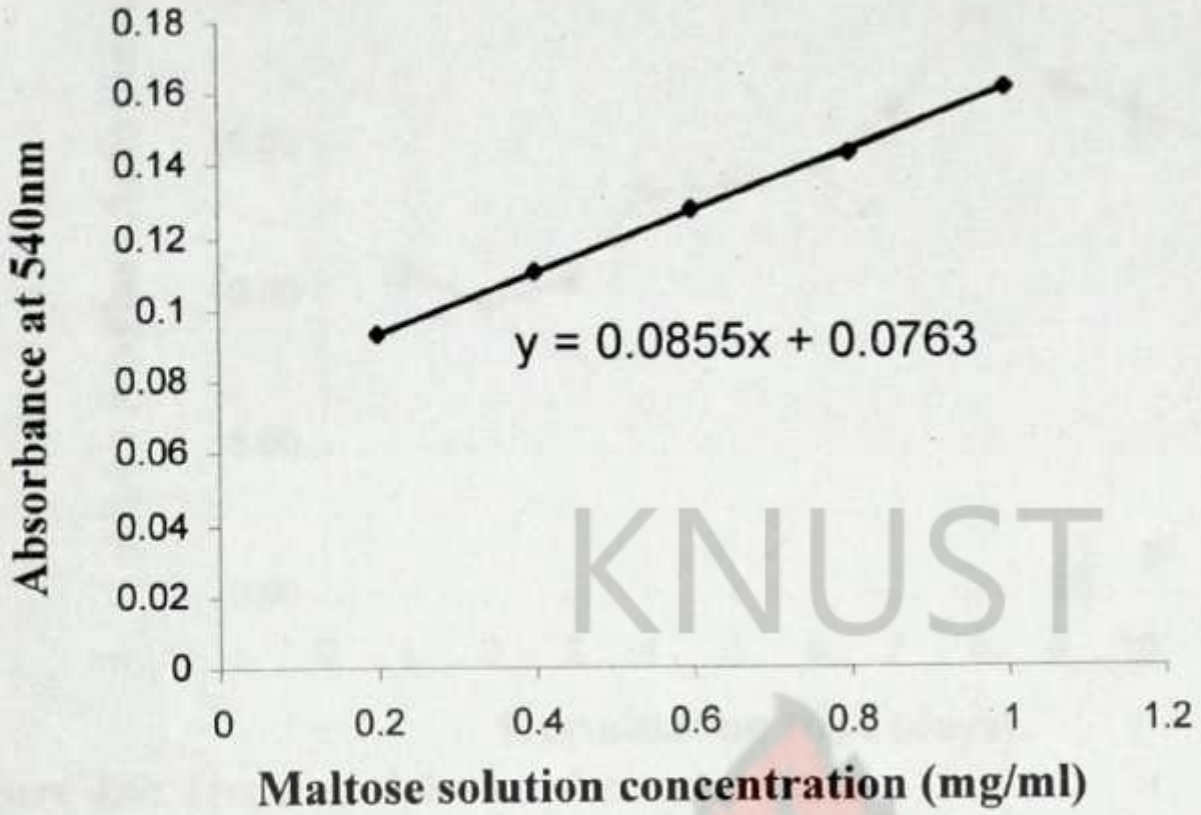


Figure 4.5 Maltose Standard Curve

4.2.3 Alpha Amylase Activities

Following the procedure outlined in section 3.2.3, the optical density of the color developed (reddish-dark) after adding DNSA reagent to the test mixture and boiling was translated into alpha amylase activities by using a modified form of the relation used by Beleia and Varriano-Marston, (1981). The trend of alpha amylase production as shown in Figure 4.6 indicates that the alpha amylase formed was parallel to the formation of reducing sugars as previously shown in Figure.4.4 The observed trend can be accounted for by the same reasons as given previously for the trend of reducing sugars formed.

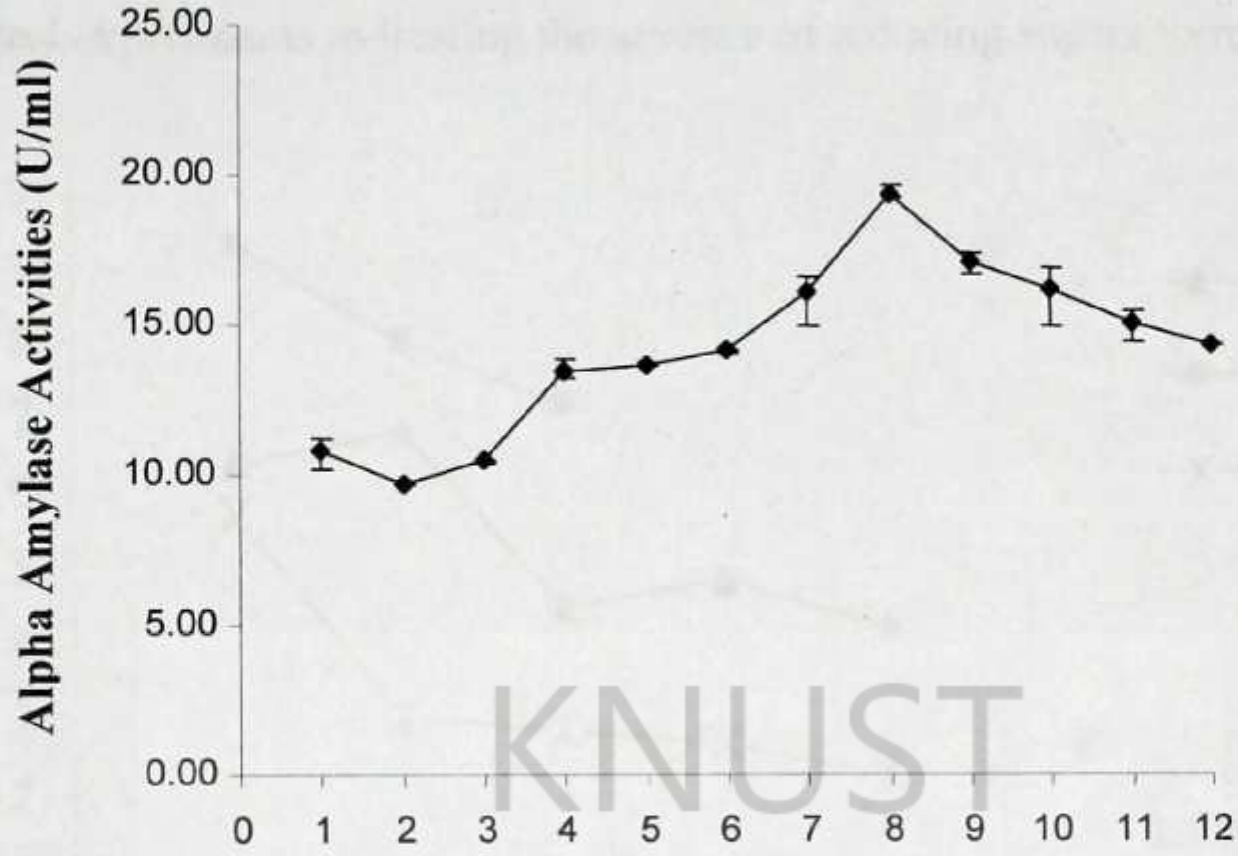


Figure 4.6: Trend of alpha amylase production.

4.3 Assessment of Reducing Sugars Production and Alpha Amylase Activities of Purified Extract obtained from varying adsorbent height to diameter ratio (L/D)

4.3.1 Reducing Sugars Production

To determine the amount of reducing sugars formed by the alpha amylase contained in the various elution fractions, 1ml of each elution fraction was added to 5ml of equilibrated starch. After 10 minutes, the reaction was terminated by adding 2ml of 0.1M NaOH solution. Two types of control experiments were conducted. In the first control experiment, 2ml of 0.1M NaOH solution was added to the equilibrated starch before the enzyme was added and in the second control experiment no enzyme was added to the equilibrated starch. Upon testing for reducing sugars using the DNSA reagent solution as described in section 3.2.3, the following trend of alpha amylase concentration in various elution fractions were obtained as shown by Figures 4.7,

4.8, 4.9, 4.10, 4.11 and 4.12. However, there was no color change in both cases for the control experiments indicating the absence of reducing sugars formation.

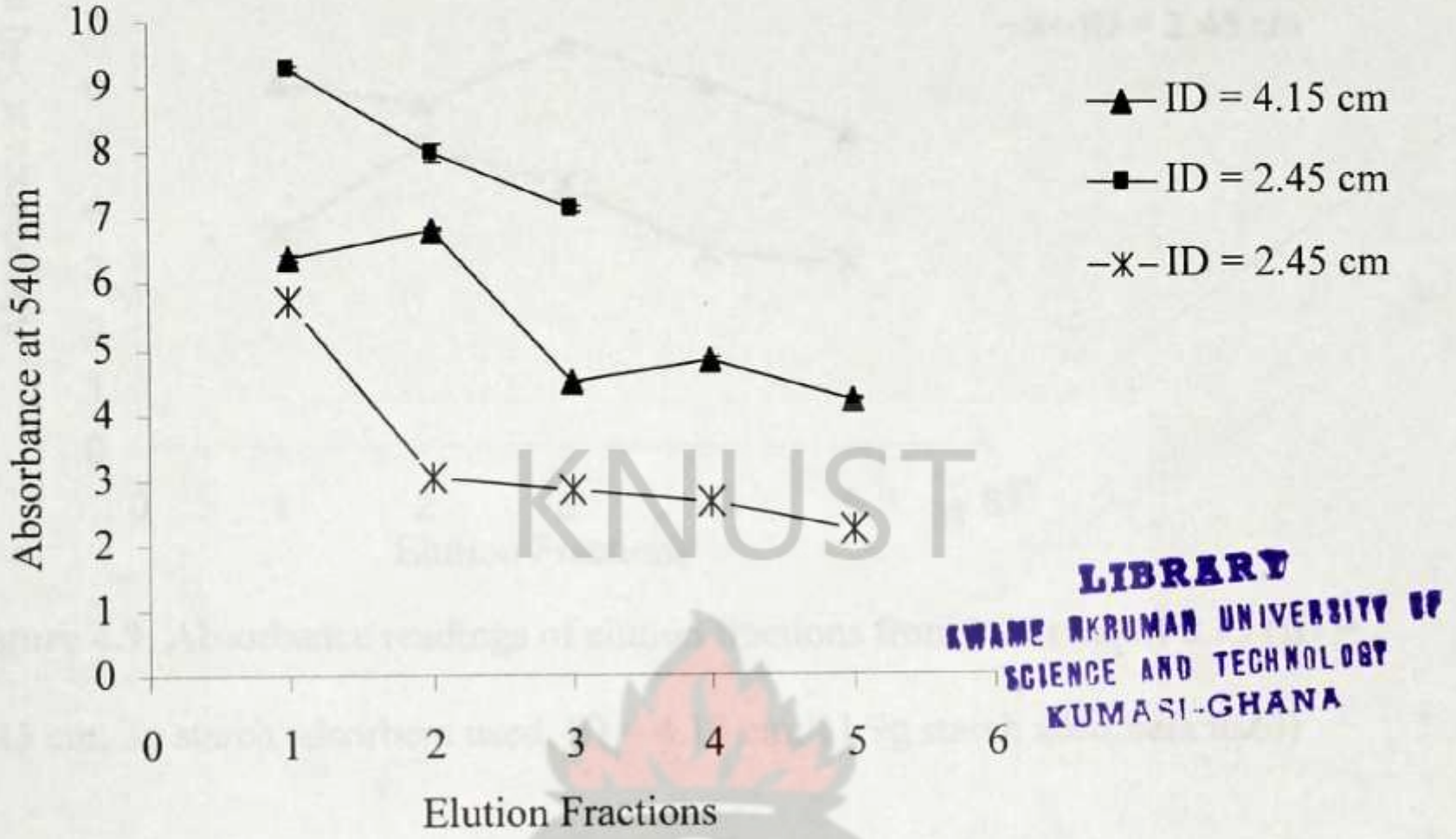


Figure 4.7: Absorbance readings of elution fractions from L/D ratio of 0.08 (ID = 2.45 cm; 1g starch adsorbent used, ID = 4.15 cm; 3g starch adsorbent used)

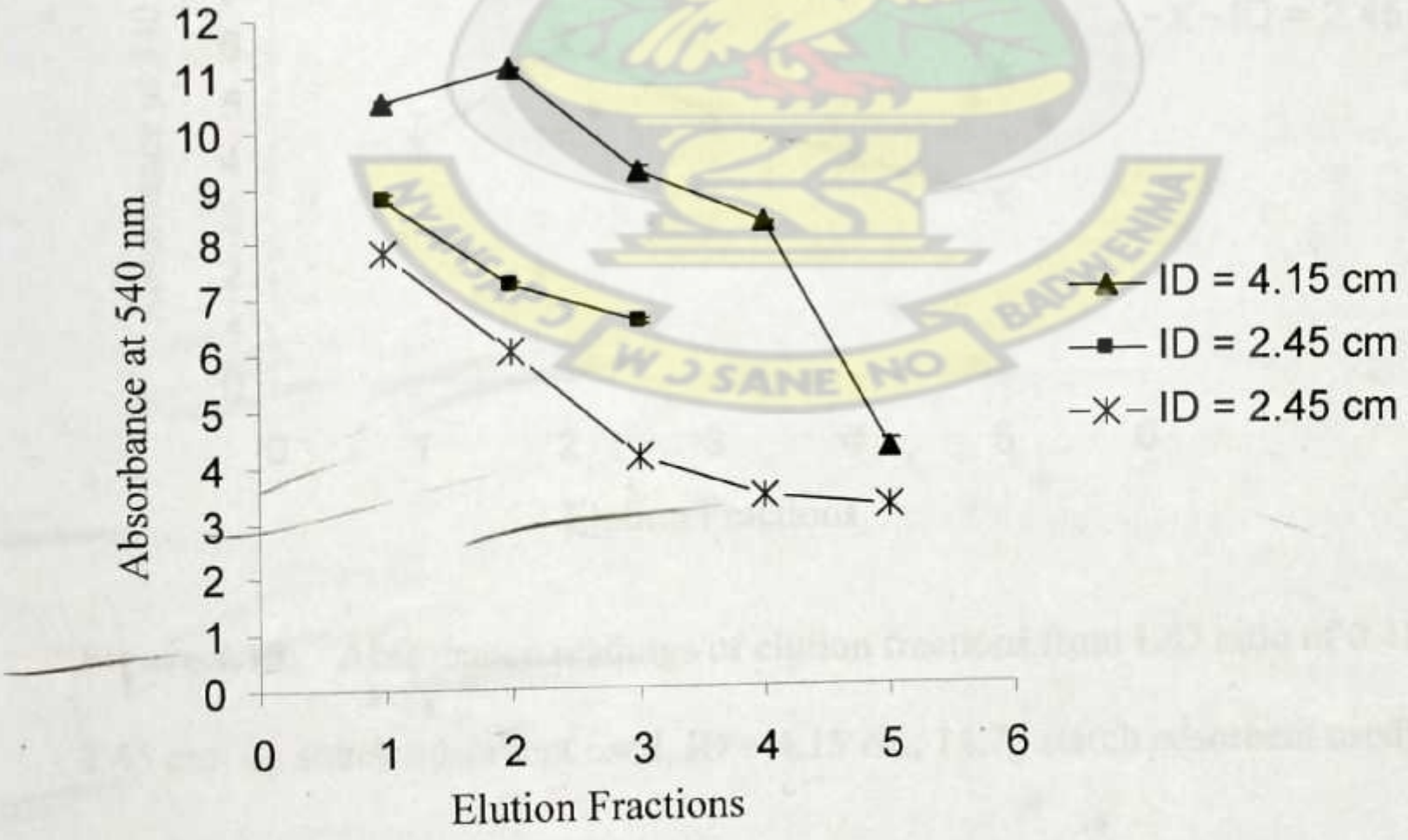


Figure 4.8: Absorbance readings of elution fractions from L/D ratio of 0.16 (ID = 2.45 cm; 2g starch adsorbent used, ID = 4.15 cm; 5.8 g starch adsorbent used)

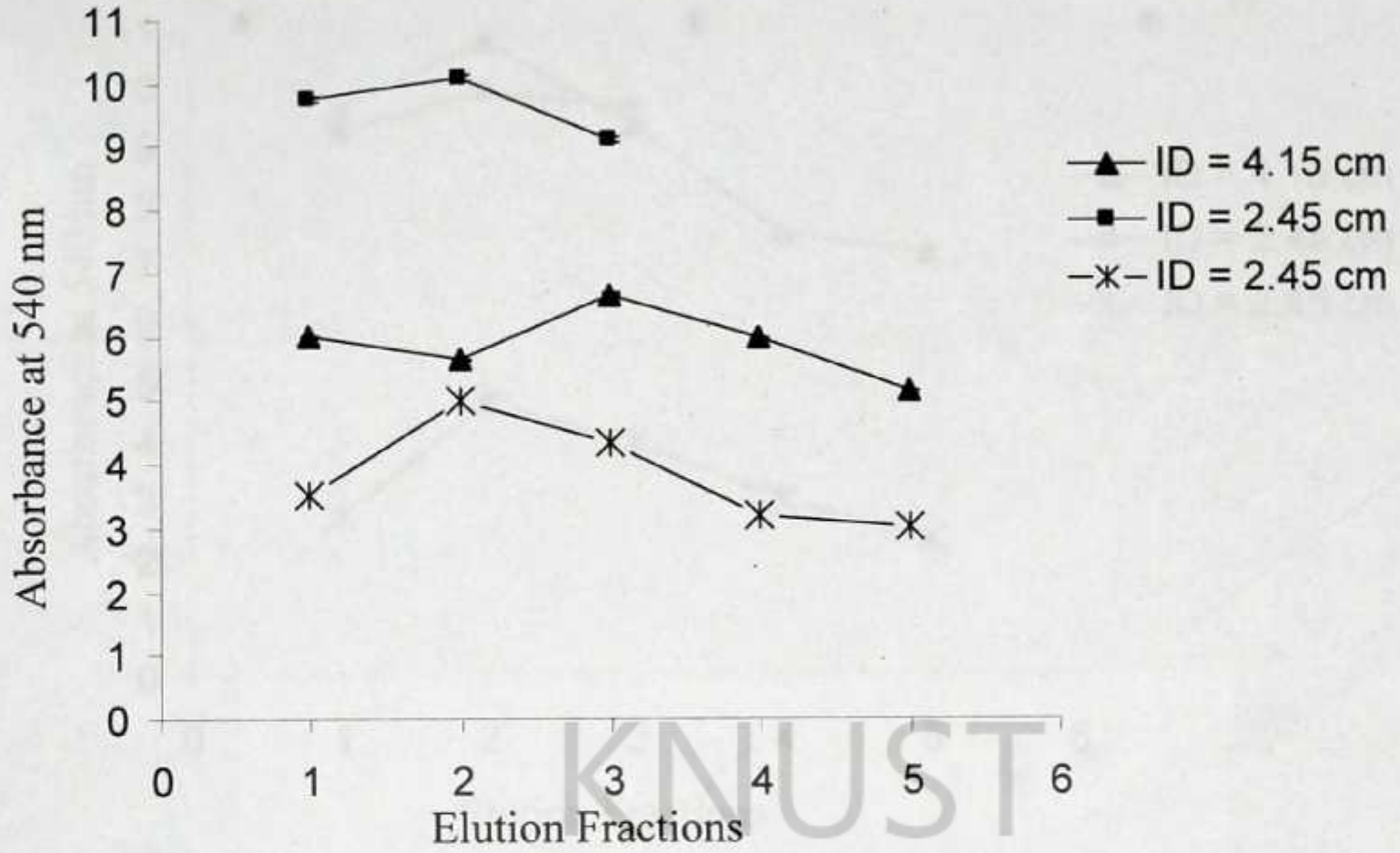


Figure 4.9: Absorbance readings of elution fractions from L/D ratio of 0.33 (ID = 2.45 cm; 3g starch adsorbent used, ID = 4.15 cm; 11.9g starch adsorbent used)

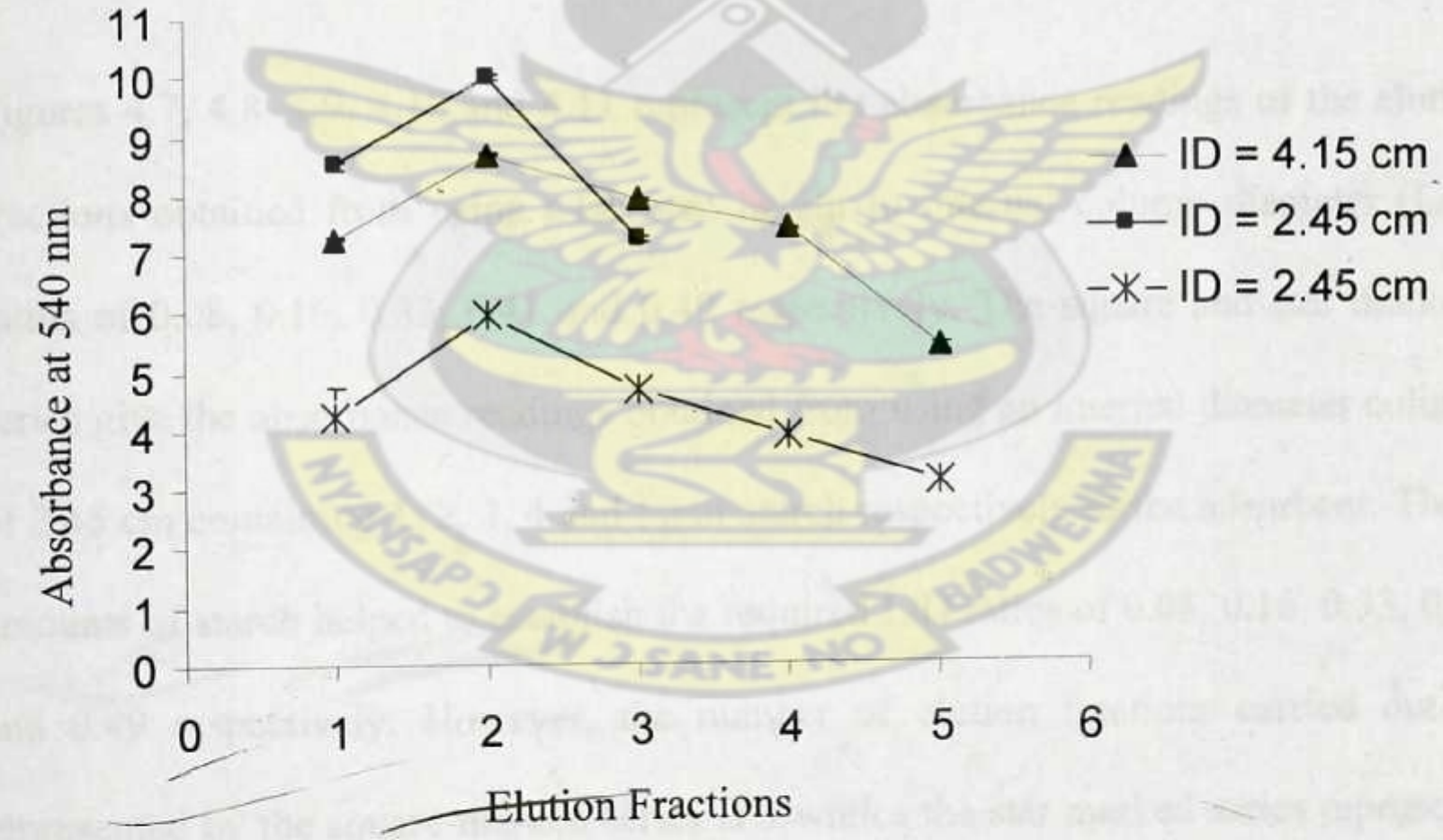


Figure 4.10: Absorbance readings of elution fractions from L/D ratio of 0.41(ID = 2.45 cm; 4g starch adsorbent used, ID = 4.15 cm; 14.7g starch adsorbent used)

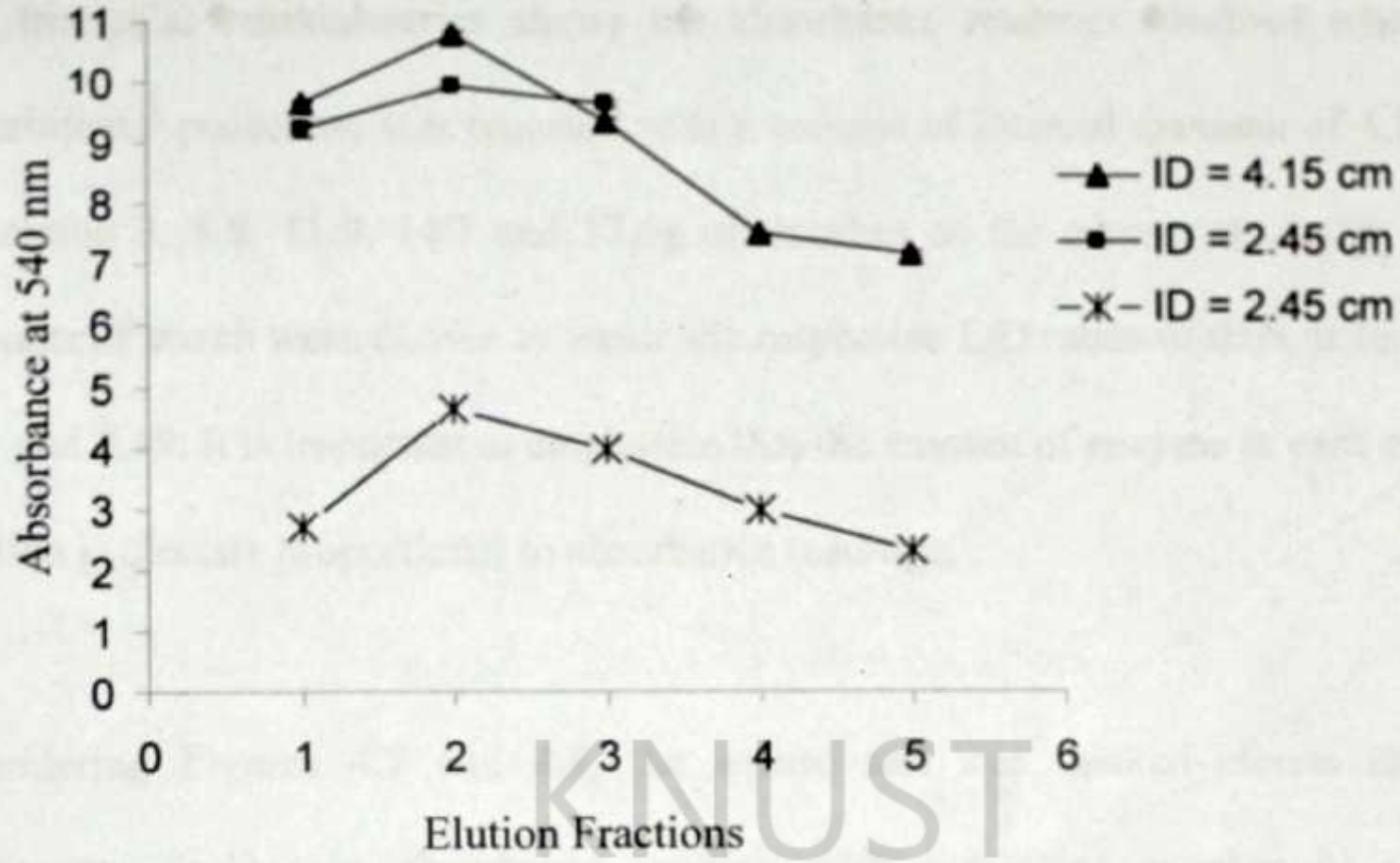


Figure 4.11: Absorbance readings of elution fractions from L/D ratio of 0.49 (ID = 2.45 cm; 5g starch adsorbent used, ID = 4.15 cm; 17.6 g starch adsorbent used)

Figures 4.7, 4.8, 4.9, 4.10 and 4.11 represent the absorbance readings of the elution fractions obtained from using adsorbent height to internal column diameter (L/D) ratios of 0.08, 0.16, 0.33, 0.41 and 0.49 respectively. The square and star marked-series give the absorbance readings obtained from using an internal diameter column of 2.45 cm containing 1, 2, 3, 4 and 5g of starch respectively as the adsorbent. These amounts of starch helped to establish the required L/D ratios of 0.08, 0.16, 0.33, 0.41 and 0.49 respectively. However, the number of elution fractions carried out as represented by the square-marked series is 3 whiles the star marked series represents 5 elutions because it was suspected most of the enzymes adsorbed in the starch would be eluted after the 5th elution.

The triangular marked-series shows the absorbance readings obtained when the experimental procedure was repeated with a column of internal diameter of 4.15 cm containing 3, 5.8, 11.9, 14.7 and 17.6g of starches as the adsorbent. Again, these amounts of starch were chosen to create the respective L/D ratios of 0.08, 0.16, 0.33, 0.41 and 0.49. It is important to emphasize that the amount of enzyme in each elution fraction is directly proportional to absorbance readings.

Considering Figures 4.7 and 4.8, the square and star marked- series show a continuous decline in absorbance reading with increasing number of elution fractions. The first elution fraction in each case showed the highest value of absorbance reading, followed by a constant decrease in absorbance reading for the 2^{nd} and 3^{rd} elutions. The reduction in absorbance reading continued with the 4^{th} and 5^{th} elutions as shown by the star marked-series in the two plots.

For the same plots with L/D ratios of 0.08 and 0.16 (Figures 4.7 and 4.8), the triangular marked-series shows an increase in absorbance reading from the first elution to the highest for the 2^{nd} elution fraction. Figure 4.7 shows a decline and an increase in absorbance reading for the third and fourth elution fractions as indicated by the triangular marked series before the lowest reading is recorded for the fifth elution. Figure 4.8, however, shows a constant decline in absorbance reading from the 3^{rd} through to the 5^{th} elution fractions.

The explanation to the trends shown by the 3 series is that, the movement of the adsorbed molecules (enzymes) located in the adsorption zone is based on the mass transfer characteristics of the starch adsorbent bed (Shuler and Kargi, 2001). The

mass transfer characteristics of the bed may depend on the porosity (available pore sizes and pore volume) and height of the bed. The bed height as reported increases the contact between biological material and adsorbent particles (Toledo *et al*., 2006). Applying 1 and 2g of starch as portrayed by the square and star marked-series to establish L/D ratios of 0.08 and 0.16 respectively, the maximum adsorbent bed height created in the column was 0.4 cm (Appendix Table C-2). As a result, the adsorption zone carrying the enzymes moves through a relatively small distance along the vertical axis of the bed. By using 5ml of elution solvent, most of the enzymes contained in the adsorption zone of the starch adsorbent are accordingly eluted during the 1st elution process. Enzymes of relatively small concentrations still contained in the adsorption zone are eluted in subsequent washing steps as indicated by the various series.

The triangular marked-series shows the trend of absorbance readings for an adsorbent bed height of 0.33 and 0.66 cm obtained from using 3g and 5.8g of starch to create the respective L/D ratios of 0.08 and 0.16. Under the present condition, more starch is used to establish the same L/D ratios and as a result, the height of adsorbent beds created is comparatively lengthier. Consequently, the adsorption zone containing the enzymes had to pass through a longer distance down the vertical axis at a rate which largely depends on the porosity of the bed (available pore sizes and pore volumes). Though, the 5ml calcium acetate solution used as the elution solvent could not elute the majority of the enzymes contained in the adsorption zone during the 1st elution, it is suspected that, most of the enzymes in the zone are relocated to a position within the bed, which favors their complete removal during the 2nd elution.

Enzyme amounts of smaller concentrations are eluted in subsequent elutions as shown in Figure 4.8.

Another important observation with the same L/D ratios worth discussing is the difference in absorbance reading (enzyme amounts) obtained when the same amount of starch adsorbents and column diameters were used as portrayed by the square and star marked-series. The square marked-series reflects higher absorbance readings in all the respective elution fractions than the readings observed for the star marked-series. It has been reported that, the amount of protein bound to raw starch adsorbents depends not only on the initial protein concentration but also on the number of modules constituting the peptide (Santiago *et al*., 2007). On this basis, the current observation can be traced to the initial protein content (enzyme solution) exposed to the starch adsorption bed before the start of the elution processes. It is possible to suspect that the amount of enzyme precipitate was relatively higher in the initial experiment as shown by the square marked-series than in the latter experiment as represented by the star marked-series. As a result, more enzymes are likely to be adsorbed and be eluted from the starch adsorbent in the former experiments than in the later experiments.

Considering the plots represented by the L/D ratios of 0.33, 0.41 and 0.49 (Figures. 4.9, 4.10, 4.11) respectively, the 3 different series generally show a rise in absorbance reading from the 1st elution to attain the highest reading for the 2nd elution. Afterwards, there is a constant decline in absorbance reading from the 3rd through to the 5th elution fractions. Relating the absorbance readings to enzyme concentrations of the various elution fractions, it can be inferred that, relatively small

amount of enzymes are usually eluted during the 1st elution whiles the 2nd elution fraction usually contains the highest amount of enzymes. The enzyme contained in subsequent elutions decrease as shown by these Figures.

The explanation to the trends observed for the 3 different series can again be given by the mass transfer characteristics of the starch adsorbent bed. As explained earlier, these characteristics depend largely on the porosity (available pore volume and pore sizes) and the bed height. The adsorbent bed height increased with increasing L/D ratios and as a result, the adsorption zone moves through a fairly long distance down the vertical height of the bed. Most of the enzymes carried in the adsorption zone could not be eluted with 5ml of elution solvent during the 1st elution due to the substantial bed height created. It is, however, able to relocate the adsorption zone containing the majority of enzymes to a point along the bed height which favored its complete removal during the 2nd elution process. Somers *et al.* (1995) observed an increase in alpha amylase adsorption level on a cross-linked starch and assumed that the observation was due to the bed matrix becoming more porous following its interaction with the enzyme.

The results obtained from the current experiment indicate that, most of the enzymes are eluted during the 2nd elution when the L/D ratios were more than 0.33 and 5ml of the elution solvent was used. However, the exact elution fraction likely to contain the highest enzyme can be determined by factors such as;

- The volume of elution solvent used as well as its ability to dislodge the adsorbed enzymes from the adsorbent surfaces.

- The mass transfer characteristics of the adsorbent which are generally dependent on the diffusion distance (the bed height) and lastly the porosity (available pore volume and pore size) of the adsorbent.

4.3.2 The Influence of Adsorbent Height to Diameter ratios (L/D) on Alpha Amylase concentration in Elution Fractions.

Figure 4.12 shows the trend of absorbance readings established from varying the amount of starch adsorbent in a column having an internal diameter of 4.15cm. The different amounts of starch adsorbent used in a constant internal diameter column created L/D ratios of 0.08, 0.16, 0.33, 0.41 and 0.49. As a result, the lowest and highest bed height is established by L/D ratios of 0.08 and 0.49 respectively. The current observation shows a general pattern of improved absorbance reading from the 1st to 2nd elution fractions. This is followed by a gradual decline from the 3rd through to the 5th elution fraction. Again, it is observed that, the amount of enzyme adsorbed and desorbed generally increased with increasing L/D ratios except for the L/D ratio of 0.16 which showed an anomaly.

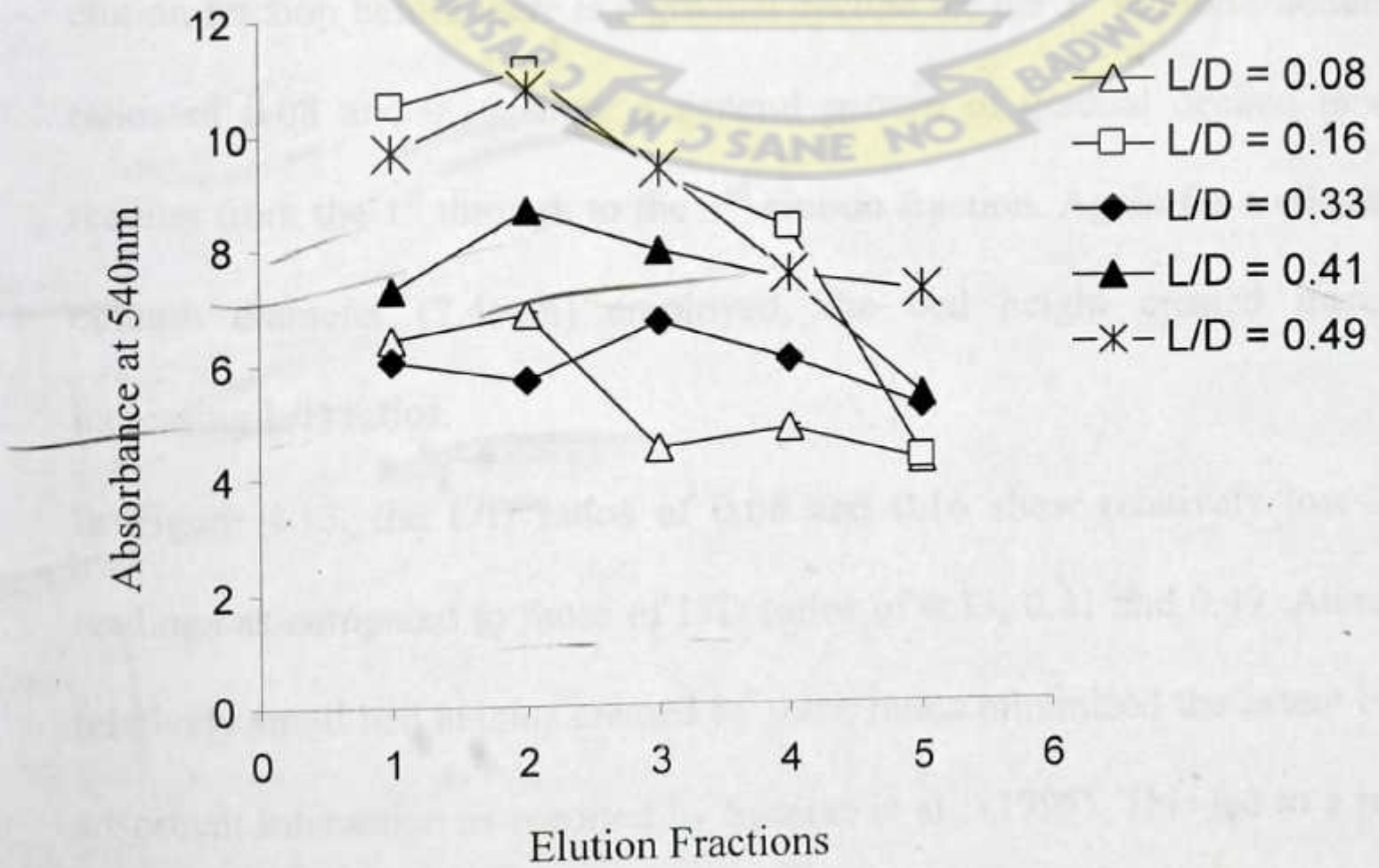


Figure 4.12: Absorbance readings of elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 4.15 cm

The 2nd elution fraction from the L/D ratio of 0.49 which had the highest bed height (2.03 cm) showed a fairly high absorbance reading of 10.805. This was followed by L/D ratios of 0.41, 0.33 and 0.08 in a decreasing order of absorbance reading. The progressive increase in bed height as reported increased the contact between the biological material and the adsorbent particles (Toledo *et al.*, 2006). As a result, more enzymes were adsorbed as they moved down along the increased height of the starch adsorbent. Though the L/D ratio of 0.16 with a relatively small bed height registered an unreasonably high absorbance reading, experimental errors, including the inability to determine the actual amount of alpha amylase precipitate that was introduced initially to adsorbent bed may have accounted for the current observation.

In Figure 4.13, the L/D ratios of 0.33, 0.41 and 0.49 for a column diameter of 2.45 cm show a general pattern of improved absorbance readings from the 1st to the 2nd elution fraction before there is a gradual decline for the 3rd elution fraction. The L/D ratios of 0.08 and 0.16 show a general pattern of gradual decline in absorbance reading from the 1st through to the 3rd elution fraction. Again for a constant internal column diameter (2.45cm) employed, the bed height created increased with increasing L/D ratios.

In Figure 4.13, the L/D ratios of 0.08 and 0.16 show relatively low absorbance readings as compared to those of L/D ratios of 0.33, 0.41 and 0.49. Admittedly, the relatively small bed heights created by these ratios minimized the extent of enzyme - adsorbent interaction as reported by Somers et al., (1995). This led to a reduction in

enzyme adsorption and subsequent elution from the starch adsorbent bed. The highest absorbance reading was recorded by the L/D ratio of 0.33, followed by the ratios of 0.41 and 0.49. The slight difference in the readings observed (10.118, 10.012 and 9.955) respectively show that, the increase in adsorbent height facilitated the enzyme adsorption and elution.

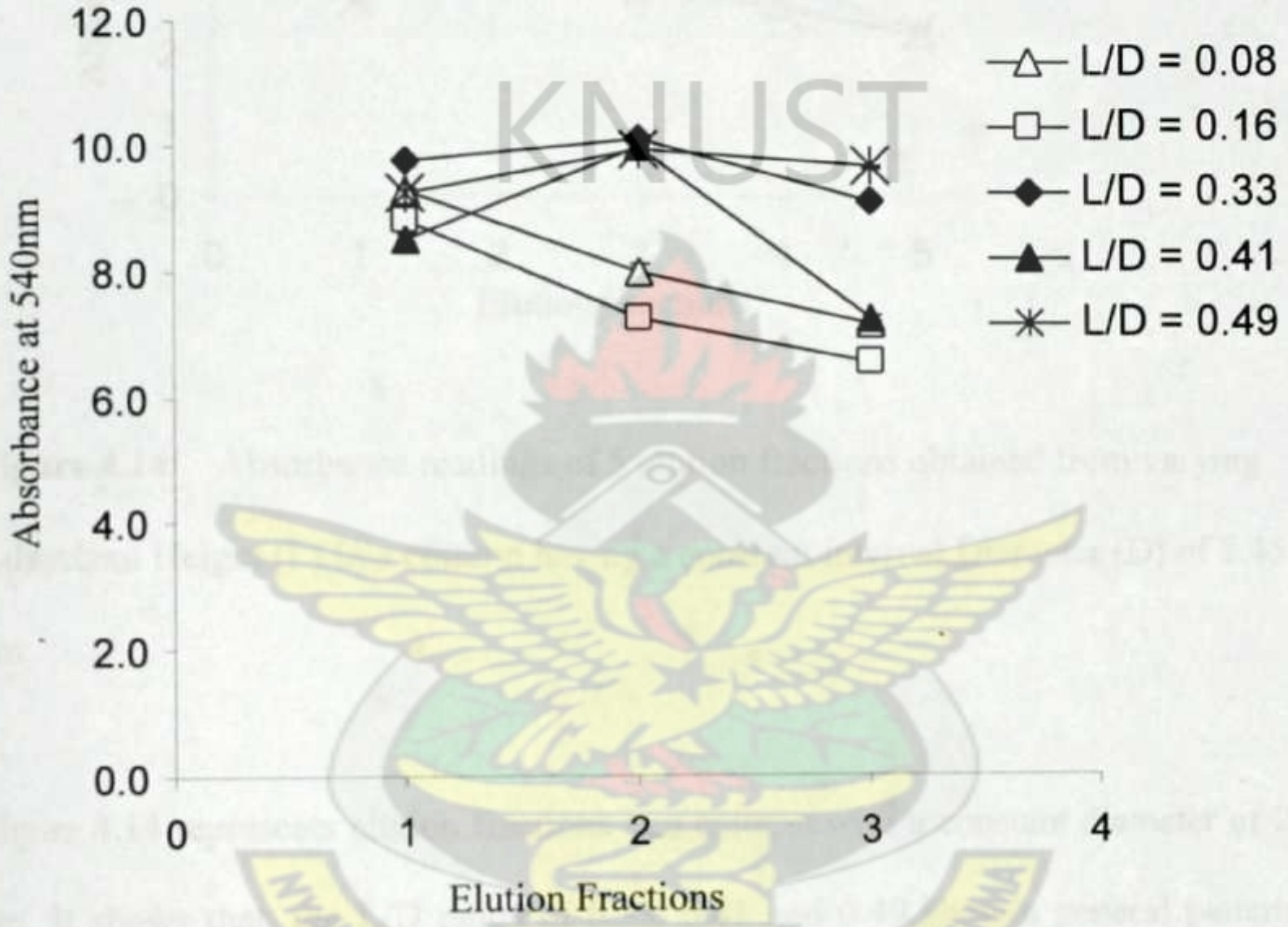


Figure 4.13: Absorbance readings of 3 elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 2.45 cm.

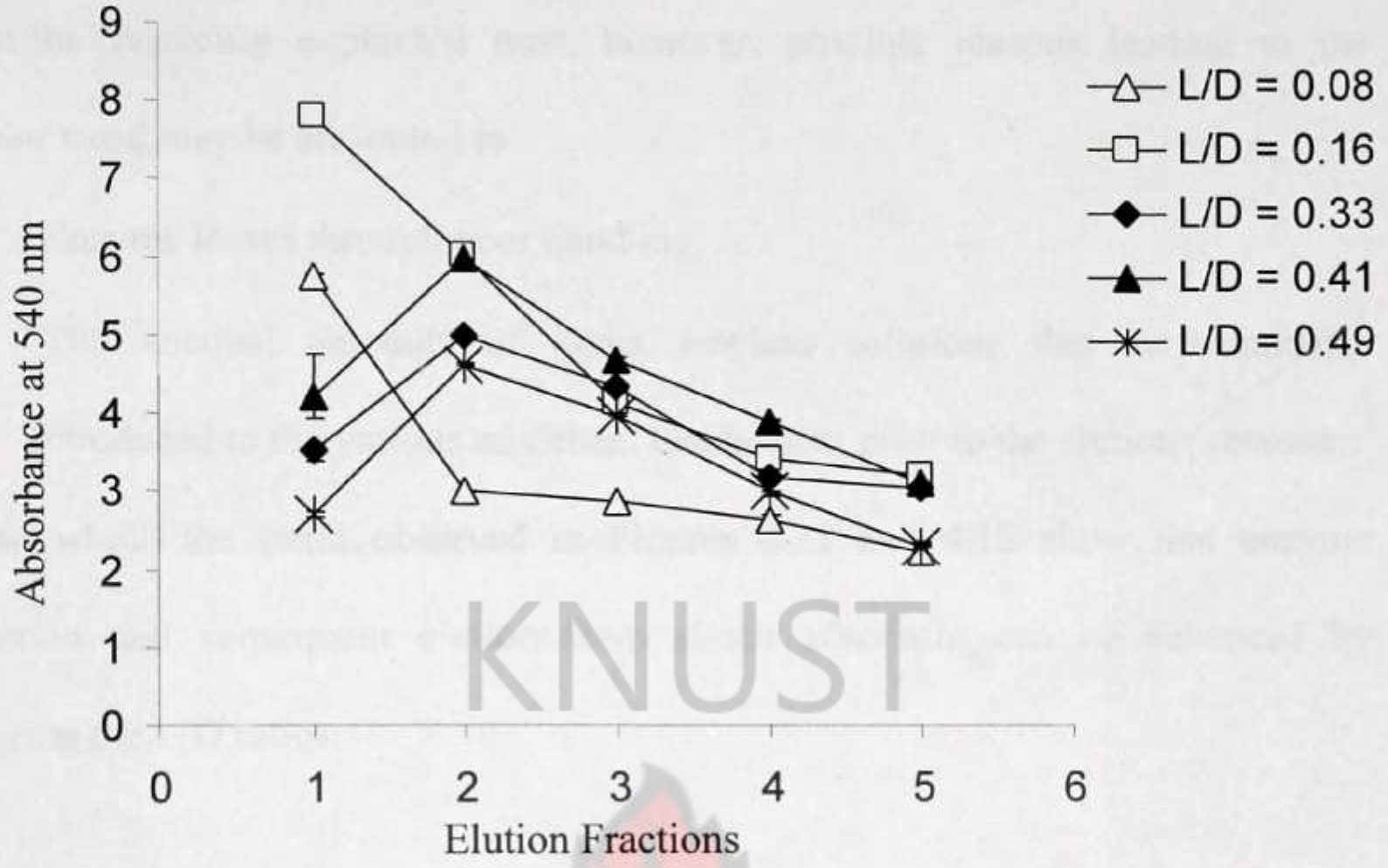


Figure 4.14: Absorbance readings of 5 elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 2.45 cm.

Figure 4.14 represents elution fractions in a column with a constant diameter of 2.45 cm. It shows that, the L/D ratios of 0.33, 0.41 and 0.49 show a general pattern of improved absorbance readings from the 1st to the 2nd elution fraction before there is a gradual decline from the 3rd through to the 5th elution fractions.

Unfortunately, the impact of L/D ratios on enzyme adsorption and elution from starch adsorbents is not clearly shown by this Figure. The L/D ratio of 0.16 with a bed height of 0.4 cm registers the highest absorbance reading. The current observation could not be explicitly accounted for on the basis of adsorbent height. This is because, the adsorbent height created by the L/D ratio of 0.16 was not enough to favor maximum enzyme - adsorbent interaction as compared to the heights

formed by the L/D ratios of 0.33, 0.41 and 0.49. It is a case of an anomaly that cannot be explicitly explained now, however, possible reasons leading to the irregular trend may be attributed to

- Enzyme losses through poor handling.
- The unequal amounts of alpha amylase solutions that were initially introduced to the various adsorbent bed heights prior to the elution processes.

On the whole the trend observed in Figures 4.12 and 4.13 show that enzyme adsorption and subsequent elution from starch adsorbent can be enhanced by increasing the L/D ratios.

4.3.3 Activities of Purified Alpha Amylase

Following the procedure given in section 3.2.3, the activities of the purified sample were determined. Since the amylase activity is directly proportional to the absorbance readings obtained for the various elutions, high and low values of absorbance reading corresponded to high and low value of alpha amylase activities respectively. As a result the highest and lowest alpha amylase activities obtained after purification were 75.529 U/ml and 14.785 U/ml respectively.

4.3.4 The Optimum Number of Elutions

The optimum number of elutions likely to be carried out depends on the height of the adsorbent bed. Considering Figures 4.7 and 4.8, the results obtained suggest that, experiments conducted for L/D ratios of 0.08 and 0.16 with the column (ID = 2.45cm), the 1st elution fraction showed the highest enzyme concentration. However, the 1st elution fraction failed to show the highest enzyme concentration when the experiment is conducted with the column (ID = 4.15). Admittedly, with relatively

small heights created by these ratios, the adsorbent bed becomes almost saturated with enzymes molecules. As a result, the 1st elution fraction is able to elute a majority of the enzyme molecules. Successive elution fractions continue to decline in enzyme concentrations through to the 5th elution.

For increased L/D ratios as expressed by the series 0.33, 0.41 and 0.49 in Figures 4.9, 4.10 and 4.11 respectively, the highest amount of enzymes were eluted during the 2nd elution fraction. The concentrations of enzymes contained in subsequent elution processes decreased through to the 5th elution fraction. Usually, enzyme adsorption zones are formed with varying concentration profiles. Their movement and subsequent complete elution from the adsorption bed will be enhanced by the volume and number of elution solvent fractions which pass through the bed.

The optimum number of fractions likely to be carried out can be estimated by comparing the initial activities of enzyme solutions prior to the elution and after the elution process. The 8th day malt had an estimated activity of 19.436 U/ml. After the elution process, the highest and lowest mean of estimated activities were 75.529 and 14.785 U/ml respectively. The highest and lowest registered mean activities were exhibited by the 2nd and 5th elutions respectively (Appendix: Tables F-2 and F-6). Consequently, the elution process can be terminated when the estimated activities of elution fractions are less than the activity of the starting enzyme solution.

4.3.5 Estimation of Enzyme Activity likely to be exhibited by One (1) g of Rice (Wita 7) malt

Following the procedure given in section 3.2.1., 3g of malted rice was dissolved in 12ml of the extraction solvent. The highest mean activity exhibited by the crude alpha amylase from the malted rice was 19.436U/ml.

Mathematically,

$\frac{3g}{12ml} = 0.25\,g/ml$ rice malt solution has an activity of $19.436U/ml$.

Using a basis of 1ml of rice malt solution, it can be inferred that, 0.25g of rice malt has an activity of 19.436 U

Accordingly, 1g of malted rice (Wita 7) can be estimated to have an activity of 77.744U as shown below

$$\frac{1g}{0.25g} \times 19.436U = 77.744U$$

After purification the same amount of alpha amylase (0.25g/ml) exhibited an activity of 75.529U/ml. Again, it can be inferred that, 1g of purified alpha amylase from malted rice (Wita 7) has an estimated activity of 302.916U as shown below

$$\frac{1g}{0.25g} \times 75.729U = 302.916U$$

From the above calculations, it can be estimated that, the crude alpha amylase from 1g of malted rice (Wita 7), has an activity of 77.744U whiles the purified form of the enzyme from the same amount has an activity of 302.916U.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

After a comprehensive laboratory work lasting for almost eleven months, the outcome of the investigation into the trend of alpha amylase and reducing sugars formation in Wita 7 variety of rice during malting revealed that, alpha amylase production peaks on the 8th day of germination performed at a temperature range of 26±3°C. The crude alpha amylase in the extract from the 8th day malt exhibited an estimated activity of 19.436 U/ml.

Considering the malting characteristics such as germination energy, malt yield as well as malt loss, the investigation revealed that, the Wita 7 rice variety can be accredited for having good germination energy since nearly 100% of the rice seeds sprouted on the 4th day of germination under the prevailing conditions. The malt yield was 57.14% with a corresponding malt loss of 42.86% on the 8th day of germination.

The purified alpha amylase expressed an estimated activity of 75.529 U/ml which shows a remarkable increase from 19.436 U/ml for the crude state. In our pursuit to investigate the various elution fractions for alpha amylase concentrations using varying adsorbent height to column diameter (L/D) ratios, the results portrayed that, for L/D ratios greater than 0.33, the 2nd elution fraction usually contained the highest enzyme concentration. Though some anomalies were registered in our attempt to investigate the effects of increasing the starch adsorbent height to diameter ratio (L/D), the general outcome suggested that, the extent of enzyme adsorption and

elution can be enhanced by increasing the L/D ratios. It must be emphasized that, the trend was established using only 5ml of 0.6% w/v calcium acetate solution as the elution solvent.

The outcome of the current work shows that, crude alpha amylase from rice malt extracts can be purified by using locally treated cassava starch as adsorbent in a column to attain higher activities.

5.2 Recommendations

The choice of the rice variety (Wita 7) used for the current work was based on a recommendation put forward by Ayernor and Ocloo, 2007 for further studies to be conducted on rice varieties available in the country for their malting characteristics. Accordingly, it is recommended that, researchers with similar ambitions will explore other locally improved varieties such as GR 19, GR 21, FARO 15, and ITA 330 for their malting characteristics.

Moreover, it is recommended that, successive works should be focused on the scale-up production of alpha amylase from rice (Wita 7) to pilot basis in other to further evaluate its feasibility as an enzyme source for starch hydrolysis.

REFERENCE

1. Agbale, M.C., Adamafio, N.A., Agyeman, K.O.G., and Sackey, S.T., (2007), Malting and Brewing Properties of Selected Cereals Cultivated in Ghana, *Journal of Ghana Science Association*. Vol. 9 No.2, pp.146-155,

2. Aiyer, P.V., (2005), Review on Amylases and their Applications, *African Journal of Biotechnology*, Vol. 4(13), pp. 1525-1529.

3. Amritkar, N., Kamat, M., and Lali, A., (2004), Expanded Bed Affinity Purification of Bacterial Alpha Amylase and Cellulase on Composite Substrate Analogue Cellulose Matrices. *Process Biochemistry,* Vol. 39, pp. 565-570

4. Awoyinka, O.A., and Adebawo, O.O., (2008), Influence of Malting time on Alpha and Beta Amylase Secretions in Nigerian Amylolytic Maize Cultivars, *Africa Journal of Agricultural Research*. Vol. 3 (1), pp. 7-12.

5. Ayernor, G.S. and Hammond, T.K., (2000), Combination of Malted Cereals and Cassava Starch in the Production of Sugar Syrup, *Journal of Ghana Science Association*, Vol.2, No.1, pp. 87-92.

6. Ayernor, G.S., and Hammond, T.K., (2001) Characteristics of Malted rice for the Production of Sugar Syrup, *Journal of Ghana Science Association*, Vol.3 No.3, pp. 91-99.

7. Ayernor, G.S., Hammond, T.K., and Graffham, A., (2002), The Combination of Rice Malt and Amyloglucosidase for the Production of Sugar Syrup from Cassava Flour, *African Journal of Science and Technology,* Vol.3, No.1, pp. 10-17.

8. Ayernor, G.S., and Ocloo, F.C.K., (2007), Physico-chemical Changes and Diastatic Activity Associated with Germinating Paddy Rice (PSB.Rc 34), *Africa Journal of Food Science*.Vol.1 pp. 37-41

9. Azakawa, T., Fukuchi, S., and Murata, T., (1968), Enzymic Mechanism of Starch Breakdown in Germinating Rice Seeds 1. Analytical Study; *Plant Physiology*, 43, pp.1899-1905

10. Azakawa, T., Nomura, T., and Kono, Y., (1969), Enzymic mechanism of Starch Breakdown in Germinating Rice Seeds 2, Scutellum as the Site of Sucrose Synthesis, *Plant Physiology*, 44, pp. 765-769.

11. Baird, J. K., Sherwood, R. F., Carr, R. G. J. and Atkinson, A., (1976), Enzyme Purification by Substrate Elution Chromatography from Procion Dye – Polysaccharide Matrices, Febs Letters, Vol. 70 (1), pp. 61-66

12. Beleia, A., and Varriano-Marston, E., (1981), Properties of Partially Purified Alpha Amylase, *Cereal Chemistry*, Vol. 58(5), pp. 433-437.

13. Beta, T., Rooney, L. W. and Waniska, R. D., (1995), Malting Characteristics of Sorghum Cultivars, *Cereal Chemistry*, Vol. 76 (6), pp. 533-538.

14. Braimoh, A.K., (2009), Agricultural Land-Use Change during Economic reforms in Ghana. [Online]. Available from: http://www.elsevier.com/locate/landusepol [Accessed 2010 February 14].

15. Cecil, J.E.,(1995), The Use of Cassava Starch in the Artisanal Production of Maltose, *In:* Transformation Alimentaire du Manioc, T. Agbor Egbe, A. Brauman, D. Griffom, S. Treche (Eds.), pp. 497-504.

16. Damadora, R.M., Ratnam, B.V.V., Purnima, A., Ayyanna, C., (2005), Affinity Chromatography of Alpha Amylase from Bacillus licheniformis, *Enzyme and Microbial Technology*, Vol. 37, pp.712-717.

17. Dewar, J., Taylor, J.R.N. and Berjak, P., (1997), Determination of Improved Steeping Conditions for Sorghum Malting, *Journal of Cereal Science*, Vol. 26, pp.129-136

18. Dicko, M.H., Hilhorst, R., and Traore, A.S., (2005), Indigenous West Africa Plants as Novel Sources of Polysaccharide Degrading Enzymes in its Application in the Reduction of the Viscosity of Cereal Porridges, *Africa Journal of Biotechnology*. Vol. 4 (10), pp. 1095-1104.

19. Japan International Cooperation Agency (JICA) and Ministry of Food and Agriculture (MOFA), (2007), Drafted Final Report on the Study on the Promotion of Domestic Rice in the Republic of Ghana, Nippon Koei Co., Limited.

20. Kranjac-Berisavljevic, G., (2001), An Overview of Rice Production in Ghana, Multi-Agency Partnerships (MAPS) for Technical Change in West African Agriculture project

21. Lin, S.C., Lin, I. P., Chou, W.I., Hsieh, C.A., Liu, S. H., Huang, R.Y., Sheu, C.C. and Chang, M.D.T., (2009), CBM21 Starch-Binding Domain: A new Purification Tag for Recombinant Protein Engineering, *Protein Expression and Purification*, Vol. 65, pp.261-266.

22. Mensah, E.O., (2008), Effects of Steeping Regimes and Giberellic Acid on Enzymatic Activity of Rice Malt for the Production of Glucose Syrup, Msc. Thesis, Kwame Nkrumah University of Science and Technology

23. Norman, B.E., Vikso-Nielsen, A., Olsen, H.S., and Pedersen, S., (2005), *Starch Processes*, United States Patent No.20050107332

24. Osman, A.M., (2002), Advantages of Using Natural Substrate-based Methods in Assessing the Roles and Synergistic and Competitive Interactions of

Barley Malt Starch Degrading Enzymes, *Journal of the Institute of Brewing*. Vol. 108 (2), pp. 204-214

25. Ranki, H., and Sopanen, T., (1984), Secretion of Alpha Amylase by the Aleurone layer and the Scutellum of Germinating Barley Grain, *Plant Physiology*. Vol. 75(3), pp 710-715

26. Rao, M.S., Reddy, N.S., Rao, G.V., and Rao, K.R.S., (2005), Studies on the Extraction and Characterization of Thermostable Alpha Amylase from the Pericarp of Borassus indica, *Africa Journal of Biotechnology*. Vol. 4(3), pp. 289-291

27. Saboury, A.A., (2002), Stability, Activity and Binding Properties studies of Alpha Amylase upon Interraction with Ca^{2+} and Co^{2+}. *Biologia, Bratislava*, 57/Suppl. 11, pp. 221-228

28. Santiago, M., Guillen, D., Linares, L., Perez, R., Morlon, J., Ruiz, B., Sanchez S., and Rodriguez-Sanoja, R., (2007), Alpha-amylase Binding Domains: Cooperative Effects of Binding to Starch Granules of Multiple Tandemly Arranged Domains, *Applied and Environmental Microbiology*. Vol. 73(12), pp. 3833-3837

29. Schwimmer, S. and Balls, A. K. (1949), Starches and their Derivatives as Adsorbents for Malt Alpha Amylase. [Online]. Available from: http://www.jbc.org.html [Accessed 2008 October 23].

30. Schwimmer, S., and Balls, A. K., (1952), *Isolation of Alpha-amylase from Malt Extract*, United States Patent No. 2,594,356

31. Schwimmer, S., (1954), Industrial Production and Utilization of Enzymes from Flowering Plants, *Economic Botany*, Vol. 8 (2) pp. 99-113.

32. Somers, W.A.C., Koenen, P.H.M., Rozie, H.J., Visser, J., Rombouts, F. M., and Riet, K.V., (1995), Isolation of Alpha Amylase on Crosslinked Starch, *Enzyme and Microbial Technology*, Vol. 17, pp. 56-92.

33. Shuler, L.M. and Kargi, F., (Eds) (2001), *Bioprocess Engineering: Basic Concepts*, 2nd ed., p: 351-354. Prentice Hall International Series in Physical and Chemical Engineering Science

34. Sujka, M. and Jamroz, J., (2007), Starch Granule Porosity and its changes by means of Amylolysis, *International Agro physics*, Vol. 21, pp.107-133

35. Toledo, A. L., Severo, J.B., Souza, R.R., Campos, E.C., Santana, J.C.C., and Tambourgi, E.B., (2007), Purification by Expanded Bed Adsorption and Characterisation of an Alpha Amylase FORILASE NTL from Aspergillus niger, *Journal of Chromatography B.*, Vol. 846, pp. 51-56

36. Vallee, B. L., Stein, E. A., Summerwell, W. N. and Fisher, E. H. (1959), Metal content of Alpha-Amylase of various origins, *Journal of Biological Chemistry*, Vol. 234 pp. 2901-2929.

37. Walker, G.J. and Hope, P.M., (1963), The Action of Some Alpha Amylase on Starch Granules, *Biochemistry Journal* Vol. 86, pp.452.

38. Wijngaard, H.H., Ulmer, H.M., Neumann, M., and Arendt, E.K., (2005), The Effect of Steeping Time on the Final Malt Quality of Buckwheat, *Journal of the Institute of Brewing*, Vol. 111(3), pp. 275-21

39. Weselake, R.J. and Hill, R.D., (1983), Inhibition of Alpha Amylase Catalyzed Starch Granule Hydrolysis by Cycloheptaamylose, *Cereal Chemistry*. Vol. 60(2), pp. 98-101.

40. Yaldagard, M., Mortazavi, S.A., and Tabatabaie F., (2008), Influence of Ultrasonic Stimulation on the Germination of Barley Seed and its Alpha Amylase Activity, *Africa Journal of Biotechnology*, Vol. 7(14), pp. 2465-2471.

41. Zawistowska, U., Sangster, K., Zawistowska, J., Langstaff, J. and Friesen, A. D., (1988), Immobilized Metal Affinity Chromatography of Wheat Alpha Amylases, *Cereal Chemistry,* Vol. 65(5), pp. 413-416.

APPENDIX A: MALTING CHARACTERISITICS OF WITA 7 RICE VARIETY

Table A-1: Water uptake rate results of steeped rice seeds

Steeping time (Hours)	Mass of water absorbed (Grams)	Water uptake rate (Grams per Hour)
0	0.000	0.000
1	0.200	0.200
2	0.200	0.100
3	0.200	0.067
4	0.200	0.050
5	0.200	0.040
6	0.300	0.050
7	0.300	0.043
8	0.300	0.038
9	0.300	0.033
10	0.300	0.030
11	0.300	0.027
12	0.300	0.025

Table A-2: Malt loss and Malt yield determination results

Day	Initial Grain dry Wt (g)	Dry malt Wt (g)	Malting loss (%)	Malting yield (%)
1	1.400	1.400	0.000	100.000
2	1.400	1.400	0.000	100.000
3	1.400	1.300	7.143	92.857
4	1.400	1.167	16.667	83.333
5	1.400	1.100	21.429	78.571
6	1.400	0.867	38.100	61.900
7	1.400	0.867	38.071	61.929
8	1.400	0.800	42.857	57.143
9	1.400	0.633	54.786	45.214
10	1.400	0.633	54.786	45.214
11	1.400	0.667	52.386	47.614
12	1.400	0.533	61.929	38.071

Table A-3: Germination energy determination results

Day	Germination Energy
1	34
2	68
3	90
4	100
5	100
6	100
7	100
8	100
9	100
10	100
11	100
12	100

APPENDIX B: ABSORBANCE AND ACTIVIITIES OF CRUDE ALPHA AMYLASE

Table B-1: Absorbance results of crude alpha amylase at 540nm

DAY	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	1.717	1.575	1.700	1.664	1.575	1.717	0.089	0.053
2	1.497	1.503	1.501	1.500	1.497	1.503	0.003	0.003
3	1.598	1.604	1.617	1.606	1.598	1.617	0.008	0.011
4	2.019	2.036	2.101	2.052	2.019	2.101	0.033	0.049
5	2.077	2.083	2.071	2.077	2.071	2.083	0.006	0.006
6	2.154	2.132	2.154	2.147	2.132	2.154	0.015	0.007
7	2.509	2.512	2.267	2.429	2.267	2.512	0.162	0.083
8	2.955	2.900	2.899	2.918	2.899	2.955	0.019	0.037
9	2.623	2.516	2.627	2.589	2.516	2.627	0.073	0.038
10	2.548	2.267	2.549	2.455	2.267	2.549	0.188	0.094
11	2.342	2.340	2.192	2.291	2.192	2.342	0.099	0.051
12	2.175	2.176	2.177	2.176	2.175	2.177	0.001	0.001

Table B-2: Activity results of crude alpha amylase

Day	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	10.859	10.251	11.222	0.363	0.609
2	9.738	9.717	9.758	0.021	0.021
3	10.463	10.408	10.538	0.075	0.055
4	13.513	13.288	13.848	0.335	0.226
5	13.684	13.643	13.725	0.041	0.041
6	14.163	14.060	14.211	0.048	0.103
7	16.092	14.984	16.659	0.568	1.108
8	19.436	19.306	19.689	0.253	0.130
9	17.186	16.687	17.446	0.260	0.499
10	16.270	14.984	16.913	0.643	1.286
11	15.148	14.471	15.497	0.349	0.677
12	14.361	14.355	14.368	0.007	0.007

Table B-3: Standard Maltose Curve Data

Standard Maltose Solution (mg/ml)	Absorbance (540nm)
0.2	0.093
0.4	0.111
0.6	0.128
0.8	0.144
1.0	0.228

APPENDIX C: STARCH ADSORBENT COLUMN

Table C-1: Column accessories data

Column ID (cm)	Mass of Cotton (g)	Starch Support Plates Diameters (cm)
2.45	0.1	2.40
4.15	0.2	4.00

Table C-2: Column data (ID = 2.45cm)

Mass Starch (g)	**Adsorbent Height (cm)**	**Cylindrical Area (cm^2)**	**Starch Volume in Cylinder (cm^3)**	**L/D Ratio**
1	0.2	4.72	0.95	0.08
2	0.4	4.72	1.89	0.16
3	0.8	4.72	3.78	0.33
4	1.0	4.72	4.72	0.41
5	1.2	4.72	5.66	0.49

Table C-3: Column data (ID = 4.15cm)

Mass Starch (g)	**Adsorbent Height (cm)**	**Cylindrical Area (cm^2)**	**Starch Volume in Cylinder (cm^3)**	**L/D Ratio**
2.97	0.33	13.53	4.91	0.08
5.75	0.66	13.53	8.98	0.16
11.86	1.37	13.53	18.53	0.33
14.74	1.70	13.53	23.02	0.41
17.61	2.03	13.53	27.52	0.49

APPENDIX D: ABSORBANCE RESULTS OF ELUTION FRACTIONS

Table D-1: Absorbance readings for L/D = 0.08 using column (ID = 4.15cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum deviation	Maximum Deviation
1	6.352	6.462	6.392	6.402	6.352	6.462	0.050	0.060
2	6.832	6.802	6.872	6.835	6.802	6.872	0.033	0.037
3	4.492	4.482	4.502	4.492	4.482	4.502	0.010	0.010
4	4.782	4.862	4.852	4.832	4.782	4.862	0.050	0.030
5	4.242	4.202	4.232	4.225	4.202	4.242	0.023	0.017

Table D-2: Absorbance readings for L/D = 0.16 using column (ID = 4.15cm)

#	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum deviation	Maximum Deviation
1	10.522	10.522	10.542	10.529	10.522	10.542	0.007	0.013
2	11.112	11.122	11.122	11.119	11.112	11.122	0.007	0.003
3	9.212	9.342	9.302	9.285	9.212	9.342	0.073	0.057
4	8.362	8.352	8.312	8.342	8.312	8.362	0.030	0.020
5	4.412	4.122	4.352	4.295	4.122	4.412	0.173	0.117

Table D-3: Absorbance readings for L/D = 0.33 using column (ID = 4.15cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	6.022	6.022	6.042	6.029	6.022	6.042	0.007	0.013
2	5.662	5.692	5.642	5.665	5.642	5.692	0.023	0.027
3	6.682	6.712	6.702	6.699	6.682	6.712	0.017	0.013
4	5.992	6.032	6.002	6.009	5.992	6.032	0.017	0.023
5	5.202	5.172	5.212	5.195	5.172	5.212	0.023	0.017

Table D-4: Absorbance readings for L/D = 0.41 using column (ID = 4.15cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	7.302	7.262	7.252	7.272	7.252	7.302	0.020	0.030
2	8.672	8.692	8.722	8.695	8.672	8.722	0.023	0.027
3	7.912	7.932	7.952	7.932	7.912	7.952	0.020	0.020
4	7.422	7.412	7.442	7.425	7.412	7.442	0.013	0.017
5	5.422	5.352	5.472	5.415	5.352	5.472	0.063	0.057

Table D-5: Absorbance readings for L/D = 0.49 using column (ID = 4.15cm)

Elution Fraction	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum deviation	Maximum Deviation
1	9.672	9.722	9.672	9.689	9.672	9.722	0.017	0.033
2	10.852	10.822	10.742	10.805	10.742	10.852	0.063	0.047
3	9.422	9.432	9.302	9.385	9.302	9.432	0.083	0.047
4	7.532	7.552	7.512	7.532	7.512	7.552	0.020	0.020
5	7.232	7.182	7.202	7.205	7.182	7.232	0.023	0.027

Table D-6: Absorbance readings for L/D = 0.08 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum deviation	Maximum Deviation
1	5.778	5.768	5.668	5.738	5.668	5.778	0.070	0.040
2	3.028	3.018	2.998	3.015	2.998	3.028	0.017	0.013
3	2.828	2.868	2.888	2.861	2.828	2.888	0.033	0.027
4	2.698	2.608	2.598	2.635	2.598	2.698	0.037	0.063
5	2.188	2.178	2.348	2.238	2.178	2.348	0.060	0.110

Table D-7: Absorbance readings for L/D = 0.16 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	7.898	7.768	7.748	7.805	7.748	7.898	0.057	0.093
2	5.988	6.098	5.988	6.025	5.988	6.098	0.037	0.073
3	4.108	4.148	4.138	4.131	4.108	4.148	0.023	0.017
4	3.368	3.448	3.428	3.415	3.368	3.448	0.047	0.033
5	3.198	3.208	3.218	3.208	3.198	3.218	0.010	0.010

Table D-8: Absorbance readings for L/D = 0.33 using column (ID = 2.45cm)

11	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	3.588	3.578	3.388	3.518	3.388	3.588	0.130	0.070
2	4.988	5.018	5.028	5.011	4.988	5.028	0.023	0.017
3	4.358	4.348	4.348	4.351	4.348	4.358	0.003	0.007
4	3.188	3.178	3.218	3.195	3.178	3.218	0.017	0.023
5	3.008	3.058	3.068	3.045	3.008	3.068	0.037	0.023

Table D-9: Absorbance readings for L/D = 0.41 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	4.758	3.938	3.958	4.218	3.938	4.758	0.280	0.540
2	5.948	5.988	6.018	5.985	5.948	6.018	0.037	0.033
3	4.648	4.728	4.688	4.688	4.648	4.728	0.040	0.040
4	3.888	3.928	3.918	3.911	3.888	3.928	0.023	0.017
5	3.118	3.138	3.098	3.118	3.098	3.138	0.020	0.020

Table D-10: Absorbance readings for L/D = 0.49 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	2.738	2.668	2.658	2.688	2.658	2.738	0.030	0.050
2	4.608	4.658	4.608	4.625	4.608	4.658	0.017	0.033
3	4.028	3.968	3.958	3.985	3.958	4.028	0.027	0.043
4	3.018	3.028	2.948	2.998	2.948	3.028	0.050	0.030
5	2.328	2.288	2.308	2.308	2.288	2.328	0.020	0.020

Table D-11: Absorbance readings for L/D = 0.08 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	9.265	9.285	9.315	9.288	9.265	9.315	0.023	0.027
2	7.985	8.135	7.865	7.995	7.865	8.135	0.130	0.140
3	7.195	7.095	7.135	7.142	7.095	7.195	0.047	0.053

Table D-12: Absorbance readings for L/D = 0.16 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	8.745	8.885	8.795	8.808	8.745	8.885	0.063	0.077
2	7.285	7.275	7.205	7.255	7.205	7.285	0.050	0.030
3	6.525	6.625	6.545	6.565	6.525	6.625	0.040	0.060

Table D-13: Absorbance readings for L/D = 0.33 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	9.705	9.805	9.755	9.755	9.705	9.805	0.050	0.050
2	10.165	10.055	10.135	10.118	10.055	10.165	0.063	0.047
3	9.175	9.125	9.105	9.135	9.105	9.175	0.030	0.040

Table D-14: Absorbance readings for L/D = 0.41 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	8.575	8.445	8.525	8.515	8.445	8.575	0.070	0.060
2	10.065	10.015	9.955	10.012	9.955	10.065	0.057	0.053
3	7.205	7.295	7.195	7.232	7.195	7.295	0.037	0.063

Table D-15: Absorbance readings for L/D = 0.49 using column (ID = 2.45cm)

Elution Fractions	OD (1)	OD (2)	OD (3)	OD (mean)	OD (min)	OD (max)	Minimum Deviation	Maximum Deviation
1	9.325	9.315	9.085	9.242	9.085	9.325	0.157	0.083
2	9.945	9.945	9.975	9.955	9.945	9.975	0.010	0.020
3	9.625	9.705	9.705	9.678	9.625	9.705	0.053	0.027

APPENDIX E: The Effects of varying Adsorbent Height to Diameter ratios (L/D) on Alpha Amylase concentration in Elution Fractions

Table E-1: Absorbance readings of Elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 4.15 cm.

	Varying L/D ratios				
Elution Fractions	**0.08**	**0.16**	**0.33**	**0.41**	**0.49**
1	6.402	10.529	6.029	7.272	9.689
2	6.835	11.119	5.665	8.695	10.805
3	4.492	9.285	6.699	7.932	9.385
4	4.832	8.342	6.009	7.425	7.532
5	4.225	4.295	5.195	5.415	7.205

Table E-2: Absorbance readings of (3) Elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 2.45 cm.

	Varying L/D ratios				
Elution Fractions	**0.08**	**0.16**	**0.33**	**0.41**	**0.49**
1	9.288	8.808	9.755	8.515	9.242
2	7.995	7.255	10.118	10.012	9.955
3	7.142	6.565	9.135	7.232	9.678

Table E-3: Absorbance readings of (5) Elution fractions obtained from varying Adsorbent Height (L) in a column having a constant internal Diameter (D) of 2.45 cm.

	Varying L/D ratios				
Elution Fractions	**0.08**	**0.16**	**0.33**	**0.41**	**0.49**
1	5.738	7.805	3.518	4.218	2.688
2	3.015	6.025	5.011	5.985	4.625
3	2.861	4.131	4.351	4.688	3.985
4	2.635	3.415	3.195	3.911	2.998
5	2.238	3.208	3.045	3.118	2.308

APPENDIX F: ACTIVITIES OF PURIFIED ALPHA AMYLASE IN ELUTIONS

Table F-1: Activities of purified alpha amylase for L/D = 0.08 using column ID = 4.15cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Mínimum deviatión)
1	43.266	42.924	43.676	0.410	0.342
2	46.228	46.002	46.481	0.253	0.226
3	30.202	30.134	30.271	0.068	0.068
4	32.528	32.186	32.733	0.205	0.342
5	28.376	28.219	28.492	0.116	0.157

Table F-2: Activities of purified alpha amylase for L/D = 0.16 using column ID = 4.15cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	71.493	71.446	71.582	0.089	0.048
2	75.529	75.481	75.549	0.021	0.048
3	62.985	62.486	63.375	0.390	0.499
4	56.535	56.330	56.672	0.137	0.205
5	28.855	27.671	29.655	0.800	1.183

Table F-3: Activities of purified alpha amylase for L/D = 0.33 using column ID = 4.15cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	40.715	40.667	40.804	0.089	0.048
2	38.225	38.068	38.410	0.185	0.157
3	45.297	45.181	45.386	0.089	0.116
4	40.578	40.462	40.735	0.157	0.116
5	35.010	34.853	35.127	0.116	0.157

Table F-4: Activities of purified alpha amylase for L/D = 0.41 using column ID = 4.15cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	49.217	49.080	49.422	0.205	0.137
2	58.949	58.792	59.134	0.185	0.157
3	53.731	53.594	53.868	0.137	0.137
4	50.263	50.174	50.379	0.116	0.089
5	36.515	36.084	36.905	0.390	0.431

Table F-5: Activities of purified alpha amylase for L/D = 0.49 using column ID = 4.15cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	65.748	65.632	65.974	0.226	0.116
2	73.381	72.950	73.703	0.321	0.431
3	63.669	63.101	63.990	0.321	0.568
4	50.995	50.858	51.132	0.137	0.137
5	48.758	48.601	48.943	0.185	0.157

Table F-6: Activities of purified alpha amylase for L/D = 0.08 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	38.724	38.246	38.998	0.274	0.479
2	20.100	19.984	20.189	0.089	0.116
3	19.047	18.821	19.231	0.185	0.226
4	17.501	17.248	17.932	0.431	0.253
5	**14.785**	14.375	15.538	0.752	0.410

Table F-7: Activities of purified alpha amylase for L/D = 0.16 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	52.862	52.472	53.498	0.636	0.390
2	40.687	40.434	41.187	0.499	0.253
3	27.733	27.576	27.849	0.116	0.157
4	22.836	22.514	23.061	0.226	0.321
5	21.420	21.352	21.488	0.068	0.068

Table F-8: Activities of purified alpha amylase for L/D = 0.33 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	23.540	22.651	24.019	0.479	0.889
2	33.752	33.595	33.868	0.116	0.157
3	29.238	29.217	29.286	0.048	0.021
4	21.331	21.215	21.488	0.157	0.116
5	20.305	20.052	20.462	0.157	0.253

Table F-9: Activities of purified alpha amylase for L/D = 0.41 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	28.328	26.413	32.021	3.693	1.915
2	40.414	40.161	40.640	0.226	0.253
3	31.543	31.269	31.816	0.274	0.274
4	26.228	26.071	26.345	0.116	0.157
5	20.804	20.668	20.941	0.137	0.137

Table F-10: Activities of purified alpha amylase for L/D = 0.49 with column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum Deviation)	U/ml (Minimum deviation)
1	17.863	17.658	18.205	0.342	0.205
2	31.112	30.996	31.338	0.226	0.116
3	26.734	26.550	27.028	0.294	0.185
4	19.984	19.642	20.189	0.205	0.342
5	15.264	15.127	15.401	0.137	0.137

Table F-11: Activities of purified alpha amylase for L/D = 0.08 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	63.005	62.848	63.190	0.185	0.157
2	54.162	53.272	55.119	0.958	0.889
3	48.327	48.006	48.690	0.363	0.321

Table F-12: Activities of purified alpha amylase for L/D = 0.16 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	59.722	59.291	60.249	0.527	0.431
2	49.100	48.758	49.305	0.205	0.342
3	44.381	44.107	44.791	0.410	0.274

Table F-13: Activities of purified alpha amylase for L/D = 0.33 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	66.200	65.858	66.541	0.342	0.342
2	68.682	68.251	69.004	0.321	0.431
3	61.959	61.754	62.232	0.274	0.205

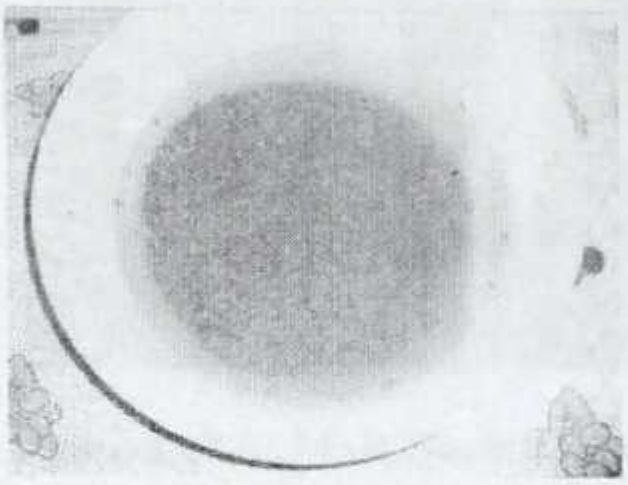
Table F-14: Activities of purified alpha amylase for L/D = 0.41 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	57.718	57.239	58.129	0.410	0.479
2	67.957	67.567	68.320	0.363	0.390
3	48.943	48.690	49.374	0.431	0.253

Table F-15: Activities of purified alpha amylase for L/D = 0.49 using column ID = 2.45cm

Elution Fractions	U/ml (mean)	U/ml (min)	U/ml (max)	U/ml (Maximum deviation)	U/ml (Minimum deviation)
1	62.691	61.617	63.258	0.568	1.074
2	67.567	67.499	67.704	0.137	0.068
3	65.673	65.310	65.858	0.185	0.363

APPENDIX G: LIST OF PROJECT PHOTOGRAPHS



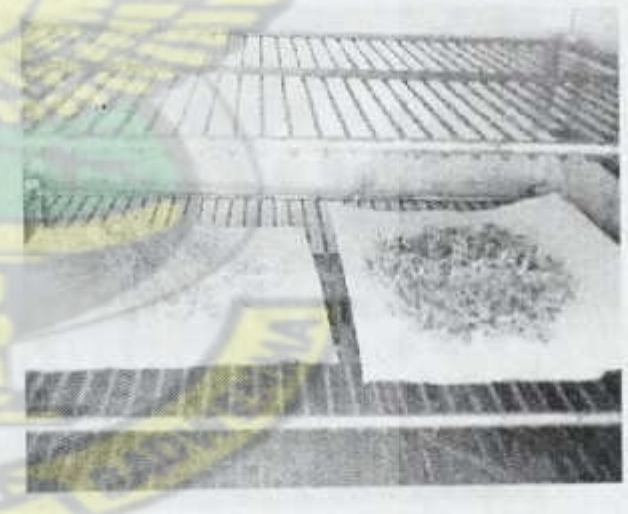
Picture 2.0: Steeping

Picture 3.0: 1st day of Germination

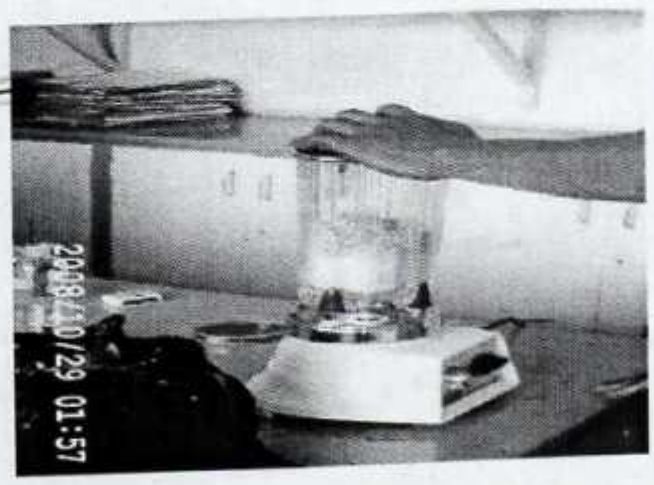
Picture 4.0: 2nd day of Germination

Picture 5.0: 3rd day of Germination

Picture 6.0: 8th day of Germination

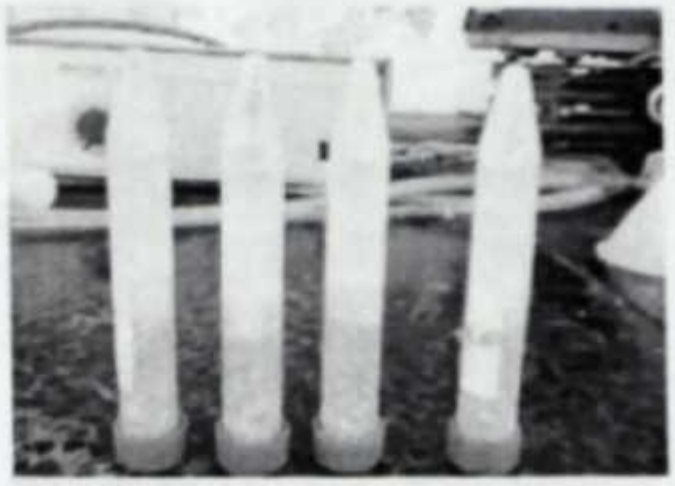


Picture 7.0: Drying

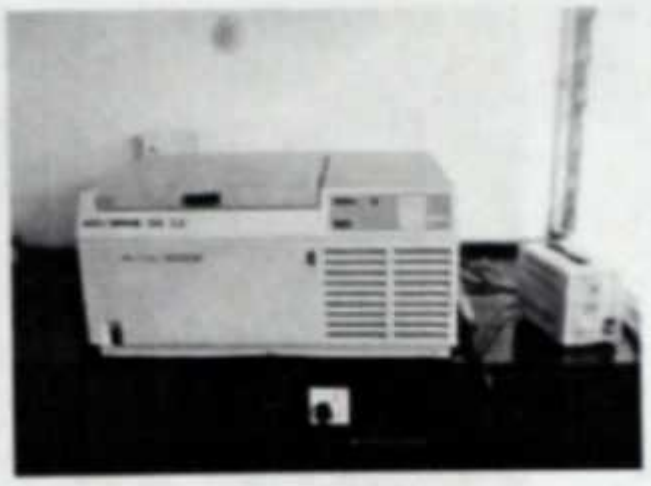


Picture 8.0: Size Reduction (blending)

Picture 9.0: Rice Malt Flour

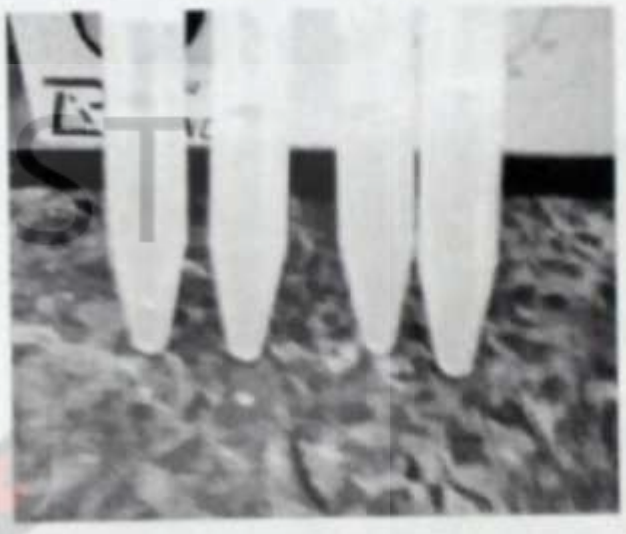


Picture 10.0: Malt flour in centrifuge tubes

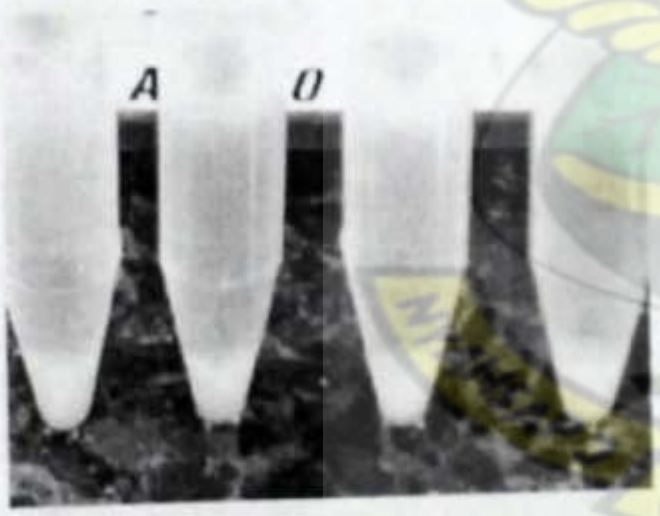


Picture 11.0: Centrifuge Machine

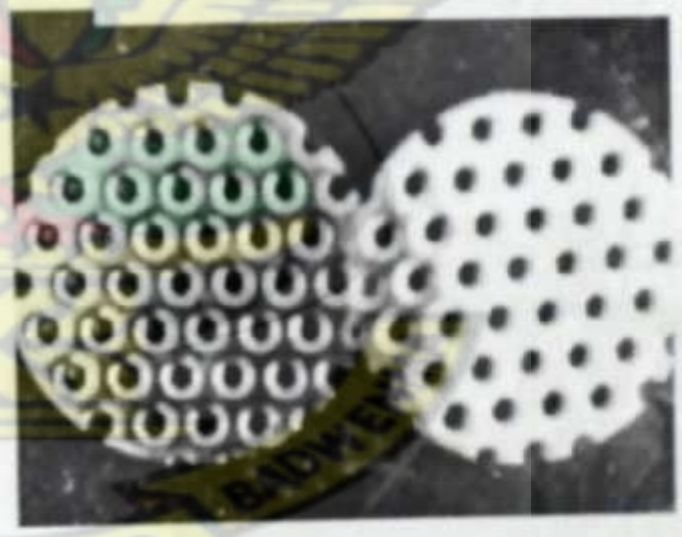
Picture 12.0: Heating of Malt extract in water bath



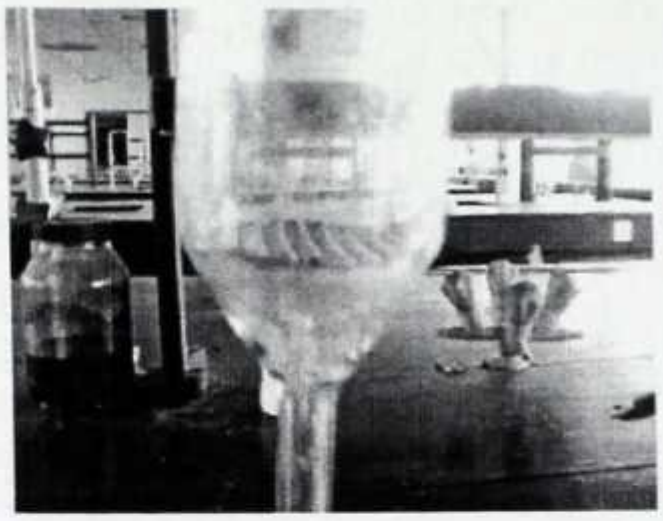
Picture 13.0: Cooled heated extract containing ammonium salt



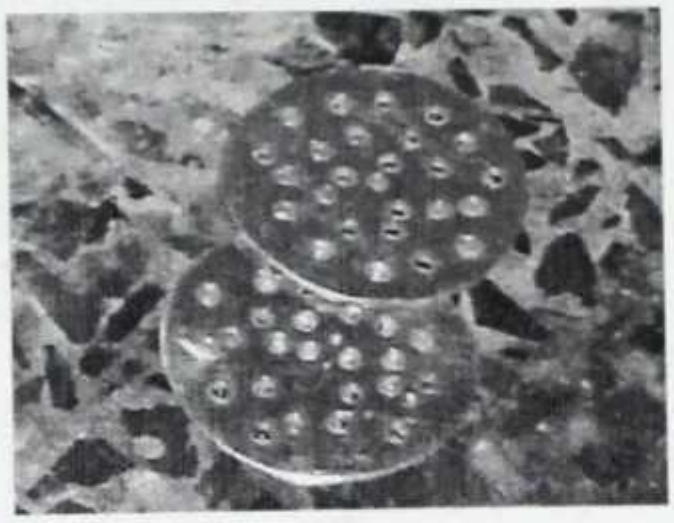
Picture 14.0: Amylase Precipitate formed from extract



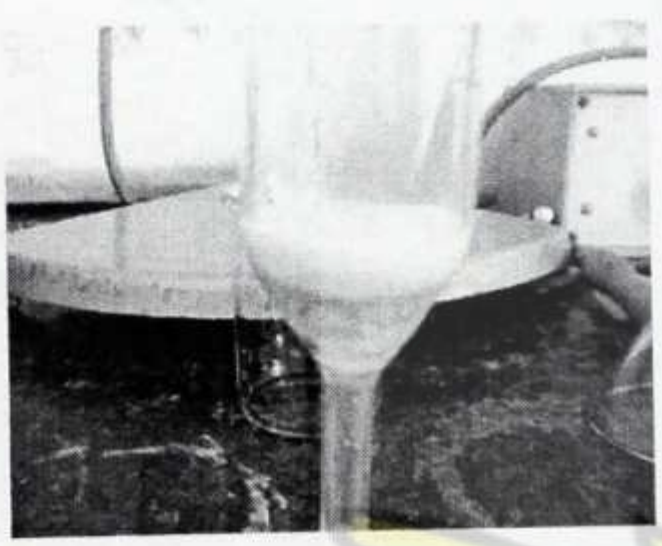
Picture 15.0: Starch Support Plates (Diameter = 2.40cm)



Picture 16.0: Support plates in column (ID = 2.45cm)



Picture 17.0: Starch Support Plates (Diameter = 4.00cm)



Picture 18.0: Treated Starch in column (ID = 2.45cm)

Picture 19.0: Support plates in column (ID = 4.15cm)

Picture 20.0: Column with enzyme solution passing through starch adsorbent

Picture 21.0: Elution and collection of Enzyme from starch adsorbent