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DEVELOPMENT OF ROUTINE CULTURE MEDIA
FROM SORGHUM

BY

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B.Sc (Hons) Microbiology, AIMLS, PGDE

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY, ZARIA,
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE
IN PHARMACEUTICAL MICROBIOLOGY

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FEBRUARY, 1997

(II)

DECLARATION

I hereby declare that the thesis has been written by me and that it is a record of my research work, carried out under the supervision of Dr. P.F. Olurinola and Dr. J.A. Onaolapo.

That, not a single part of this work has been presented in any previous applications for a degree. The work of other investigators is acknowledged and referred to accordingly.



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CERTIFICATION

This thesis entitled DEVELOPMENT OF ROUTINE CULTURE MEDIA FROM SORGHUM, submitted by Izebe, Kasimu Salihu meets the regulations governing the award of the degree of MASTER OF SCIENCE (PHARMACEUTICAL MICROBIOLOGY) OF AHMADU BELLO UNIVERSITY, and is approved for its contribution to science and literary.


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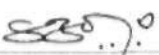
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DEDICATION

I thank Allah for His GUIDANCE during the study. I want to use this opportunity to remember my late father, Alhaji Salihu Izebe who was not able to witness the completion of my course.

ACKNOWLEDGEMENT

I wish to acknowledge with profound gratitude the immense contributions of Dr. P.F. Olurinola, who saw to the successful completion of this work through his meticulous supervision, criticism, guidance and kind encouragement. My thanks must go to Dr. J.A. Onalapo for his role not only as co-internal examiner, but also for his other innumerable helpful contributions towards the successful completion of this course.

I wish to acknowledge with due respect the encouragement given by Dr. Y.K.E. Ibrahim, Dr. I. Kolo and Dr. Osuji (NIPRD, Abuja).

I wish to also express my sincere appreciation to Daniel Adedire, Muhammed Sanusi and the entire staff of faculty of Pharmaceutical Sciences Ahmadu Bello University Zaria and National Institute for Pharmaceutical Research and Development Abuja, for creating the workable environment for the accomplishment of this work.

It is with great joy that I remember the following friends and associates, Lawal M.K, Akpulu I, Oburo, Martin Baker, Edame G.E, Joseph L, Martins S, Alhaji Raji, Sani and Family, Abdulummini and family, Boyi and family, Late Okaro, and B. Umugo and family, Obawede and Family for their contribution and encouragement towards the success of this study.

I am also grateful for their financial and moral support by my parents, brothers and sisters. I say thanks for all you did for me. To Miss Sefietu Momoh for typing the project at difficult hours.



(VI)

ABSTRACT

The nutritive value of sorghum grains (Short Kaura(SK) variety) was explored in the development of culture media.

The sorghum (SK) starch and proteineous materials were extracted, dried and subjected to amylase and pepsin hydrolysis respectively. Dried powder each of bean husk, soybean, spent yeast and fish was treated with pepsin hydrolysis to obtain growth supplements. Sorghum starch, protein digests and growth supplements were reconstituted into varied media were used in liquid and solid forms to grow Staphylococcus aureus (NCTC 6571), Bacillus subtilis (NCTC 8241), Escherichia coli (NCTC 10418), Pseudomonas aeruginosa (NCTC 6750) and Candida albicans (ATCC 10231). The growth of these micro-organisms were better in commercially prepared Nutrient broth than the sorghum broth. 20% (0.103g/L glucose) sorghum broth gave the best growth and 40% (0.206g/L glucose) sorghum broth was the least.

Sorghum broths with growth supplements performed better in supporting the growth of the test micro-organisms than the commercially prepared Nutrient broth. 35% (3.78/L protein) fish supplement in sorghum broth gave better growth than the other formulations. On solidifying the formulated sorghum media with one percent agar, the resultant media also proved better in supporting the growth of the test micro-organisms than the commercially prepared Nutrient agar.

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CHAPTER ONE

1.0 INTRODUCTION

Micro-organisms are abundant in nature, present virtually everywhere, and are of a wide variety. Micro-organisms are of great importance to the human species, because of their value as sources of many antibiotics, drug products and chemicals; but also as sources of danger, as many are pathogenic. Micro-organisms are also valuable as scientific research tools.

Micro-organisms in their natural habitat are however rarely sufficiently available in populations adequate for their industrial or research needs, hence the need to 'grow' them under controlled experimental conditions, and feeding them with artificial microbial foods, known as culture media.

Culture media are used routinely for the cultivation of a variety of bacteria, fungi, protozoa and viruses for the purpose of studying their cultural morphology, identification, classification and microbiological assay of antibiotics.

Culture media used routinely contain basically carbon (energy source), nitrogen, inorganic sources, essential growth factors and ideal temperature for the growth of micro-organisms.

1.1 VARIETIES OF CULTURE MEDIA

1.1.1 Routine Culture Media

Routine culture media are classified into three groups based on their physical state and usage, viz: liquid, semi-solid and solid media.

- (a) Liquid media are employed or used mainly as general growth, enrichment media, and blood culture. They are useful in the preparation of vaccines, antibiotics and specific amino acids in small and large scale production.

Liquid media can be used for microbiological assays and biochemical reactions or tests. Liquid media can also be used to estimate the population of bacteria using turbidimetric method, since growth in liquid medium is shown by increased turbidity in the medium. Examples of liquid media are Nutrient broth, a general purpose medium for growth of non-fastidious micro-organisms

Peptone water sugar is used in carbohydrate identification test for differentiating the enteric pathogens.

Selenite-F broth is an enrichment medium for isolating Salmonella sp in faeces and urine.

Thioglycollate broth is a medium for culturing anaerobic microorganisms in blood.

Todd-Hewitt broth is a medium for culturing beta-haemolytic streptococci and other bacteria.

- (b) Semi solid media can be prepared by adding a small amount of agar (0.4-0.5%^{w/v}) to liquid media. Semi solid media are used as transport media which contain substances that prevent overgrowth of undesired micro-organisms. These media are also used in motility test, conservation and propagation of the preserved stock cultures.

Viral transport medium for example is a transport medium for viruses.

Amies transport medium is a transport medium for Neisseria gonorrhoea and other pathogens.

Cary-Blair transport medium is used to transport faecal pathogens (*Salmonella* spp, *Shigella* Spp, *Vibrio* Spp, *Yersinia* Sp and *Campylobacter* Spp).

Alkaline peptone water is a transport medium for Vibrio cholerae

- (c) Solid media contain solidifying agent such as agar or gelatin incorporated into components of liquid media. Solid media play an important role in the identification of micro-organisms in which the identifying micro-organisms show discrete colonies with distinct colonial morphology. Others may show notable changes in the surroundings of the growing organisms, such as haemolytic reactions, pigment formation and colour changes in the media.

Solid media are also employed in microbiological assays of antibiotics and also in conducting sensitivity tests.

Blood agar for example is used as enriched medium for culturing a wide range of pathogens and to detect haemolytic bacteria.

MacConkey agar is used to differentiate the lactose fermenters from non-lactose fermenting bacteria.

Mannitol salt agar is also a differential and selective medium for Staphylococcus aureus.

Sabouraud dextrose agar is used to culture fungi.

Xylose lysine Deoxycholate is selective and differential medium for Salmonella Sp and Shigella Sp.

1.1.2 Defined Media

They are prepared from pure chemical substances and their exact compositions are known. The ingredients are of analytical reagent quality and are dissolved in distilled or demineralized water. Defined media are used for various experimental purposes and are classified as simple, synthetic and complex synthetic media.

- (a) Simple synthetic media contain carbon and energy sources such as glucose or lactose, nitrogen source in the form of ammonium chloride; phosphates or sulphates as inorganic salts in a buffered aqueous solution.
- (b) Complex synthetic media contain the carbon source, nitrogen source, inorganic salts and in addition certain amino acids, purines and pyrimidines and other growth factors are added. Complex synthetic media are therefore used for the growth of fastidious bacteria.

1.1.3 Routine Laboratory Media

These are used for the study of pathogens or commensals of human body. These media should provide nutrients and pH approximate to those of the tissue and body fluids. The most popular routine laboratory media include Basal, Enriched, Selective and Indicator media.

- (1) Basal media are simple nutrient and peptone water broths, which can be used as routine culture media for general purpose media.
- (2) Enriched media are prepared to meet the nutritional requirements of fastidious bacteria by addition of substances such as blood, serum and egg to a basal medium.
- (3) Selective Media are media that contain certain substances that inhibit all but a few types of bacteria. The media facilitate the isolation of a particular species from a mixed inoculum or broth.
- (4) Indicator media contain some substances which are incorporated into the media to show characteristic visible change as a result of metabolic activities of particular bacteria.

1.1.3.1 Basic or Basal Media

These are media that can be used to grow ordinary bacteria which are mainly non-selective or exact in their nutritional requirements. Such media have no specific action against any bacteria. Examples of such media include nutrient broth, nutrient agar, czapek-dox media and sabouraud dextrose media.

- (a) Nutrient broth: This is commercially available as Oxoid nutrient broth No.2 in dehydrate powder form and it is usually reconstituted by addition of specified volume of distilled water per gramme before use.

TABLE 1: COMPOSITION OF NUTRIENT BROTH

Constituents	Quantity used
Peptone	6.0gm
Yeast extract	3.0gm
Beef extract	1.5gm
Dislled Water to	1000ml

pH 6.8 and it is sterilized by autoclaving at 121°C for 15 minutes.

(b) Nutrient agar Medium: It is nutrient broth solidified by addition of 1.5-2% w/v agar.

(c) Nutrient gelatin medium: This is nutrient broth solidified by addition of 10-15% w/v gelatin.

(d) Sabouraud Glucose fluid Medium

TABLE 2: COMPOSITION OF SABOURAUD GLUCOSE FLUID MEDIUM

Constituents	Quantity used
Glucose	40.gm
Oxoid mycological peptone	10.gm
Distilled water to	1000ml

PH 5.4 and it is sterilized by autoclaving at 121°C for 15 minutes.
The medium is used for growing fungi.

- (e) Sabouraud glucose agar medium: This can be prepared from the sabouraud glucose fluid medium by addition 1.5 to 2.0% w/v agar to solidify the medium.

- (f) Peptone Water

TABLE 3: COMPOSITION OF PEPTONE WATER

Constituents	Quantity used
Peptone	10.0gm
Sodium chloride	5.0gm
Water to	1000ml

PH 6 and sterilize by autoclaving at 121°C for 15 minutes.

Czapek - Dox medium: This is a synthetic medium used for the cultivation of fungi.

TABLE 4: COMPOSITION OF CZAPEK - DOX MEDIUM

Constituents	Quantity used
Sodium nitrate	2.0gm
Potassium chloride	0.5gm
Ferrous sulphate	0.01gm
Magnesium glycerophosphate	2.5gm
Potassium sulphate	8.35gm
Sucrose	30.0gm

Oxoid agar No.3	12.0gm
Distilled water - to -	1000ml

Adapted from the Oxoid manual (1971)

PH 6.8 and it is sterilized by autoclaving.

1.1.3.2 Enriched Medium

These are media developed by modifying the environmental conditions that would guarantee the growth and survival of the microbial cells in a mixed population of bacteria. The design of these media is to meet the nutritional requirements of fastidious bacteria, that is those which cannot be grown on ordinary media but require complex organic body fluids to supplement the deficiency in the routine media. Examples of enriched media include Loffler's serum, Blood agar, serum agar and chocolate agar.

- (a) Loffler's Serum Medium is made of three parts of horse or Ox-blood mixed with 1 part of broth containing 1% glucose. This is sterilized at 75°C for 24 hours on two successive days. It may be poured into plates or used as slope cultures and can be employed in cultivation of Corynebacterium Sp.
- (b) Blood agar medium is an enriched medium prepared by melting nutrient agar medium at 100°C and cooling to 55°C. Then 5-10% sterile defibrinated blood of sheep, OX, horse or human being is mixed well with the melted nutrient agar.
- (c) Serum agar medium is prepared in the same way as blood

agar medium but sterile serum is used instead of sterile blood.

- (d) Chocolate agar medium is prepared from blood agar which is melted at 55°C. The temperature is raised to 100°C for 2 minutes and poured into sterile plates or used as slant culture for the cultivation of Haemophilus Sp.

1.1.3.3 Selective Media

These are routine media to which selectively inhibitory chemicals have been added to suppress or kill all but few types of bacteria. They are used to aid in the isolation of a specific bacterium from mixed culture. Examples include cetrimide agar, Lowenstein Jensen and MacConkey agar media

- (a) Cetrimide agar medium is prepared in the same way as nutrient agar but 0.03% cetrimide (Cetyltrimethylammonium bromide) is added to the nutrient agar. It is used as a selective medium for the isolation of Pseudomonas aeruginosa
- (b) MacConkey agar medium contains peptone as a nutritive; bile salt (sodium taurocholate as an inhibitor of non-intestinal bacteria), Lactose as a test sugar and neutral red as an indicator. It is used to select coliforms especially Escherichia coli which ferments lactose. Such bacteria produce rose pink colonies while non-lactose fermenters such as Salmonella and Shigella Spp produce yellow colonies.
- (c) Lowenstein Jensen Medium contains mineral salts, asparag-

ine, glucose and beaten eggs. It also contains 0.03% malachite green to suppress the growth of other organisms other than Mycobacterium Sp.

1.1.3.4 Indicator Media

These contain added specific substrate or indicator which changes colour with the metabolic activities of particular bacteria and may result from a change in appearance of the medium or the PH or of the redox potential in the area of growth. Examples are MacConkey medium, Macleoud's tellurite medium, Litmus milk medium and methyl red and Voges - Proskauer Medium.

- (a) Litmus milk medium is used in the rapid litmus milk reduction test to identify enterococci and also used to demonstrate the stormy clot reaction of Clostridium perfringens.
- (b) Methyl red-Voges - Proskauer (MRVP) is used in identifying enterobacteriaceae,

1.1.3.5 Media for Anaerobes These media are made in such a way that they contain very little or no dissolved oxygen. The oxygen present may be removed by use of special apparatus or heating the media in a water bath for sometime with the lid tightly closed. The media usually contain reducing substances with a great affinity for oxygen. Examples are Robertson's cooked meat medium, thioglycollate medium, Ascorbic acid medium and Glutathione medium.

- (a) Robertson's cooked meat medium when prepared in 28ml McCartney bottle will contain 10ml nutrient broth with minced meat added to a depth of 2-5cm of the bottle. Extra nutrient broth is necessary if all the meat is to be used. Reducing conditions are provided in the depth of the medium by the reducing substances such as cysteine and glutathione present in the meat. It is recommended that the medium be heated for about 30 minutes before use to drive out dissolved oxygen. A layer of sterile liquid paraffin to cover 1-cm layer is sometime added to the surface to prevent oxygen diffusing into the medium.
- (b) Fluid thioglycolate medium is prepared by dissolving the other ingredients other than sodium thioglycollate and resazurin solution by steaming at 100°C. Thioglycollate is added and the pH adjusted to 7.3. If there is precipitate, the preparation is heated without boiling, filtered solution is added, mixed thoroughly, distributed into containers (20ml) sterilized at 121°C for 15 minutes and stored in the dark preferably used for sterility testing, and for cultivation of anaerobic bacteria.



TABLE 5: COMPOSITION OF FLUID THIOLYCOLLATE MEDIUM (BREWER'S MEDIUM)

Constituents	Quantity required
Yeast extract, water soluble	5.0
Casein hydrolysate pancreatic digest	15.0gm
Glucose	5.5gm
L-cysteine	0.5gm
Agar	0.75gm
Sodium chloride	2.5gm
Sodium thiolglycollate	0.5gm
Resazurin sodium solution freshly prepared (1:1000 strength)	1.0ml
Water - to -	1000ml

1.2 GENERAL COMPOSITION OF CULTURE MEDIA

Most standard culture media contain the following ingredients in appreciable proportions.

1.2.1 Water

Water is an important constituent, making up about 80-90 percent of the total weight of micro-organism. Water used in culture media must be free from any inhibitory substances that might affect the growth of organisms. The commonly used water is deionized or distilled water. Copper distilled water is

not allowed in media because of the presence of traces of copper which is highly inhibitory to bacteria growth.

1.2.2 Peptone

Peptone is obtained from the break down of animal and plant proteins. These proteins are from meat, milk and soyabeans meal, which are hydrolysed by acids or by enzymes such as papain, pepsin and trypsin. The important constituents of these proteins are peptones, proteoses, amino acids and a variety of inorganic salts. The salts include phosphates, potassium, magnesium and certain accessory growth factors such as nicotinic acid and riboflavin. Peptone mainly provides nitrogen source for micro-organisms as building bricks for protein construction or they may be utilised as a source of energy, forming compounds such as hydrogen sulphide, indole amines, etc. In diagnostic tests, peptones may be selected which have predominant virtues. Thus peptones rich in tryptophan are selected for indole tests. Peptones with high sulphur content may be used for hydrogen sulphide tests.

Apart from the standard grades of bacteriological peptone, some manufacturers supply special grades of peptone recommended for particular purpose. Examples, Neopeptone, proteose peptone and mycological peptone. Table 6 shows the constituent compositions of Oxoid peptone.

TABLE 6 CHEMICAL COMPOSITION OF BACTERIOLOGICAL PEPTONE (OXOID
MANNUAL 1976)

Constituents	Quantity present
Total nitrogen	14.5 percent
Total proteose nitrogen (Saturated 2_nSO_4)	1.9
Primary Proteose nitrogen	0.14
Amino acid nitrogen (Formoltitration)	1.7
Tryptophane	1.2
Ash	5.5
Chloride	1.0
Phosphate (as P_2O_5)	1.3
Calcium	0.13
Magnesium	0.07
Copper	0.001
Iron	0.0075
Zinc	0.0025
Sulphur (total)	0.68
Ether-soluble extract	0.03
Nicotinic acid	75mg per gm
Riboflavin	50mg per gm
Carbohydrate	Nil
Moisture	Less than 5%

1.2.3 Meat Extract

Meat extract supplements amino acids with essential growth factors such as thiamine, nicotinic acid, riboflavin, pyridoxine, pantothenic acid and choline. Others are vitamins and mineral salts including phosphates, sulphates and sodium chloride.

1.2.4 Yeast Extract

Yeast extract is a common constituent of standard medium mainly as a growth stimulant. It contains a wide range of amino acids (amounting to nearly fifty percent of its mass), growth factors (especially of the vitamin B group) and inorganic salts such as potassium and phosphates. Over ten percent of its constituent is made up of carbohydrate such as glucogen, trehalose and pentoses. Yeast extract may be substituted for meat in culture media.

1.2.5 Carbohydrates

Carbohydrates are important in the formulation of culture media and a component of a standard culture medium. Carbohydrates provide carbon content which is fifty percent of the total mass of the bacterium.

Carbohydrates can be in form of simple or complex sugars or starch added into the medium.

Generally, carbohydrate nutrients are directly catabolized by the appropriate enzymes possessed by the microorganisms and their products are absorbed into the cell constituents.

Carbohydrates also supply the energy requirements of the cell. The carbon in the form of organic substrates such as glucose enter the pathways for energy yielding metabolism and is eventually excreted again from the microbial cells as carbon (iv) oxide. Thus, carbohydrate nutrients play a dual role in micro-organisms' culture media requirements, serving as carbon and energy sources.

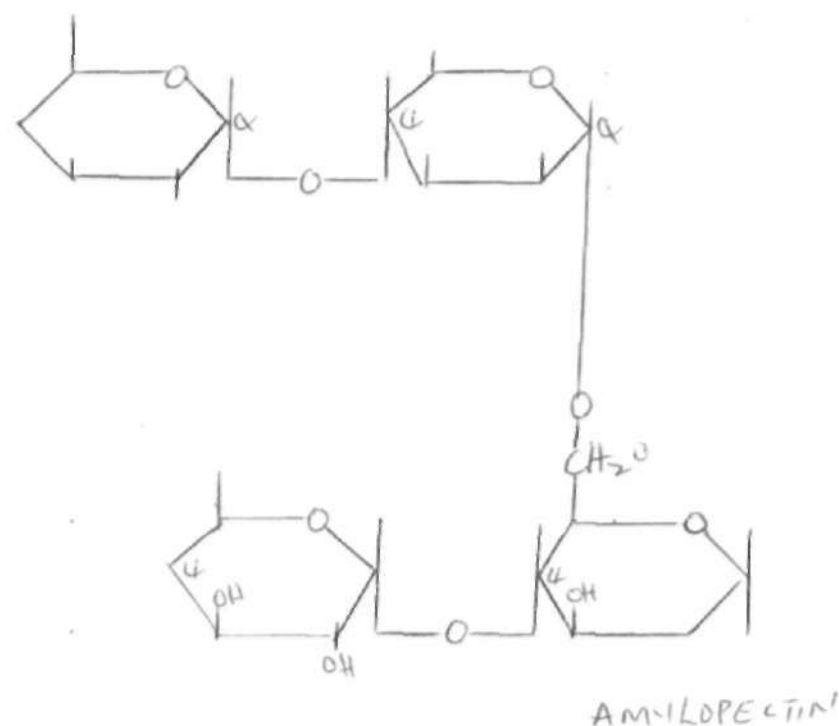
Starch is the commonest source of carbohydrate. Most of the food crops notably the cereals, root and stem tubers have high quantity of starch.

(1) Biochemistry of starch

Starch is a mixture of amylose and amylopectin.

- (a) Amylopectin or 4-amylose is the main constituent of most starches constituting about eighty percent is amylopectin. It is made up of branched chains of 20-26, Q-1,4 linked glucose residue. There are several hundreds of these chains linked by α - 1, 6 glycosidic bonds to neighbouring chains. Amylopectin is more complex than δ - amylose and is responsible for starch paste formation.

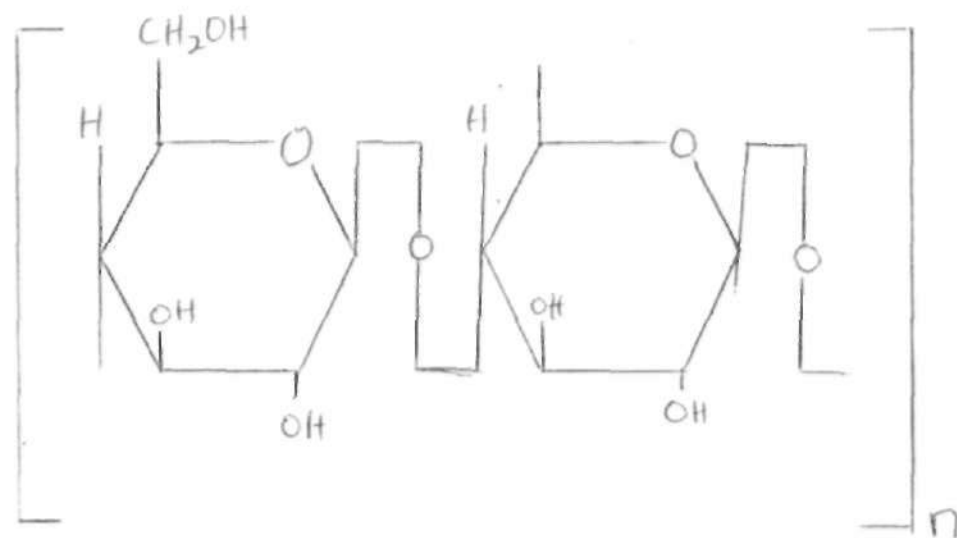
Amylopectin gives reddish colour with iodine. On hydrolysis with B - amylase, amylopectin gives maltose to the extent of about fifty percent and the remains being dextrin.



- (b) Amylose or B-amylase constitutes up to twenty percent of starch and sometime it can be totally absent. Amylose consists of linear chains of 4-1,4 linked glucose residue. There are several thousand linkages that constitute a chain.

Amylose gives deep blue colour with iodine and much deeper than that given by the whole starch. On hydrolysis of amylose with 4-amylase yields maltose and maltotriose. Amylose is degraded by alkaline solution to release D-glucoisosaccharinic acid.

Generally, starch can be hydrolysed by enzymes to yield mainly maltose.



AmyLOSE

1.2.6 Mineral Salts

Ions of potassium, magnesium, calcium and iron are required in significant amount (1.35-4.2%) in culture media. These ions or salts constitute macronutrients, and function in cell material formation.

Micronutrients are salts present in small amounts (0.025-0.2%w/v) in routine culture media and they function in enzymatic activities of micro-organisms. Such salts include manganese, cobalt, molybdenum and zinc. Some micro-organisms require heavy impregnation of the cell wall with silica, thus silicon is specifically required and should be incorporated into the culture medium for their growth to occur.

The importance of the various ions for the growth of micro-organisms are summarized in table 7.

TABLE 7: GENERAL PHYSIOLOGICAL FUNCTIONS OF THE PRINCIPAL ELEMENTS

Element	Physiological function
Hydrogen	- Constituent of cellular water, organic cell materials.
Oxygen	- Constituent of cellular water, organic cell material as molecular oxygen, electron acceptor in respiration of aerobes.
Carbon	- Constituent of organic cell material
Nitrogen	- Constituent of protein, nucleic acid, co-enzymes.
Phosphorus	- Constituent of nucleic acids, phospholipids
Potassium	- One of the Principal cations in cells, cofactor some enzymes.
Magnesium	- Important cellular cation, inorganic co-factor for very many enzymes reactions including those involving ATP, functions in binding enzymes to substrates.
Manganese	- Inorganic cofactor for some enzymes.
Calcium	- Important cellular cation, cofactor for some enzymes (for examples proteinases).
Cobalt	- Constituent of Vitamin B ₁₂ and its co-enzymes.
Iron	- Constituent of cytochromes.
Copper	)
Zinc	) Inorganic constituents of special enzymes.
Molybdenum	

Adapted from Stanier et al (1981).

1.2.7 Growth Factors

Growth factors are organic compounds that cannot be synthesized using simpler carbon source by micro-organisms, but are required as precursor or constituents of their organic cell materials.

Growth factors must be provided as nutrients in the media. Growth factors fulfil specific needs in biosynthesis and are required in small quantities as precursors of cell carbon. Growth factors are classified into three classes in terms of chemical structure and metabolic functions.

- (a) Amino acids: These are required as constituents of protein. Most micro-organisms can synthesize their amino acids need. However, a few amino acids cannot be synthesized by some micro-organisms, but are added externally into their culture media.
- (b) Purines and Pyrimidine: These organic compounds are required as constituents of nucleic acids. The purine group consists of adenine, and guanine, while the pyrimidine includes cytosine, thymine and uracil. These five compounds enter into the structure of nucleic acids.
- (c) Vitamins: These are diverse collection of organic compounds that form parts of the prothetic groups or active centers of certain enzymes. For example, nicotinic acid (Niacin) is incorporated into pyrimidine

nucleotide coenzymes (nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) which function in dehydrogenation reaction. The list of vitamins and their possible function is shown in table 8.

TABLE 8 FUNCTION OF VITAMINS

Vitamin	Coenzyme	Function/reaction
Biotin	Biotin coenzyme	Carboxy/transfer, deamination Carbon (iv) oxide fixation.
Pantothenic acid	Coenzyme	Acyl/transfer or exchange
Folic acid	Tetrahydrofolate	Transfer of one carbon units methylation, purine and pyrimidine synthesis
Thiamin (B ₁)	Thiamin Pyrophosphate	Accepts Carbonyl/groups from decarboxylation of keto acids. Tricentric acid cycle, lipid metabolism.
Riboflavin(B ₂)	1. Flavin Mononucleotide (FMN)	Dehydrogenation, electron transfer - oxidations
	2. Flavine Adenine dinucleotide (FADN)	Dehydrogenation
Nicotinamide(B ₃)	Nicotinamide adenine dinucleotide (NAD).	Dehydrogenation, energy generation
	NADP	Dehydrogenation, hydrogen acceptor, synthesis of fatty acids.
Pyridoxine (B ₆)	Pyridoxy/ phosphate	Deamination, racemization transamination decarboxylation of amino acids.

Cobamide (B ₁₂)	Cobamide coenzyme deoxyladenosyl(B ₁₂)	Transfer of methyl groups
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Adapted From Free, 1966.

1.2.8 Solidifying Agents

These are substances which when added to liquid nutrient media produce firm gels called solid media. These substances include gelatin, agar and inspissation of serum and egg media.

- (a) Gelatin: This was used by the early bacteriologist to develop the first solid media. For bacteriological use, an edible grade of gelatin is preferred; since this is free from preservatives and inhibitory amounts of heavy metals. The proportion of gelatin used in culture medium is about twelve (12) percent. Gelatin is decomposed by proteolytic bacteria.
- (b) Serum and Egg: With the use of the inspissator apparatus, proteins in serum or egg in culture media can be made to solidify by maintaining a temperature between 75° and 85°C. Loeffler serum and Lowenstein Jensen media are solidified by this method.
- (c) Agar: Agar is commonly used as a solidifying agent in culture media. Agar is prepared from a variety of seaweeds such as Gelidium Spp, Eucheuma Spp and Pterocladia Sp. The method of extraction from the dried seaweeds is by hot water process. The product is clarified, dried and finally supplied as dried strands or

powder. Agar consists of two main polysaccharides, an agarose (70-75%) and agaropectin (30-25%). Agar forms gel firmly at concentration of 1.5% w/v with high melting point of 90-95°C and setting temperature of 32°C and 39°C, which is safe for the addition of whole blood at 45° to 50°C.

1.3 PHYSICAL REQUIREMENTS FOR BACTERIAL AND FUNGAL GROWTH

- 1.3.1 (a) Temperature: Temperature is one of the most important environmental factors that regulate the growth of micro-organisms. Temperature is related to the ability of organisms to grow and survive. Temperature of the environment also has an effect on cell size, metabolic products such as pigment and toxins, nutritional requirements, enzyme reactions and the chemical composition of the microbial cells.

At an optimum temperature of incubation, micro-organisms grow best. For most routine culture for human pathogens, the optimum temperature selected is between 35 to 37°C.

The optimum temperature of growth for most pathogenic fungi is between 25° to 30°C. Dimorphic fungi, such as Histoplasma Sp produces yeast forms at 35° to 37°C and mycelial forms at 25° to 30°C. Campylobacter Sp when mixed with enterobacteriaceae

are best isolated at a higher temperature of 42°C from clinical specimen containing non-pathogenic enterobacteriaceae.

- (b) pH: Adequate alkaline and acidic requirements vary with micro-organisms. Most human pathogens grow best in pH 6.8 to 7.4. Glucose containing medium produce organic acids by the growing micro-organisms during fermentation process. Whereas, the decomposition of proteins and amino acids will result in increased alkaline in the medium. These varied acidic and alkaline concentration can inhibit the growth and survival of micro-organisms. The pH variation in the medium can be prevented when buffer solution is incorporated. Phosphate buffer is widely used in medium preparation. In recent times others such as Tris HCl buffer and Tri (hydroxymethy) aminomethane (Cruickshank et al 1980) have been found more effective, especially for chemically defined media.

Fungi grow at a pH range of 5.2 to 5.6 a pH range which inhibit the growth of many bacterial species. Yeast have a pH tolerance ranging from 1.5 to 8.5.

- (c) Gaseous Requirement: Micro-organisms vary in their need for oxygen and use of it as a means of producing energy. Aerobes require free oxygen, for

example *Pseudomonas* Sp.

Obligate anaerobes cannot grow and survive in an atmosphere containing oxygen, for example *Clostridium* Spp.

Facultative anaerobes can grow with or without free oxygen. Most human pathogens such as *Staphylococcus* Spp are facultative anaerobes.

Micro-aerophilic micro-organisms can grow only in traces of free oxygen. *Campylobacter* Spp requires an oxygen tension of about 5-10 percent.

Carboxyphilic microbes require carbon (iv) oxide enriched atmosphere. *Neisseria* Spp and *Brucella* Spp grow best in an atmosphere of 5 to 10 percent carbon (iv) oxide.

- (d) Moisture Content: Most micro-organisms require a minimum of available free water determined as water activity for growth. Generally bacteria require 0.91 and 0.95 for Gram negative and positive bacteria respectively. Yeasts require 0.88, although *Aspergillus* Spp can grow in water activity of 0.61.
- (e) Osmotic Pressure: This phenomena exhibited by micro-organisms is due to their possession of a semi-permeable cytoplasmic membrane. They can thus, tolerate the osmotic pressure change in their environment and culture medium, with varying compo-

sitions of sugars, salts and other solutes (5%w/v and 10%w/v). High salt concentrations may cause plasmolysis, while too low salt concentration results to leakage of the cytoplasmic contents, sudden transference of bacteria to a hypotonic solution may cause plasmoptysis. Marine forms of bacteria require a high salt concentration and therefore called halophilic bacteria. Organism such as Vibrio parahaemolyticus can tolerate 8% w/v sodium chloride, while Vibrio alginolyticus requires 10%w/v sodium chloride for growth.

1.4 HISTORICAL DEVELOPMENT OF CULTURE MEDIA

The invention of simple microscope by Anthony Van Leeuwenhoek (1632-1723) in 1675, and the descriptions of animalcules viz protozoa, algae, yeasts and bacteria began the era of a new specialized field of biology called microbiology. The improvements in the compound microscope and Amici's discovery of oil immersion lens allowed for further exploration of the microbial world (Baker, 1980).

The pioneer work in the development of culture media dated to 1857 when Louis Pasteur (1822-1895) grew and isolated Lactobacillus sp in defective wine in Lille with sugar solution which contained chalk (Stanier 1980).

In 1876, Robert Koch (1843-1910) cultured and identified Bacillus anthrax. He cultured the causative agent of anthrax

by drops of particles of infected spleen from a diseased animal into a sterile serum. In 1881, Koch published the method of producing pure cultures of bacteria by growing them on the surface of translucent solid medium. The medium Koch devised was a meat infusion broth solidified with gelatin and by the use of this method, many pathogenic bacteria were isolated (Baker, 1980). Similarly, Koch (1881) developed the first nutrient broth which was formulated from meat infusion, meat extract and digest broth. In 1882, Koch presented his discovery and described the culture of tubercle bacilli to the Berlin Society, which finally established that tuberculosis was an infectious disease (Stanier, 1980).

For more nutritionally exact micro-organisms such as Leptospiral Sp, Treponema Sp and Borellia spp, only Leptospiral sp will grow readily in routine culture media. Noguchis medium developed in 1912 was used for the isolation of pathogenic Borreliae and non-pathogenic Treponema sp. Hartley (1922) introduced Hartley broth in which trypsin was the proteolytic enzyme used for the hydrolysis of the lean meat, ox heart or beef. The broth was economical and no peptone added. The broth was used to grow fastidious micro-organisms such as Bucella Sp Dobell and Laidlaw (1926) developed standard medium for the isolation and propagation of Entamoeba histolytica Benham (1931) developed cornmeal agar used for the investigation of yeast - like culture for the production of Chlamydospores in Candida albicans.

The discovery of Goodpasture (1931) that many viruses could be propagated in the embryonated hen's egg suggested new methods of cultivation of viruses.

In 1933, Wright developed improved meat infusion broth with 0.15 percent glucose added and the medium was especially good for the cultivation of Pneumococcus species.

Wilson (1938) developed the Wilson and Blair medium which depended on the reduction of sulphite to sulphide in the presence of glucose by Salmonella typhi and Salmonella paratyphi B. The inhibition of Escherichia coli by brilliant green and bismult sulphite in Wilson and Blair medium was because of excess sodium sulphite.

In 1940, Gladstone and Filde developed casein-yeast-lactate glucose medium (CYLG) which was a simple culture medium for general use without meat extract or peptone. They used yeast autolysate and casein digest in the medium formulation.

Christie and Keogh (1940) developed milk agar medium for growing Staphylococcus aureus, which gave large characteristic colonies of staphylococci which appeared within 24 hours with marked pigmentation. The medium facilitated the recognition of weekly golden colonies.

Anderson (1944) devised Anderson's medium which was selective and preserved for the microscopic morphology of Corynebacterium diphtheriae.

Lowbury and Collins (1955) found that at 0.03% cetrimide added to Lemco agar, had proved a useful selective agent for the isolation of Pseudomonas aeruginosa.

Thayer and Martin (1966) developed Thayer and Martin's medium. The medium was used to isolate gonococci from contaminated specimen. The medium consists of special basal medium enriched with haemoglobin and yeast supplements and made selective with vancomycin, colistin and nystalin. Also trimethoprin (5mg/ml) was added to inhibit Proteus Sp.

Advances in the formulation and preparation of culture media for most micro-organisms have been made over the years. Oxoid investigated into peptone as a major component of culture media. In 1924, Oxoid commenced the manufacturing of peptone and hence the bedrock of most culture media.

1.5 EFFORTS TOWARDS DEVELOPMENT OF CULTURE MEDIA IN NIGERIA

The poor storage conditions of commercially prepared culture media in the tropic such as in Nigeria and the expensive nature of these culture media have led scientists in Nigeria to research into locally prepared media intensified.

Olurinola and Nasiru in 1986 started into preliminary investigations on the possibility of developing routine culture medium from various varieties of locally produced sorghum grains. In their investigation, they used 5%, 10%, 20% extracts of short kaura and Farafara varieties. These were used to compare the growth of three strains each of

Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli, using the viable cell count method. They found that short Kaura variety supported growth better than the Farafara variety.

Further investigation by Olurinola and Ojabo (1989) had shown that sorghum grains extracts in concentration of 20% w/v and 10%w/ have the ability to support bacterial growth to a good extent. The growth supporting ability shown by these media when inoculated with standardized cultures of Staphylococcus aureus (NCTC 6571 and wild strain) as well as Escherichia coli (NCTC 10418 and a wild strain) were less than what observed for the Nutrient broth. They concluded that with sufficient enrichment of the 10%w/v sorghum extracts may provide a good and economically viable result than the 20%w/v sorghum grains extracts.

Olurinola and Guyit (1990) reported the use of 10% ^{w/v} of sorghum malt extract. The malted extract was degelatinized by adding 1% amylase obtained from Bacillus subtilis and 0.1w/v peptone was added and the sterilized broth was used to grow Escherichia coli and Pseudomonas aeruginosa. The result was significant and comparable in terms of growth of the test organisms with nutrient broth.

Similar works was carried out by Olurinola and Zakari (1990) to develop routine culture media from malted sorghum extracts. They used 10%^{w/v} malted sorghum extract treated with 1% crude extract of 4-amylase obtained from Bacillus Sp and

0.1% w/v of industrial powder of Rohalase M-Fungal α -amylase. The 10% w/v of degelatinized malted extract supplemented with 1% peptone powder were used to grow Staphylococcus aureus and Candida albicans (wild strain) using the colorimetric method, the growth supporting ability of 10% malted sorghum grain extract appeared better in performance than in 10% malted sorghum grain extract plus 1% peptone and nutrient broth on the test micro-organisms.

Other workers (Jassin, 1988, and Osazuwa 1989) have contributed immensely in the development of culture media from other local sources such as fish and coconut water.

Jassin et al (1988) reported the use of trypsin degraded fish waste as a substitute for the more expensive beef extract.

Osazuwa (1988) attempted to use coconut water (sap) in the place of conventional nutrient broth for the propagation of micro-organisms.

Oloke and Famurewa (1991) reported the use of different media prepared from cassava (Mannihot esculenta), maize (Zea mays) and potato (Solanum tuberosum) to propagate Staphylococcus aureus, Bacillus subtilis, Serratia Marcescens, Micrococcus luteus, Pseudomonas fluorescens, Trichophyton mentarophyte and Aspergillus niger. They had shown that potato medium supplemented with bean husk digest and fish (waste) digest compared, favourably well with the conventional nutrient broth when used to culture Serratia marcescens, Pseudomonas

fluorescens and Staphylococcus aureus.

This work is an extension of what other investigators have done in the formulation of routine culture media using short, Kaura variety of sorghum. However, in this investigation, the carbohydrate and protein fractions of short Kaura variety are subjected to amylase and peptin hydrolysis respectively. These hydrolysates constitute the broths. Bean Husk, fish, spent yeast and soymilk are subjected to pepsin hydrolysis and these digests constitute their growth supplements.

These formulations in liquid and solid media are used to cultivate Pseudomonas aeruginosa, Escherichia coli, Bacillus Subtilis, Staphylococcus aureus and Candida albicans.

1.6 SORGHUM

Sorghum is a carbohydrate rich cereal and in some ways similar to barley, rice, wheat and maize.

Sorghum belongs to the Family Andropogaceae. Sorghum is commonly called 'guinea corn and is the fourth most important cereal crop in the world surpassed only by rice, wheat and maize respectively.

Sorghum has a relatively high level of carbohydrate with appreciable protein level which serves as an energy source for the teeming millions all over the world. (Axtell et al, 1972). Other uses of sorghum include

- a. Soft drinks which is locally called Kunu by the people of

Northern Nigeria. Kunu is rich nutritionally.

- b. Alcoholic production: Substantial amount of sorghum grains is being used in brewery industries for the production of alcoholic drinks such as Star beer, Gulder etc People of the Northern Nigeria use the grains to produce local alcoholic drinks such as pito and Burukutu.
- c. Animal Feeds: It is also used to feed domestic animals by providing the sorghum grains or their waste to the animals.
- d. Fencing: The dried sorghum plant stalks are used for making fence around houses.
- e. Pharmaceutical industry: Sorghum starch is found suitable as a binder and disintegrant in tabletting as reported by Bangudu, Garr and Akande (1988).

The West Africa variety of sorghum is cultivated in the coastal, mixed savannah in the south through the savannah region in the north. However, production is concentrated in the Guinea and Sudan Savannah zones of the sub-regions (Cutis, 1965).

In Nigeria, improved varieties with improved nutritional value have been developed by the Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria. Based on their properties and characters, the improved sorghum varieties are classified as

- i. Farafara variety
- ii. Short Kaura variety

iii. and Yar gauki variety

When harvested, sorghum can be stored for a very long time. To prevent insect attack, the grains are sprayed with chemicals such as Acetellic before storage.

1.6.1 Chemical Composition of Sorghum

The chemical composition of sorghum reveals great potentials in its ability to support bacterial growth because of the high number and variability in the chemical constituents as has been published by some workers (Miller m(1958) Matz and Kramar (1969), Eka (1971), and Axtell and Oswalt (1972).

PROXIMATE ANALYSIS OF SORGHUM GRAINS

Table 9

<u>Constituents</u>	<u>Range Percentage</u> (Dry basis)	<u>Average Percentage</u> (Dry basis)
Water (%Weight basis)	8.00-20.00	15.5
Starch	50-77	74.10
Protein	6.60-18.00	11.20
Fat (CCl ₄ extract)	1.40-6.10	3.7
Ash	1.20-7.10	2.5
Fibre (crude)	0.40-15.40	2.6
Sugars	0.50-2.50	1.8
Tannis	0.003-0.17	0.10

Reported by Miller (1958)

COMPOSITION OF SORGHUM GRAINS.

TABLE 10.
Constituents

<u>Mean Value/(Percentage basis)</u>	
Moisture	15.5%
Protein	11.2
Fat	3.7
Ash	1.5
Reducing sugars	1.8
Starch	74.1
Crude fibre	2.6
Tannin	0.1
Wax	0.3
Phosphorus	2.5

Reported by Kramar and Matz (1969)

TABLE 11: CHEMICAL COMPOSITION OF SORGHUM GRAINS IN 100gm OF EDIBLE PORTION.

<u>Constituents</u>	<u>Quantity present</u>
Energy	339 calories
Moisture	11.5%
Protein	9.8
Fat	3.1gm
Carbohydrate	75.6gm
Fibre	2.3gm
Ash	2.0gm
Calcium ions	3.0gm
Phosphorus	318mg
Iron	15.6gm
Ascabic acid	12.6iu
B-carotene	10.0iu
Thiamine	0.29mg
Riboflavin	0.28mg
Niacin	3.7iu
Tryptophan	154iu

Reported by Eka (1971)

TABLE 12: PROTEIN AND AMINO ACID CONTENT OF SORGHUM GRAINS.

<u>Constituents</u>	<u>Mean value (Percentage Basis)</u>
Protein	12.61
Lysine	2.14
Histidine	2.01
Arginine	3.59
Aspartic acid	7.83
Threonine	3.26
Serine	4.52
Glutamic acid	23.22
Proline	8.16
Glycine	3.07
Alarine	9.89
Cystine	1.50
Valine	5.35
Methiomine	1.80
Isoleucine	3.08
Tyrosine	4.50
Phenylalamine	5.19
Tryptophan	1.31

Reported by Axtell and Oswalt (1972).

1.7 AIMS AND OBJECTIVE

The aim of this investigation is to compound suitable culture media using locally available materials. This work is to explore the potentials of sorghum (short Kaura variety) in the media development.

The protein and starch extracts of sorghum will be digested and compounded into media.

Bean husk, fish, yeast and soymilk will be digested and used as growth supplements.

The media will be used both in liquid and solid forms with and without growth supplements respectively to grow Staphylococcus aureus (NCTC 6571), Bacillus subtilis (NCTC 8241), Escherichia coli (NCTC 10 418), Pseudomonas aeruginosa (NCTC 6750) and Candida albicans (ATCC 10231).

The absorbance values (OD) will be determined in static and shaken cultures. The growth curves of the test micro-organisms will be determined and compared with growth in standard media (NB).

The need for cheap, functional, convenient and available culture media in Nigeria and other developing countries has prompted the continued search for alternative media from sorghum.

CHAPTER TWO

2.0 MATERIALS AND GENERAL METHODS

2.1 The test micro-organisms

The test micro-organisms used were Staphylococcus aureus (NCTC 6571), Bacillus subtilis (NCTC 8241), Escherichia coli (NCTC 10418), Pseudomonas aeruginosa (NCTC 6750) and Candida albicans (ATCC 10231). These standard strains of bacterial and fungal species were initially obtained from National Collection of Type Cultures, London, but were directly obtained from the Laboratory stock cultures of the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria and National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. All the micro-organisms were adapted to grow on agar slope bottles and stored in the refrigerator at 2° to 8°C until required for use.

2.1.2 The Sorghum Grains

The variety of sorghum used was short Kaura (SK 5912) obtained from the Institute of Agricultural Research Ahmadu Bello University Zaria Nigeria.

2.1.3 The bean husk

The variety of beans (Vigna unguiculata) was obtained from Samaru market Zaria Nigeria.

2.1.4 The Soymilk

The soybeans (Glycine Max L) was obtained from Samaru market Zaria Nigeria.

2.1.5 The fish (Clupeid Species)

The variety of dried fish used was obtained from Samaru market, Zaria, Nigeria.

2.1.6 The spent yeast (Sacchromyces Sp)

The spent yeast was obtained from the Internatinal Beverages and Brewery Industries (IBBI) Kaduna, Nigeria.

2.1.7 Media

These include Nutrient agar (Oxoid) NA, Nutrient broth (Oxoid) NB, MacConkey agar (Oxoid) (MCA), Mannitol Salt Agar (Oxoid) MSA, and Sabourand Dextrose agar (Oxoid) SDA.

2.1.8 Colony Counter (CNW - 300 - 010m Mode)

This was manufactured by A. Gallenkamp and Co Limited London England.

2.1.9 Bioscreen C (200 wells)

For automation of absorbance values. Biolink Laboratories Finland.

2.1.10 Spectrophotometer (Unical Sp 500)

This was used for the measurement of absorbance of the various micro-organisms in various media at regular time intervals. (GallenKamp and Co Limited, London).

2.1.11 Electronic Ultra Model Spectrophotometer

This was used for the measurement and printing of the absorbance values. (Shimidu Laboratories Ltd Japan).

2.1.12 Shaker bath (6B 8528C Model)

This was used for the shaking of cultures (to effect proper mixing and aeration) at 37°C. It was also used for protein hydrolysis at 55°C for four hours. A. (Gallenkamp and Co limited, London).

2.2 GENERAL STERILISATION METHODS

Petridishes, pipettes (glass types) and other glass wares were sterilized by dry heat at 160°C for one hour. Solutions and plastic tips of automatic pipettes were sterilized by autoclaving at 121°C for 15 minutes. The inoculating loops were sterilized by flaming in an open Bunsen flame and allowed to cool before use.

2.3 Preparation of culture media and other solutions.

2.3.1 Preparation of Nutrient broth

Thirteen (13.0) grams of dehydrated nutrient (NB) broth powder was dissolved in one litre of distilled water and dispensed in 10ml volumes into 28ml McCartney bottles and 50ml volumes in 250ml Conical flasks. They were then sterilized by autoclaving at 121°C for 15 minutes and stored in a cold place until required for use.

2.3.2 Preparation of Nutrient agar

Dehydrated nutrient agar powder (28.0g) was suspended in one litre of distilled water and boiled with constant stirring to facilitate the dissolution of the agar. The dissolved solution was then dispensed in 20ml volumes into McCartney bottles, sterilized by autoclaving at 121°C for 15 minutes.

2.3.3 Preparation of MacConkey agar

Dehydrated MacConkey agar powder (51.5g) was suspended in one litre of distilled water, boiled to dissolve the agar and then dispensed in 20ml volumes into 28ml McCartney bottles. They were sterilized by autoclaving at 121°C for 15 minutes and then stored in a cold place (2°to 8°C) until required for use.

2.3.4 Preparation of mannitol salt agar

A hundred and eleven grams (111.0gm) of dehydrated mannitol salt agar powder was suspended in one litre of distilled water and boiled with constant stirring to facilitate the dissolution of the agar. The solution was then dispensed in 20ml volumes into 28ml McCartney bottles, sterilized at 121°C for 15 minutes and stored in a refrigerator until required for use.

2.3.5 Preparation of 0.03% cetrimide agar

The procedure for preparation of nutrient agar was repeated and three (3.0g) grams of cetrimide was added to one litre of nutrient agar. The sterilization and storage procedure was essentially the same with that of the nutrient agar.

2.3.6 Preparation of Sabourand Dextrose agar

Sixty-two (62.0g) grams of dehydrated powder was suspended in one litre of distilled water and similar procedure was repeated as in mannitol salt agar.

2.3.7 Preparation of normal saline (0.9%^{w/v})

Powdered sodium chloride crystal (9.0g) was dissolved in one litre of distilled water and dispensed in varied amounts in bottles and sterilized by autoclaving at 121°C for 15 minutes.

2.4 ISOLATION AND AUTHENTICATION OF THE TEST MICRO-ORGANISMS

2.4.1 Isolation *Staphylococcus aureus*

The culture of standard strain of *Staphylococcus aureus* (NCTC 6571) was subcultured into sterile nutrient broth and incubated for 18 hours at 37°C. The same procedure was repeated for two consecutive days.

From the activated culture, a loopful of 18hours culture was subculture into sterile mannitol salt agar (MSA) and nutrient broth, incubated at 37°C for 18 hours.

The following tests were carried out on the discrete colonies on mannitol salt agar.

Gram reaction:- Gram staining technique was performed on colonies obtained from MSA. Observed for Gram positive cocci in short chains was made.

Biochemical tests

- (1) Catalase test: Catalase test was performed on colonies obtained from MSA. Observed for the gas evolution was made.
- (2) Coagulase test: With sterile wire loop, colonies from MSA was introduced into 0.02ml of human serum. Observed for the coagulations of the cells was made.

2.4.2 Isolation of *Escherichia coli*

The activation of *Escherichia Coli* (NCTC 10418) was repeated the same way as for that of *Staphylococcus aureus*.

The activated culture of Escherichia coli was subcultured into sterile MCA and NB incubated at 37°C for 18 hours. The following tests were carried out on the discrete colonies obtained from MCA showing pink colouration.

- (1) Gram reaction:- Gram staining technique was performed on lactose fermenting colonies obtained from MCA and observed for Gram negative bacilli.
- (2) Catalase test: Discrete colonies obtained from MCA were introduced into 0.02ml of H₂O₂. Observed for gas evolved.
- (3) Indole test: A drop (0.02ml) of indole reagent was added into 18 hours culture in NB. Observed for pink colouration on the surface of NB.
- (4) Motility test: 0.02ml of 18 hours NB culture was placed on a cleaned slide and covered with coverslip, viewed, under the microscope. Observed for movement.

2.4.3 Isolation of Pseudomonas aeruginosa

The stock culture of Pseudomonas aeruginosa (NCTC 6750) was subcultured into sterile NB and incubated for 18 hours at 37°C. The same procedure was repeated for two consecutive days. From the activated culture, a loopful of the 18 hours culture was subcultured into 0.03% cetrimide agar, NA and NB incubated at 37°C for 18 hours.

- (1) Gram reaction: Gram staining technique was performed on colonies obtained from cetrimide agar and NA. Observed for Gram negative bacilli.
- (2) Oxidase test: Colonies obtained from cetrimide agar and NA were exposed to oxidase reagent stained filter paper. Observed for pink colouration on the filter paper.
- (3) Motility test: A drop of 18 hours NB culture was screened for motility.

2.4.4 Isolation of Bacilli subtilis

The activation procedure was repeated for the stock culture of Bacillus subtilis (NCTC 8241). The activated culture of Bacillus subtilis was subcultured into NA and NB incubated at 37°C for 18 hours. The following tests were carried out on discrete colonies obtained from NA after 18 hours incubation.

- (1) Gram reaction: Gram staining technique was performed on the colonies obtained from NA. Observed for Gram positive large bacilli in chains.
- (2) Presence of capsule: Capsule was sort for in the Gram stained bacilli.
- (3) Spore formation: On Gram stained bacilli, observation for centrally positioned spores were made.
- (4) Motility test: A drop of 18 hours NB culture on a slide and covered with coverslip and view for motility.

- (5) Pathogenicity for mouse: 0.5ml of 24 hours NB culture was inoculated subcutaneously into the thigh of a mouse. The mouse was observed for 3 days.

2.4.5 Isolation of Serratia:

The activation procedure was repeated for the stock culture of Serratia marcescens (wild strain). The activated culture of Serratia marcescens was subcultured into two plates of NA and NB. One NA and NB were incubated at 37°C for 18 hours. The other NA plate was incubated at 20°C (room temperature) for 18 hours.

Pigment formation: The discrete colonies in NA incubated at 20°C were screened for red pigment formation.

- (1) Gram reaction: Gram staining technique was performed on colonies obtained from NA plates at 20° and 37°C incubation.
- (2) Motility test: 18 hours NB culture was screened for motility.
- (3) Indole test: A drop of indole reagent was added to 18 hours NB culture. Observed for pink colour.
- (4) Oxidase test: Colonies on NA were exposed to oxidase reagent stained filter paper. Observed for pink colouration on the filter paper.

2.4.6 Isolation of Candida albicans

A sterile inoculating loop was used to transfer colonies obtained from the activated stock culture of Candida albicans (ATCC 1023) into corn meal agar and SDA and incubated at 37°C for 36 hours.

- (1) Gram reaction: Gram staining technique was performed on discrete colonies obtained from corn meal agar medium. Observed for gram positive cocci showing budding were made.
- (2) Germ tube test: With sterile loop, colonies obtained from corn meal medium was inoculated into tube containing 1.0ml of human serum and incubated at 37°C. At interval of 30 minutes, 0.02ml of the mixture was viewed under the microscope. Observation for pseudohyphae formation.

CHAPTER THREE

3. FORMULATION OF SORGHUM CULTURE MEDIA AND DETERMINATION OF THEIR GROWTH PROPERTIES

3.1 PREPARATION OF SORGHUM STARCH AND PROTEIN MATERIALS

Sorghum grains were cleaned by sieving to remove the impurities, broken seeds and foreign seeds. Two kilograms of the cleaned grains were steeped in distilled water for twenty four (24) hours.

The steeped grains were subjected to coarse grinding to remove the pericarp and free the germs from the endosperm. The germs were removed by floatation method.

The underflow mixture of fibres, starch and protein were finely ground in a blender.

The fibres were removed by sieving using calico muslin. The fine suspension were allowed to settle down for four(4) hours and water decanted.

The starch sediment was centrifuged at 9000 revolution per minute for fifteen (15) minutes. The starch sedimented first, followed by proteinous material at the uppermost layer. The proteinous material was removed mechanically using spatula. The protein and starch materials were then air dried separately at room temperature (25°C) for three(3) days.

3.1.1 Enzymatic Hydrolysis of Starch in Cereal (Baur and Alexander, 1979)

Ten grams (10.0gm) of sorghum starch was added to 100ml of distilled water and mixed with a stirrer. The mixture was heated and maintained at 70°C.

5.0ml of 0.1%, α -amylase (Hitempase) was added to the starch suspension. The suspension was stirred continuously for 30 minutes.

The temperature was lowered to 60°C and 1.0ml of glycoamylase (fungamyl) was added to the starch suspension and stirred for further 30 minutes.

The suspension was allowed to cool and centrifuged at 9000 revolution per minute for 5 minutes. The clear solution was collected into 250ml flask. The deposited starch was weighed and from it, the amount digested determined, this was converted in to glucose concentration.

(i) Test for starch: 0.1ml of the digested carbohydrate suspension was dropped on a white tile. 0.02ml of iodine solution was added.

(ii) Glucose oxidase method to estimate glucose (Trinder, 1969)

2.5ml of phenol reagent and 2.5ml of glucose oxidase were added to 0.05ml of sorghum starch suspension and 0.05ml of standard glucose solution (10mM/l). 0.05ml of distilled water as blank. The solutions were read at 510nm after 20 minutes incubation at 37°C.

3.1.2 Enzymatic hydrolysis of protein (adopted from methods in Medical Microbiology by Cruickshank et al; 1983).

Sorghum Protein hydrolysis

<u>Ingredient</u>	<u>Concentration g/l</u>
Sorghum protein	50.0gm
Pepsin	1.0gm
Conc hydrochloric acid	6.0ml
Distilled water - to -	1L

100ml of distilled water was dispensed into 250ml flask. 0.6ml of concentrated hydrochloric acid (HCL) was added to the flask. The flask was placed in water bath at 37°C for 30 minutes. 0.1gm of pepsin was added into the flask and mixed to obtain uniform suspension. 5.0gm of sorghum protein was added into the pepsin suspension in the flask. The flask was placed in water-bath shaker at 52°C for four(4) hours.

At the end of the four hours of hydrolysis, the digested sorghum protein was centrifuged at 9000 revolution per minute for 5 minutes. The supernatant was filtered using Whatman filter paper. The filtrate constituted the sorghum digest or the protein base.

3.1.2.1 Determination of Protein by Biuret method

0.1ml of the digest was added to 5.9ml of the Biuret solution. 0.1ml of the standard solution (6.0gm/L) was added to 5.9ml of Biuret solution. The solution was incubated at 37°C for 10 minutes. The solutions were read at 540nm using distilled water as control.

3.2 SOYMILK PREPARATION

Three hundred grams (300gm) of Soybeans was screened for stones and damaged seeds. The soybean was steeped in 1000ml of distilled water for 10 minutes. The seed coats were removed machanically by rubbing the seeds against the hands continuously.

The cleaned seeds were ground using wet milled blender. The fibres were removed by sieving using calico muslin. The filtrate was boiled for 10 minutes and cooled. This is soymilk.

3.3 PREPARATION OF OTHER DIGESTS

The preparation of other digest such as Bean husk digest, fish digest soymilk digest and yeast digest were essentially similar to that of the procedure for the sorghum protein digest. However, the details of the concentrations of various components of the digest varied slightly according to the material being digested. Formulation concentrations of these digests were as shown below.

(1) Bean husk hydrolysis formulation

<u>Ingredient</u>	<u>Quantity</u>
Ground dried Bean husk	50.0gm
Pepsin	1.0gm
Conc hydrochloric acid	6.0ml
Distilled water - to -	IL

(2) Fish hydrolysis formulation

<u>Ingredient</u>	<u>Conc g/l</u>
Ground dried fish	50gm
Pepsin	1.0gm
Conc hydrochloric acid	6.0ml
Distilled water - to -	IL

(3) Soymilk hydrolysis formulation

<u>Ingredient</u>	<u>Conc g/l</u>
Soymilk	50ml
Pepsin	1.0gm
Conc hydrochloric acid	6.0ml
Distilled water - to -	IL

(4) Spent yeast hydrolysis formulation

<u>Ingredient</u>	<u>conc g/l</u>
Washed spent yeast	50gm
Pepsin	1.0gm
Conc hydrochloric acid	6.0ml
Distilled water - to -	IL

3.4 PREPARATION OF COMPLETE MEDIA

(1) Formulation I BATCH I

<u>Ingredient</u>	<u>Varied concentrations (100ml)</u>						
Sorghum starch digest (ml)	05	10	15	20	25	30	40
Sorghum protein digest (ml)	95	90	85	80	75	70	60

For each formulation, the ingredients were added into 250ml

flask. The pH was adjusted to 7.4 with 0.2m Hydrochloric acid (HCl) and 0.2m NaOH, using pH meter.

The mixture was centrifuged and filtered. The clear broth was dispensed in 10ml amount into 28ml McCartney bottles and 49ml amount in 250ml flasks. The bottles and the sealed flasks were sterilized by autoclaving at 121°C for 15 minutes. The cooled broth bottles and flasks were stored in a refrigerator 2° to 8°C until required.

- (2) Formulations, II, III and IV: Details of ingredients and their proportion differed as indicated in the respective formulation tables. For these formulations the sorghum starch proportion was fixed at 5%^{w/v}.

Formulation II

<u>Ingredient</u>	<u>Concentration</u> (100ml)		
	Sorghum fish broth	Sorghum Beanbroth	Sorghum Milk broth
Sorghum starch digest (ml)	5.0	5.0	5.0
Sorghum protein digest (ml)	47.5	47.5	47.5
Fish digest (ml)	47.5	-	-
Bean digest (ml)	-	47.5	-
Soy milk digest (ml)	-	-	47.5

Formulation III

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum fish broth	Sorghum bean broth	Sorghum milk broth
Sorghum starch digest (ml)	5.0	5.0	5.0
Sorghum protein digest (ml)	35.0	35.0	35.0
Fish digest (ml)	60.0	-	-
Bean digest (ml)	-	60.0	-
Soy milk digest (ml)	-	-	60.0

Formulation IV

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum fish broth	Sorghum Bean milk	Sorghum Mil broth
Sorghum starch digest (ml)	5.0	5.0	5.0
Sorghum protein digest (ml)	60.0	60.0	60.0
Fish digest (ml)	35.0	-	-
Bean digest (ml)	-	35.0	-
Soy milk digest (ml)	-	-	35.0

- (3) Formulation VII to XI, the sorghum starch digest content was varied from 10% to 30% systematically, to determine the effect of particular fixed sorghum starch digest on varying concentration of enrichment additives on the growth patterns of the test bacterial and fungal species. Details of these contents and their proportions are as

indicated in the formulation tables. Otherwise the procedures are essentially the same as the previous formulations.

Formulation V

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	10.0	10.0	10.0
Sorghum protein digest (ml)	45.0	45.0	45.0
Fish digest (ml)	45.0	-	-
Bean digest (ml)	-	45.0	-
Soymilk digest (ml)	-	-	45.0

Formulation VI: BATCH II

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	10.0	10.0	10.0
Sorghum protein digest (ml)	30.0	30.0	30.0
Fish digest (ml)	60.0	-	-
Bean digest (ml)	-	60.0	-
Soymilk digest (ml)	-	-	60.0

Formulation VII

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	10.0	10.0	10.0
Sorghum protein digest (ml)	60.0	60.0	60.0
Fish digest (ml)	30.0	-	-
Bean digest (ml)	-	30.0	-
Soymilk digest (ml)	-	-	30.0

Formulation VIII

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	15.0	15.0	15.0
Sorghum protein digest (ml)	42.5	42.5	42.5
Fish digest (ml)	42.5	-	-
Bean digest (ml)	-	42.5	-
Soymilk digest (ml)	-	-	42.5

Formulation IX

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	20.0	20.0	20.0
Sorghum protein digest (ml)	40.0	40.0	40.0
Fish digest (ml)	40.0	-	-
Bean digest (ml)	-	40.0	-
Soymilk digest (ml)	-	-	40.0

Formulation X

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	25.0	25.0	25.0
Sorghum protein digest (ml)	37.5	37.5	37.5
Fish digest (ml)	37.5	-	-
Bean digest (ml)	-	37.5	-
Soymilk digest (ml)	-	-	37.5

Formulation XI

<u>Ingredient</u>	<u>Concentration (100ml)</u>		
	Sorghum	Sorghum	Sorghum
	Fish broth	bean broth	milk broth
Sorghum starch digest (ml)	30.0	30.0	30.0
Sorghum protein digest (ml)	35.0	35.0	35.0
Fish digest (ml)	35.0	-	-
Bean digest (ml)	-	35.0	-
Soymilk digest (ml)	-	-	35.0

3.5 DETERMINATION OF MICROBIAL GROWTH IN BROTHS

18 hours broth culture of standard strains of Staphylococcus aureus (NCTC 6571), Bacillus subtilis (NCTC 8241), Pseudomonas aeruginosa (NCTC 6750), Escherichia coli (NCTC 10418), Candida albicans (ATCC 10231) and Serratia Marcescens (wild strain) were tested.

0.02ml of overnight of each organism was used to inoculate 10ml NB and 10ml of each formulated broths. (Sorghum broth (SB), Sorghum bean broth (SBB), Sorghum yeast broth (SYB), Sorghum milk broth (SMB) and Sorghum fish broth (SFB). All the inoculated culture bottles were incubated along with its negative controls of the formulation and NB at 37°C for 18 hours.

The level of growth for each microbial species was observed after 18 hours. The result is as depicted in tables 13 and 14.

3.6 DETERMINING THE GROWTH IN BROTH MEDIA USING SPECTROPHOTOMETER

18 hours broth culture of the standard strains of Staphylococcus aureus (NCTC 6571), Bacillus subtilis (NCTC 824), Pseudomonas aeruginosa (NCTC 6750), Escherichia coli (NCTC 10418) and Candida albicans (ATCC 10231) and their respective negative control broths were used.

An electronic ultra spectrometer was adjusted to a wavelength of 540nm. NB was used to zero the optical density (OD). Similarly control sorghum broths and sorghum broth with

growth supplements were read and values recorded.

2.0ml of 18 hours static cultures of NB and formulated broths for each test organism was withdrawn into the cleaned cuvette one at a time and OD readings taken. The various readings were recorded as shown in tables 15, 16, 17 and 18.

3.7 DETERMINING OF GROWTH CURVES OF THE TEST ORGANISMS

Bioscreen C method:- Bioscreen C (200 wells) was adjusted to wavelength 540nm and ready for use.

For Nutrient broth:- Overnight broth cultures of the test organisms each was centrifuged at 3000rpm for 5 minutes. Then each pellet was aseptically resuspended in fresh NB maintained at 37°C for 20 minutes. From the subcultured broth, 2.0ml of the broth culture aseptically was transferred into 18.0ml fresh NB maintained at 37°C and placed in water bath shaker at 50rpm for 4 hours.

Meanwhile, 300ul of fresh sterile NB maintained at 37°C was dispensed into Honey comb wells in the inoculation hood. At the end of the 4 hours, shaking culture of the test organisms were withdrawn each with 50 l amount into the labelled and duplicated wells.

For formulated broths: For each test organism in the formulated broth, the procedure was essentially the same as in NB in activation, dispensing, and inoculation into labelled honey comb wells.

Obtaining readings from Bioscreen:- The inoculated 100 to 200 wells tray were transferred into the incubation section of the Bioscreen C analyser and all parameters fed into the PC software.

Readings of the OD of the sample was done at zero minute.

After each reading, the enclosed honey comb wells tray was aseptically transferred to a shaker maintained at 37°C and shaken at 20rpm at the interval of 60, 120.... 540 minutes.

The results of each OD readings were recorded. The results are shown in Appendice I, II and II.

3.8 STANDARDIZATION OF THE TEST ORGANISM

The pour plate method was used for each of the micro-organism used. A serial dilution was carried out in 9.0ml of sterile peptone water (0.1%^{w/v}) by adding 1.0ml of the culture to obtain a 1:10 dilution. This was thoroughly mixed with a vortex mixer before withdrawing another 1.0ml of the 1:10 diluted culture, transferring aseptically to another 9.0ml peptone water and mixed to get a 1:100 dilution. This process was repeated until a 1:10⁶ dilution was made. From 1:10⁶ dilution 1:10⁸, 1:10¹⁰, 1:10¹², 1:10¹⁴, 1:10¹⁶, 1:10¹⁸, and 1:10²⁰ dilutions were made depended on the turbidity 1.0ml of the final dilution was transferred into 19.0ml melted NA at about 40° to 45°C in McCartney bottle. This was then rubbed within the palm and poured into sterile plate. The plate was then rocked gently to effect a good dispersion of the organisms.

This was then allowed to cool and solidify. All inoculated plates were incubated at 37°C for 24 hours and the number of the colonies observed were counted using the colony counter. The number of viable cells on colony forming units (cfu) per ml of the culture was determined as shown in tables 22 and 23.

3.9 SOLIDIFYING THE FORMULATED BROTHS

Agar-agar was added into each of the best growth supporting formulation on the test organisms. Their respective colonial morphology and changes in the medium exhibited by the test organisms were observed.

3.10 STATISTICAL ANALYSIS

Statistical analysis was carried out with analysis of variance (ANOVA) which examined the variations within the whole group of sample means computer.

CHAPTER FOUR

4.0 RESULTS

4.1 ISOLATION AND AUTHENTICITY OF THE TEST ORGANISMS

4.1.1 Staphylococcus aureus

The strain of Staphylococcus aureus (NCTC 6571) was non-motile. The colonies obtained on MSA were yellowish with surrounding yellow zones. The strain showed catalase and coagulase positive. The organisms were Gram positive cocci with short chains or clusters.

4.1.2 Escherichia coli

The strain of Escherichia coli (NCTC 10418) was rod-shaped, motile and Gram negative bacterium. They produced pink colonies with diffuse zones surrounding them on MCA medium. The strain was catalase, indole, methyl red positive and produced acid and gas in Lactose fermentation test.

4.1.3 Pseudomonas aeruginosa

The strain of Pseudomonas aeruginosa (NCTC 6750) was rod shaped, non-sporing Gram negative bacterium. The colonies obtained on 0.03%^{w/v} cetrimide agar were off-white in colour and compact. They were catalase and oxidase positive

4.1.4 Bacillus subtilis

The strain of Bacillus subtilis (NCTC 8241) was large rod shaped in chains and non-capsulated Gram positive bacillus. They were motile and spore centrally positioned.

4.1.5 Serratia marcescens (wild strains)

The strain of Serratia marcescens produced pigment (red) on NA at 20°C. They were motile and Gram negative coccobacilli. They were indole and oxidase negative.

4.1.6 Candida albicans

The strain of Candida albicans (ATCC 10231) was concave shaped colonies on SDA. They gave Gram positive large cocci with budding forms showed. They gave positive germ tube test.

4.1.7 Test for the presence of starch

The result showed yellow colouration which indicated absence of starch in the digested sorghum starch suspension.

4.1.8 Determination of sorghum starch digest

The starch digest was found to contain 516.8mg/l of glucose.

4.1.9 Determination of protein digest

<u>Digested protein</u>	<u>Concentration (g/l)</u>
Sorghum	3.0
Bean husk	6.0
Soymilk	4.5
Fish	10.50
Nutrient broth	6.0

4.2 DETERMINATION OF MICROBIAL GROWTH IN BROTHS

After 18 hours static incubation at 37°C of the growth Staphylococcus aureus (NCTC 6571) Bacillus subtilis (NCTC 8241), Pseudomonas aeruginosa (NCTC 6750), Escherichia coli (NCTC 10418), Serratia marcescens (wild strain) and Candida albicans (ATCC 10231) in both NB and formulated broths were observed. It was observed that there was turbidity in all the cultures of the broths. These results are shown in tables 13 and 14. There was matching of controls of NB, sorghum broth and sorghum broth with growth supplements with the overnight growth culture of each organism by reading printed letters through the suspensions.

Table 3 illustrates the various degree of visualized growths of test micro-organisms after 18 hours static incubation. In table 13, two of the formulations namely 15% and 20% sorghum broths vigorously support the growth of the test micro-organisms and perform as good as NB. 10% sorghum broth supported the growth of the test micro-organisms better

than 5%, 25%, 30% and 40% sorghum broths. Generally, there was better performance of Candida albicans in all the formulations than NB.

TABLE 13: VISUALIZED GROWTH OF ESCHERICHIA COLI (NCTC 10418), STAPHYLOCOCCUS AUREUS (NCTC 6571), PSEUDOMONAS AERUGINOSA (NCTC 6750), BACILLUS SUBTILIS (NCTC 8241), SERRATIA MARCESCENS (WILD STRAIN) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND SORGHUM BROTHS, STATICALLY INCUBATED AT 37^{OC} FOR 18 HOURS

SB Media +	SSD SPD (ML)	<u>Esch</u> <u>Coli</u>	<u>Staph</u> <u>aureus</u>	<u>Pseud</u> <u>aeruginosa</u>	<u>Bac</u> <u>Subtilis</u>	<u>Serratia</u> <u>Sp</u>	<u>Candida</u> <u>albicans</u>
5%SB	5 : 95	+	++	+	++	++	+++
10%SB	10 : 90	++	++	++	+++	+++	+++
15%SB	15 : 85	+++	+++	+++	+++	+++	+++
20%SB	20 : 80	+++	+++	+++	+++	+++	+++
25%SB	25 : 75	+	++	+	+++	+++	+++
30%SB	30 : 70	++	+++	++	+++	++	+++
40%SB	40 : 60	++	++	++	++	++	+++
NB	-	+++	+++	+++	+++	+++	++

Key

+++ - vigorous growth
 ++ - moderate growth
 + - scanty growth
 SB - Sorghum broth
 NB - Nutrient broth
 *SSD - Sorghum starch digest
 SPD - Sorghum protein digest
 + - See appendix I

TABLE 14: VISUALIZED GROWTH OF ESCHERICHIA COLI (NCTC 10418) STAPHYLOCOCCUS AUREUS (NCTC 6571), PSEUDOMONAS AERUGINOSA (NCTC 6750) BACILLUS SUBTILIS (NCTC 8241) SERRATIA MARCESCENS (WILD STRAIN) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND ENRICHED SORGHUM BROTHS STATICALLY INCUBATED AT 37°C FOR 18 HOURS.

Media	SSD: SPD: GS (ML)	Esch. Coli	Staph aureus	Pseud aeruginosa	Bacillus subtilis	Serratia Sp	Candida albicans
SMB	5 : 47.5:47.5	+++	+++	+++	+++	+++	+++
SFB	5 : 47.5:47.5	+++	+++	+++	+++	+++	+++
SYB	5 : 47.5:47.5	++	++	+	++	++	+
SBB	5 : 47.5:47.5	+++	+++	++	+++	+++	++
NB		+++	+++	+++	+++	+++	++

Key

+++	-	vigorous growth	SMB	Sorghum milk broth
++	-	moderate growth	SFB	Sorghum fish broth
+	-	scanty growth	SYB	Sorghum yeast broth
			SBB	Sorghum bean broth
			NB	Nutrient broth

Table 14 depicts the growth of test micro-organisms in sorghum broths enriched with soymilk fish, yeast and bean husk digests. Sorghum broth enriched with fish and soymilk digest were the highest growth supporting media when compared with NB and other formulations. The performance of sorghum bean broth was poorer than that of NB. The least growth supporting medium was sorghum yeast broth.

TABLE 15: ABSORBANCE (OD) VALUES FOR ESTHERICHA COLI (NCTC 10418), STAPHYLOCOCCUS AUREUS (NCTC 6571), PSEUDOMONAS AERUGINOSA (NCTC 6750), BACILLUS SUBTILIS (NCTC 8241) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND SORCHUM BROTHS, STATICALLY INCUBATED AT 37°C FOR 18 HOURS AT 540NM

Media	Blank (OD)	<u>Esch Coli</u>	Staph aureus	Pseud aeruginosa	Bacillus subtilis	Candida albicans
5%SB	0.012	0.218	0.356	0.325	0.422	0.905
10%SB	0.012	0.498	0.520	0.388	0.510	1.120
15%SB	0.013	0.590	0.551	0.389	0.571	1.230
20%SB	0.013	0.740	0.520	0.411	0.375	1.326
25%SB	0.013	0.322	0.438	0.278	0.551	1.316
30%SB	0.014	0.342	0.660	0.315	0.457	1.292
40%SB	0.016	0.379	0.345	0.242	0.384	1.253
NB	0.040	0.702	0.670	0.650	0.478	0.487

Key

SB - Sorghum broth
 NB - Nutrient broth
 SSD - Sorghum starch digest
 SPD - Sorghum protein digest

Table 15 illustrates the OD of the test organisms in formulations and NB incubated (static) at 37°C for 18 hours. The following observation was made after 18 hours. The growth of Escherichia coli in 20% sorghum broth (0.740) compared with NB (0.702), therefore the 20% sorghum broth performed better than NB in the growth supporting ability of Escherichia coli.

Staphylococcus aureus in 30% sorghum broth (0.660) compared with NB (0.670). The growth of Staphylococcus aureus in 30% sorghum broth was equally as good when compared with NB.

The growth of Bacillus subtilis in 15% sorghum broth (0.571) when compared with NB (0.478) showed better growth supporting

ability than NB.

The growth of Candida albicans in all the formulations was better than Nutrient broth.

The absorbance values Candida albicans in 20% sorghum broth (1.326) was better than Nutrient broth (0.478).

Table 16 depicts the growth of test micro-organisms in NB and sorghum broth enriched with varied concentrations of growth supplements, the following observation was made.

The growth of Escherichia coli in sorghum broth with 43% fish digest (0.803), 38% fish digest (0.792) and 48% fish digest (0.789) showed significant growth when compared with NB (0.489). These enriched sorghum media showed better growth supporting ability than NB.

The growth of Staphylococcus aureus in Sorghum broth enriched with 43% fish digest (0.835) was the best closely followed by 48% fish digest (0.809) and 38% fish digest (0.748) when compared with growth in NB (0.530).

The growth of Pseudomonas aeruginosa in Sorghum broth supplemented with 45% bean digest (0.513) was significantly poor when compared with growth in NB (0.601).

The growth of Bacillus subtilis in Sorghum broth enriched with 48% milk digest (0.561) was better when compared with NB (0.337). Candida albicans also performed better growth wise in the supplemented sorghum media than Nutrient broth. Sorghum broth supplemented with 38% fish digest (1.300) being the best when compared with NB (0.391) which was also the least growth supporting

medium.

TABLE 16: ABORBANCE (OD) VALUES FOR ESCHERICHIA COLI (NCTC 10418) STAPHYLOCOCCUS AUREUS (NCTC 6571) PSEUDOMONAS AERUGINOSA (NCTC 6750), BACILLUS SUBTILIS (NCTC 8241) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND ENRICHED SORGHUM BROTHS INCUBATED (STATIC) AT 37°C FOR 18 HOURS AT 540NM.

+Media	Conc(100ml) SSD : SPD : GS	Esch Coli	Staph aureus	Pseud aeruginosa	Bacillus subtilis	Candida albicans
5%SBB 0.916	5 : 47.5 : 47.5		0.559 0.600	0.469	0.457	
10%SBB	10 : 45 : 45	0.661	0.532	0.513	0.543	0.708
15%SBB	15 : 42.5 : 42.5	0.640	0.625	0.444	0.471	0.852
20%SBB	20 : 40 : 40	0.723	0.615	0.376	0.459	0.840
25%SBB	25 : 37.5 : 37.5	0.581	0.642	0.369	0.518	0.903
30%SBB	30 : 35 : 35	0.698	0.573	0.325	ND	0.837
5%SMB	5 : 47.5 : 47.5	ND	0.625	0.322	0.561	0.896
10%SMB	10 : 45 : 45	ND	0.586	0.313	ND	1.086
15%SMB	15 : 42.5 : 42.5	0.567	0.498	0.380	0.464	0.928
20%SMB	20 : 40 : 40	0.409	0.400	0.222	0.249	ND
25%SMB	25 : 37.5 : 37.5	0.470	0.386	0.452	ND	0.707
30%SMB	30 : 35 : 35	0.376	0.469	0.271	0.292	0.931
5%SFB	5 : 47.5 : 47.5	0.789	0.809	0.393	ND	0.910
10%SFB	10 : 45 : 45	ND	0.712	0.263	0.317	1.169
15%SFB	15 : 42.5 : 42.5	0.803	0.835	0.449	0.282	0.857
20%SFB	20 : 40 : 40	ND	ND	0.249	ND	1.300
25%SFB	25 : 37.5 : 37.5	0.792	0.748	0.364	0.356	0.683
30%SFB	30 : 35 : 35	0.533	0.544	ND	0.238	0.700
NB		0.489	0.530	0.601	0.337	0.391

Key: SBB - Sorghum bean broth ; SMB - Sorghum milk broth
 SFB - Sorghum fish broth NB - Nutrient broth
 SSD - Sorghum starch digest; SPD - Sorghum protein digest
 GS - Growth supplement ND - Not Done
 +Blank (OD) values: SBB (0.175); SMB (0.039); SFB (0.032) NB (0.039).

relatiaon to protein content
 e.g 15% SFB = 43%FD.

TABLE 17: ABSORBANCE (OD) VALUES FOR ESCHERICHIA COLI (NCTC 10418) STAPHYLOCOCCUS AUREUS (NCTC 6571), PSEUDOMONAS AERUGINOSA (NCTC 6750), BACILLUS SUBTILIS (NCTC 8241) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND ENRICHED SORGHUM (SSD FIXED AT 5%) BROTHS INCUBATED (STATIC) AT 37°C FOR 18HOURS AT 540NM

Media	Conc(100ml)		Esch	Staph	Pseud	Bacillus	Candida
	SSD : SPD : GS		Coli	aureus	aeruginosa	subtilis	albicans
35%SBB	5 : 60 : 35		0.691	0.672	0.451	0.726	0.844
48%SBB	5 : 47.5 : 47.5		0.787	0.625	0.365	0.672	1.048
60%SBB	5 : 35 : 60		0.696	0.699	0.455	0.648	1.029
35%SMB	5 : 60 : 35		0.787	0.765	0.473	0.877	1.080
48%SMB	5 : 47.5 : 47.5		1.029	0.833	0.546	0.894	1.043
60%SMB	5 : 35 : 60		0.946	0.919	0.701	0.984	1.060
35%SFB	5 : 60 : 35		0.881	0.699	ND	0.406	1.080
48%SFB	5 : 47.5 : 47.5		0.851	0.819	0.461	0.476	1.138
60%SFB	5 : 35 : 60		0.930	0.774	0.423	0.472	0.868
NB	-		0.740	0.667	0.799	0.477	0.487

Key:

SBB - Sorghum bean broth; SMB - Sorghum milk broth
 SFB - Sorghum fish broth; NB - Nutrient broth
 SSD - Sorghum starch digest; SPD - Sorghum protein digest
 GS - Growth supplement ND - Not done

+ Blank (OD) value SBB (0.174); SMB (0.037); SFB (0.033) ; NB (0.040).

Table 17 compares the growth of test organisms in NB and sorghum broth enriched with varied concentrations of growth supplements, the following observation was made;

The growth of Escherichia coli in sorghum broth enriched with 48%, milk digest (1.029) compared with NB (0.740), the formulation performed best. The growth of Escherichia coli in other formulations such as 60% sorghum milk broth (0.946) and 60% sorghum fish broth (0.930) were even higher than growth in NB.

The growth of Staphylococcus aureus in sorghum broth enriched with 60% milk digest (0.919) was the best, followed closely by 48% sorghum milk broth (0.833) and 48% sorghum fish broth (0.819) when compared with NB (0.667).

The growth of Pseudomonas aearuginosa in sorghum broth enriched with 60% milk digest (0.701) was poor when compared with NB (0.799).

The growth of Bacillus subtilis in 60% sorghum milk broth (0.984) was better than Nutrient broth (0.477).

The growth of Candida albicans was good enough in all the formulations. The formulation enriched with 48% fish digest (1.138) gave the highest growth.

TABLE 18: ABSORBANCE VALUE (OD) FOR ESCHERICHIA COLI (NCTC 10418), STAPHYLOCOCCUS AUREUS (NCTC 6571), PSEUDOMONAS AERUGINOSA (NCTC 6750), BACILLUS SUBTILIS (NCTC 8241) AND CANDIDA ALBICANS (ATCC 10231) IN NUTRIENT AND ENRICHED SORGHUM (SSD FIXED AT 10%) BROTHS INCUBATED (STATIC) AT 37°C FOR 18 HOURS AT 540NM.

⁺ Media	Conc(100ml) SSD : SPD : GS	<u>Esch</u> <u>Coli</u>	<u>Staph</u> <u>aureus</u>	<u>Pseud</u> <u>aeruginosa</u>	<u>Bacillus</u> <u>subtilis</u>	<u>Candida</u> <u>albicans</u>
30%SBB	10 : 60 : 30	0.629	0.995	0.981	0.911	1.148
45%SBB	10 : 45 : 45	0.783	0.805	0.502	0.670	0.823
60%SBB	10 : 30 : 60	0.890	0.978	0.695	0.914	1.352
30%SMB	10 : 60 : 30	0.843	0.722	1.090	0.807	1.038
45%SMB	10 : 45 : 45	0.810	0.753	0.728	0.790	1.007
60%SMB	10 : 30 : 60	0.775	0.721	0.627	0.883	1.053
30%SFB	10 : 60 : 30	0.775	0.800	ND	0.543	0.971
45%SFB	10 : 45 : 45	ND	0.890	0.433	0.531	0.838
60%SFB	10 : 30 : 60	1.112	1.048	0.618	0.609	1.087
NB	-	0.729	0.687	0.853	0.543	0.690

Key

SBB - Sorghum bean broth; SMB - Sorghum milk broth
SFB - Sorghum fish broth; NB - Nutrient broth
SSD - Sorghum starch digest; SPD - Sorghum protein digest
GS - Growth supplement ND - Not done

+ Blank (OD) value; SBB (0.174); SMB (0.036); SFB (0.032) NB(0.030)

Table 18 shows the absorbance (OD) values of the test organisms at a fixed 10% sorghum starch digest and varied concentrations of growth supplements such as bean, fish and milk digests. The following observations was made:

The growth of Eschericia coli in two sorghum broths enriched with 60% fish digest (1.112) and 60% bean digest (0.890) gave better growth than NB (0.729).

Similarly, the growth of Staphylococcus aureus in sorghum broth supplemented with 60% fish digest (1.048) and 30% bean digest (0.995) gave better growth than NB (0.687).

The growth of Pseudomonas aeruginosa in sorghum broth enriched with 30% milk digest (1.090) and 30% bean digest (0.981) gave better growth than NB (0.853).

The growth of Bacillus subtilis in NB (0.543) showed significantly poor growth when compared the growth in sorghum broth enriched with 60% bean digest (0.914) and 30% bean digest (0.911).

The growth of Candida albicans in all the formulations were best with sorghum broths enriched with 60% beana digest (1.352) was the best followed by 30% bean digest (1.148). Growth in Nutrient broth (0.690) was the worst.

4.3 TABLE 19A: ANALYSIS OF VARIANCE

Source of variation	Degree of freedom	Sum of squares	Mean	F	P.>F
Model	12	2.39539857	0.19961655	29.60	0.0001
Error	92	0.62050962	0.00674467		
Total	104	3.01590819			
Nutrient	2	0.30361156	0.15180578	22.51	0.001
Organisms	4	0.01282429	0.00320607	0.48	0.7537
Time	6	2.07896272	0.34649379	51.32	0.0001

TABLE 19B: LEVEL OF GROWTH SUPPORTING ABILITY OF THE SORGHUM AND NUTRIENT BROTHS.

Media	Mean	Grouping
Nutrient broth	0.4290	A
20% Sorghum broth	0.3719	B
15% Sorghum broth	0.2972	C

TABLE 19C: THE LEVEL OF GROWTH PERFORMANCE OF EACH TEST MICRO-ORGANISMS

Micro-organisms	Statistic rating Mean	Grouping
<u>Escherichia coli</u>	0.3787	A
<u>Staphylococcus aureus</u>	0.3740	A
<u>Pseudomonas aeruginosa</u>	0.3705	A
<u>Candida albicans</u>	0.3599	A
<u>Bacillus subtilis</u>	0.3479	A

TABLE 20A: ANALYSIS OF VARIANCE

Source of variations	Degree of freedom	Sum of squares	Mean squares	F	Pr>R
Model	27	18.02983129	0.66777153	61.4	0.0001
Error	602	6.58627297	0.01094065		
Total	629	24.61610426			
Nutrient	17	5.35945369	0.31526198	28.82	0.0001
Organism	4	1.93814228	0.48453557	44.29	0.0001

TABLE 20B: LEVEL OF GROWTH SUPPORTING ABILITY OF ENRICHED SORGHUM AND NUTRIENT BROTHS

Media	Conc(100ml)	Statistical	Grouping
	SSD : SPD : ES	rating	
48%SFB	5 : 47.5 : 47.5	0.4491	A
NB	-	0.4290	A
43%SFB	15 : 42.5 : 42.5	0.4170	A,B
35%SFB	30 : 35 : 35	0.3745	B
20%SB	20 : 80 : -	0.3719	B
15%SB	15 : 85 : -	0.2977	C
40%SFB	20 : 40 : 40	0.2825	C,D
48%SMB	5 : 47.5 : 47.5	0.2584	C,D,E
43%SBB	15 : 42.5 : 42.5	0.2405	D,E
45%SMB	10 : 45 : 45	0.2340	D,E
48%SBB	5 : 47.5 : 47.5	0.2309	E
40%SBB	20 : 40 : 40	0.2133	E,F
43%SMB	15 : 42.5 : 42.5	0.2132	E,F
45%SBB	10 : 45 : 45	0.2112	E,F
40%SMB	20 : 40 : 40	0.1812	F,G
35%SMB	30 : 35 : 35	0.1777	F,G
35%SBB	30 : 35 : 35	0.1679	F

KEY NB Nutrient broth; SFB sorghum fish broth
SMB: Sorghum milk broth; SBB Sorghum bean broth

TABLE 20C: THE LEVEL OF GROWTH PERFORMANCE OF EACH TEST MICRO-ORGANISMS

Micro-organism	Statistica rating	Grouping
<u>Candida albicans</u>	0.3364	A
<u>Escherichia coli</u>	0.3098	B
<u>Staphylococcus aureus</u>	0.3033	B
<u>Bacillus subtilis</u>	0.2063	C
<u>Pseudomonas aeruginosa</u>	0.2049	C

TABLE 21A: ANALYSIS OF VARIANCE

Source of Variations	Degree of freedom	Sum of squares	Mean squares	F	Pr>F
Model	20	21.67935604	1.08396780	68.67	0.0001
Error	379	5.982271746	0.01578553		
Total	399	27.66207350			
Nurtrient	9	1.2154667	0.13505074	8.56	0.0001
Organism	4	3.44807949	0.86201987	54.61	0.0001

TABLE 21B: LEVEL OF GROWTH SUPPORTING ABILITY OF FORMULATIONS COMPARED WITH NUTRIENT BROTH

Media	Statistical rating Value	Grouping
60%SFB	0.4321	A
NB	0.4172	A
35%SFB	0.3906	A,B,C
35%SMB	0.3625	B,C,D
35%SBB	0.3435	D,E
28%SBB	0.3174	D,E
60%SBB	0.2835	E
28%SMB	0.2808	E
60%SMB	0.2658	E

Key

NB Nutrient broth, SFB sorghum fish broth SMB - sorghum milk broth, SBB = Sorghum

TABLE 21C: THE LEVEL OF GROWTH PERFORMANCE OF EACH TEST MICRO-ORGANISM

Micro-organism	Statistical rating	
	Mean value	Groupping
<u>Candida albicans</u>	0.5009	A
<u>Escherichia coli</u>	0.3732	B
<u>Staphylococcus aureus</u>	0.3382	B
<u>Bacillus subtilis</u>	0.2932	C
<u>Pseudomonas aeruginosa</u>	0.2214	D

The statistical analysis of the data (appendix II) is shown in Tables 19A and 19B indicate that Nutrient broth (0.4290) had better growth supporting ability of the test microorganisms when compared with 20% Sorghum broth (0.3719) and 15% Sorghum broth (0.2977). Based on the groupings, Nutrient broth belongs to group A while 20% and 15% sorghum broths belong to B and C respectively.

The Nutrient broth showed the best growth supporting medium, followed by 20% and 15% sorghum broths respectively.

Table 19C indicates the growth performance of the test micro-organisms in the media, Escherichia coli (0.3787) grew best in the media, Staphylococcus aureus (0.3740) was next. Bacillus subtilis grew next to Staphylococcus aureus. All the test micro-organisms grew well in Nutrient broth (A) and sorghum broths (A).

The statistical analysis of data (appendix III) is shown in Tables 20A and 20B indicate that the 48% sorghum fish broth (0.4491) was the best growth supporting medium when compared with Nutrient broth (0.4290). 43%, Sorghum fish broth (0.4170) showed better growth medium than 35% Sorghum fish broth (0.3745) and 20% sorghum broth (0.3719).

For the grouping of the formulations and Nutrient broth, 48% Sorghum fish broth (A) and Nutrient broth (A) showed Best growth

significantly while growth in 43%, 35% sorghum fish broths and 20% sorghum broth were significant though slightly poorer than the Nutrient broth.

Table 20C indicates that Candida albicans (0.3364) was the best grown species in the formulations and Nutrient broth. Staphylococcus aureus (0.3033) was next to Escherichia coli (0.3098). The growth of Pseudomonas aeruginosa (0.2049) was the least to grow well in the media.

Based on the grouping which indicate the level of growth performance, Candida albicans (A) and Staphylococcus aureus (B). While Bacillus subtilis (C) and Pseudomonas aeruginosa (C) were the least.

The statistical analysis of data (appendix IV) is shown in Tables 21A and 21B indicate that sorghum broth enriched with 60% fish digest (0.4321) gave better growth than Nutrient broth (0.4172) as growth supporting medium. 35% sorghum milk broth (0.3906) was equally a good medium.

Based on grouping of the level of performance of each medium on test micro-organisms, 60% sorghum fish broth (A), Nutrient broth (A) and 35% sorghum fish broth (A,B and C) showed the same level of media performance.

Table 21C indicates the level of growth performance of the test micro-organisms on the formulations and Nutrient broth. The growth of Candida albicans (0.5009) was best while Escherichia coli (0.3732) was next best. The growth of Pseudomonas aeruginosa (0.2214) was the least in the media.

The growth of Candida albicans (A), Escherichia coli (B) and staphylococcus aureus (B) gave the order of growth performance.

Figure I illustrate the growth curves of Escherichia coli in NB, 60% and 35% Sorghum fish broths. A slight ag phase was observed in all the growth curves. The log phase commenced in all the media after one hour and continued until after 3 hours. The decline phase set in immediately. The mean generation time of Escherichia coli in Nutrient broth (60 minutes) and 60% sorghum fish broth (60 minutes) were the same and slight different in 35% sorghum fish broth (66 minutes).

Figure 2 illustrates the growth curves of Staphylococcus aureus in Nutrient broth, 60% and 35% sorghum fish broths. The growth curve followed the same pattern as that of Escherichia coli. The mean generation time of Staphylococcus aureus was highest in 35% sorghum fish broth (69 minutes), followed by Nutrient broth (63 minutes) and 60% sorghum fish broth (60 minutes).

Figure 3 illustrates the growth curves of Pseudomonas aeruginosa in Nutrient broth, 60% and 35% sorghum fish broths. The mean generation time of Pseudomonas aeruginosa in all the media were high with 35% sorghum fish broth (81 minutes) being the highest followed by Nutrient broth (72 minutes) and the least was in 60% sorghum fish broth (69 minutes).

Figure 4 illustrates the growth curves of Bacillus subtilis in Nutrient broth, 60% and 35% sorghum fish broths. The mean generation time of Bacillus subtilis in Nutrient broth (54 minutes) was the lowest when compared with 60% (75 minutes) and 35% (78

minutes) sorghum fish broths respectively.

Figure 5 illustrates the growth curves of Candida albicans in Nutrient broth, 60% and 35% sorghum fish broths. The mean generation time of Candida albicans in Nutrient broth (78) minutes) was the highest compared to 35% (75 minutes) and 60% (69 minutes) sorghum fish broths.

Table 22 illustrates the populations of Staphylococcus aureus at different absorbance values with time in Nutrient broth, 20% sorghum broth and 60% sorghum fish broth. The population of Staphylococcus aureus at absorbance values of 0.02 (Nutrient broth), 0.06 (20% sorghum broth) and 0.4 (sorghum fish broth) at zero hour were 2.63×10^4 , 1.01×10^5 and 7.21×10^4 cfu/ml respectively. At 5 hours growth period, the absorbance values of 0.82 (Nutrient broth), 0.58 (20% sorghum broth) and 1.24 (sorghum fish broth) were obtained and the population of Staphylococcus aureus were 3.9×10^{10} , 2.23×10^{10} and 6.3×10^{12} cfu/ml respectively. The highest population of Staphylococcus aureus was obtained from 60% sorghum fish broth, followed by Nutrient broth and the least was 20% sorghum broth.

Table 23 illustrates the populations of Escherichia coli at different absorbance values with time in Nutrient broth, 20% sorghum broth and 60% sorghum fish broth. The populations of Escherichia coli at one hour with the absorbance values of 0.09 (Nutrient broth), 0.16 (20% sorghum broth) and 0.107 (60% sorghum fish broth) were 3.2×10^7 , 4.07×10^9 and 1.77×10^8 cfu/ml respectively. The population of Staphylococcus aureus in 20%

sorghum broth had doubled after one hour period of growth.

At 5 hours growth period, the absorbance values were obtained as 0.84 (Nutrient broth), 0.60 (20% sorghum broth) and 0.83 (60% sorghum fish broth) which gave the population of Escherichia coli as 7.76×10^{19} , 3.23×10^{16} and 5.24×10^{19} cfu/ml respectively. There was no significant difference between the populations of Escherichia coli in Nutrient and 60% sorghum fish broths.

Figures 6 and 7 show the typical growth curves of log cells count/ml against time for Staphylococcus aureus and Escherichia coli.

TABLE 22: DETERMINATION OF CHANGE IN ABSORBANCE VALUES WITH TIME
FOR STAPHYLOCOCCUS AUREUS IN NUTRIENT, 20% SORGHUM AND 60%
SORGHUM FISH BROTHS.

22.1 NUTRIENT BROTH

TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.02	2.63×10^4	4.42
1	0.059	3.02×10^6	6.48
2	0.20	1.5×10^{10}	10.18
3	0.59	5.01×10^{16}	16.70
4	0.74	7.9×10^{18}	18.90
5	0.82	3.9×10^{19}	19.59

22.2 20% SORGHUM BROTH

TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.06	1.01×10^5	5.004
1	0.15	5.24×10^9	9.72
2	0.26	1.58×10^{11}	11.19
3	0.38	2.51×10^{13}	13.40
4	0.54	2.57×10^{15}	15.41
5	0.58	2.23×10^{16}	16.35

22.3 60% SORGHUM FISH BROTH

TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.04	7.21×10^4	4.86
1	0.06	1.41×10^5	5.15
2	0.11	1.31×10^9	9.12
3	0.54	1.99×10^{15}	15.30
4	1.20	2.5×10^{22}	22.40
5	1.24	6.3×10^{22}	22.80

TABLE 23: DETERMINATION OF CHANGE IN ABSORBANCE VALUES WITH TIME FOR ESCHERICHIA COLI IN NUTRIENT, 20% SORGHUM AND 60% SORGHUM FISH BROTHS

23.1 NUTRIENT BROTH

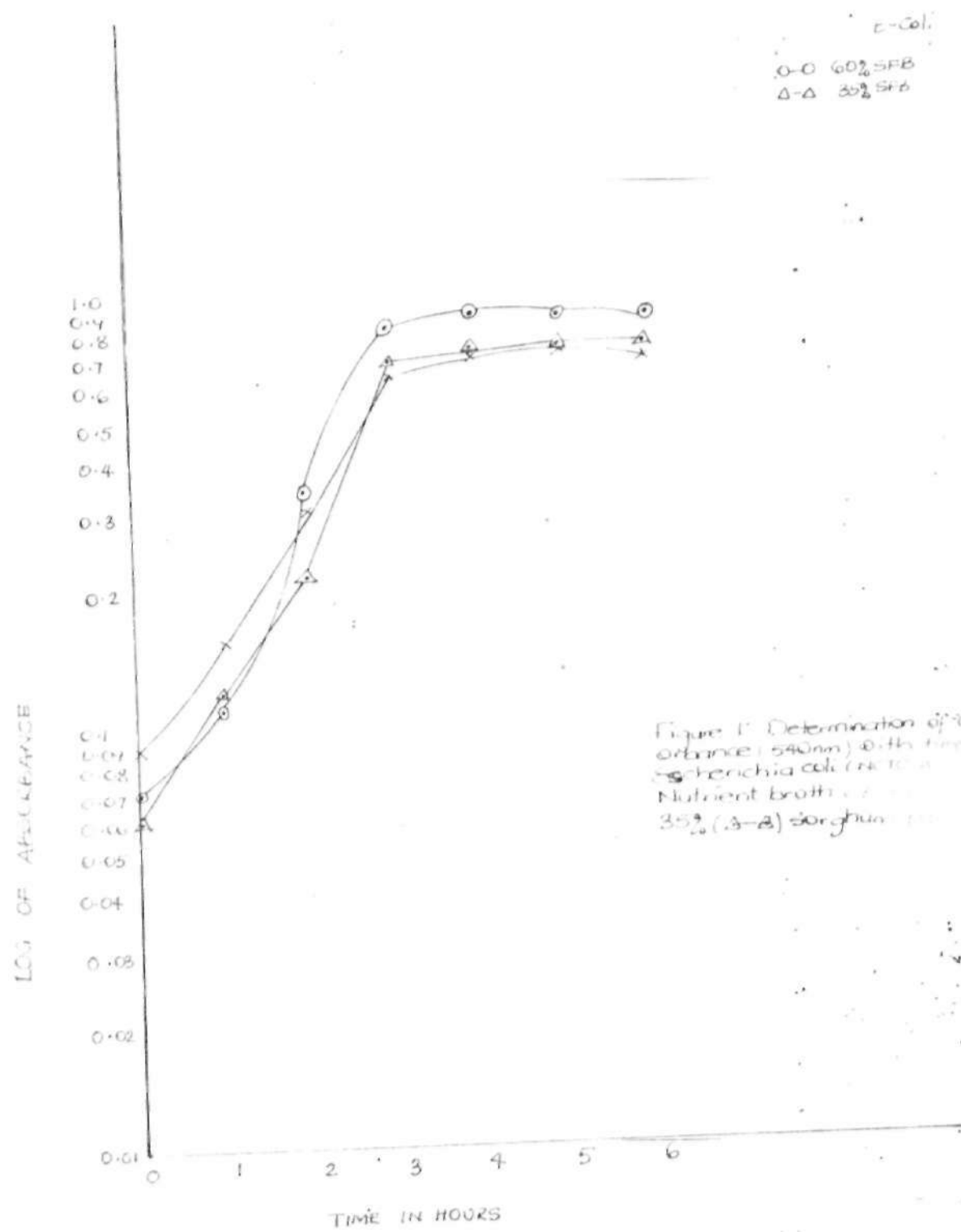
TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.02	1.2×10^4	4.08
1	0.09	3.2×10^7	7.51
2	0.30	3.16×10^{11}	11.50
3	0.50	6.31×10^{14}	14.80
4	0.74	1.58×10^{18}	18.20
5	0.84	7.76×10^{19}	19.89

23.2 20% SORGHUM BROTH

TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.05	1.3×10^5	5.13
1	0.16	4.07×10^6	9.61
2	0.29	3.98×10^{11}	11.60
3	0.48	6.31×10^{14}	14.80
4	0.58	2.02×10^{16}	16.31
5	0.60	3.23×10^{16}	16.51

23.3 60% SORGHUM FISH BROTH

TIME (HOURS)	OPTICAL DENSITY	CELLS COUNT/ML	LOG CELLS COUNT/ML
0	0.05	2.11×10^5	5.32
1	0.07	1.77×10^8	8.25
2	0.22	4.46×10^{10}	10.65
3	0.54	1.62×10^{16}	16.21
4	0.72	1.54×10^{19}	19.19
5	0.83	5.2×10^{19}	19.72



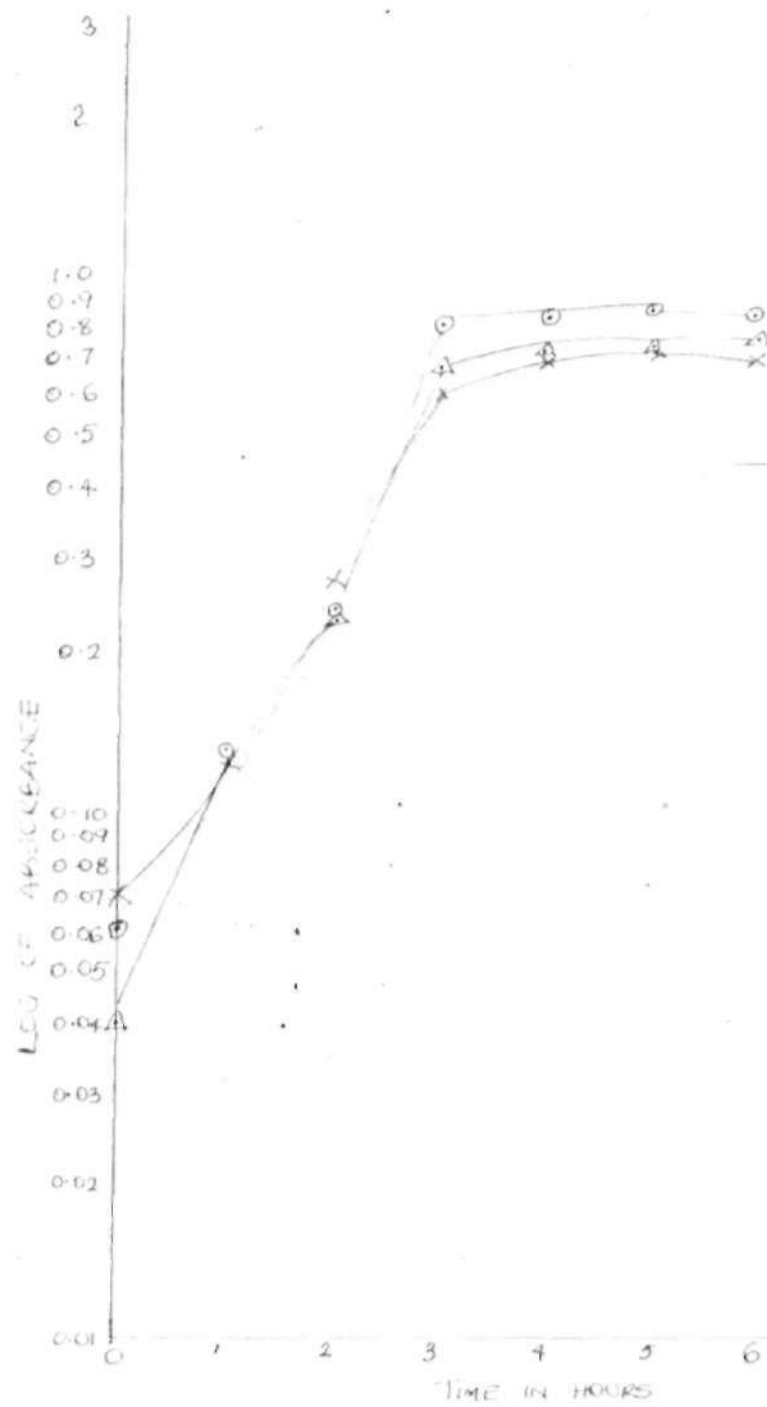


Figure 2: Determination of change in absorbance (540nm) with time of *Staphylococcus aureus* (ATCC 6511) growing in Nutrient broth (x-x), 60% (o-o) and 35% (delta-delta) sorghum broths respectively.

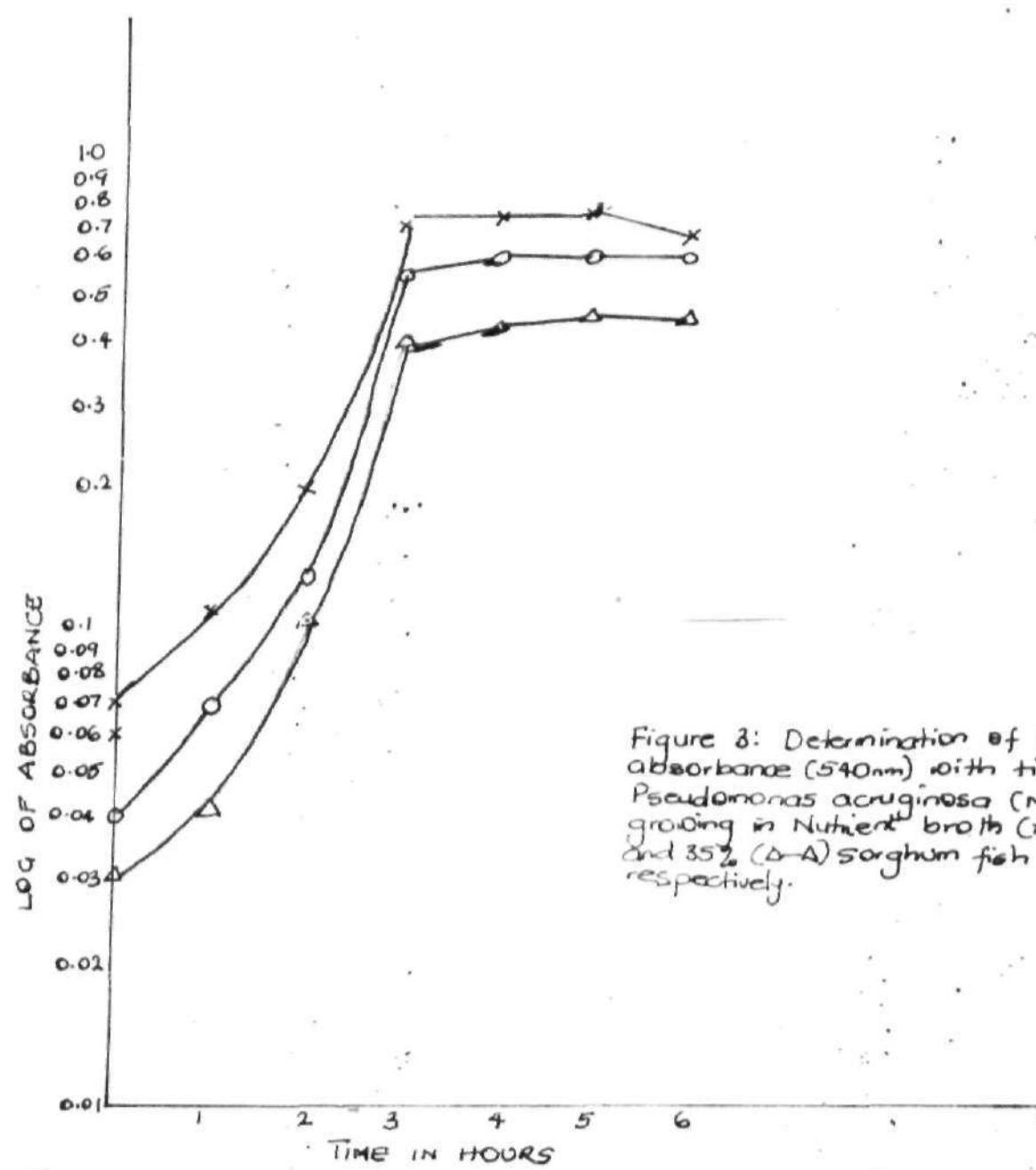
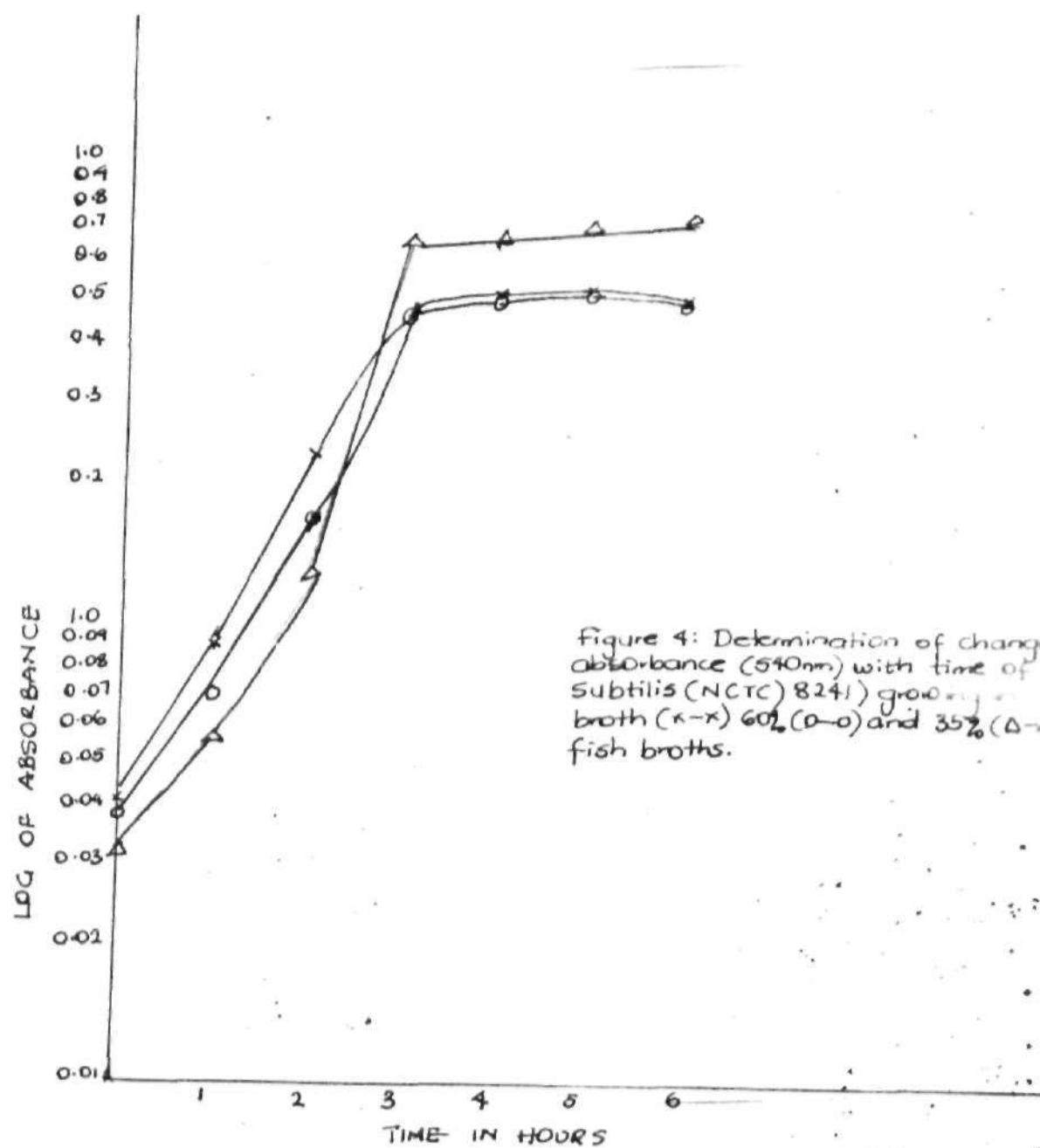
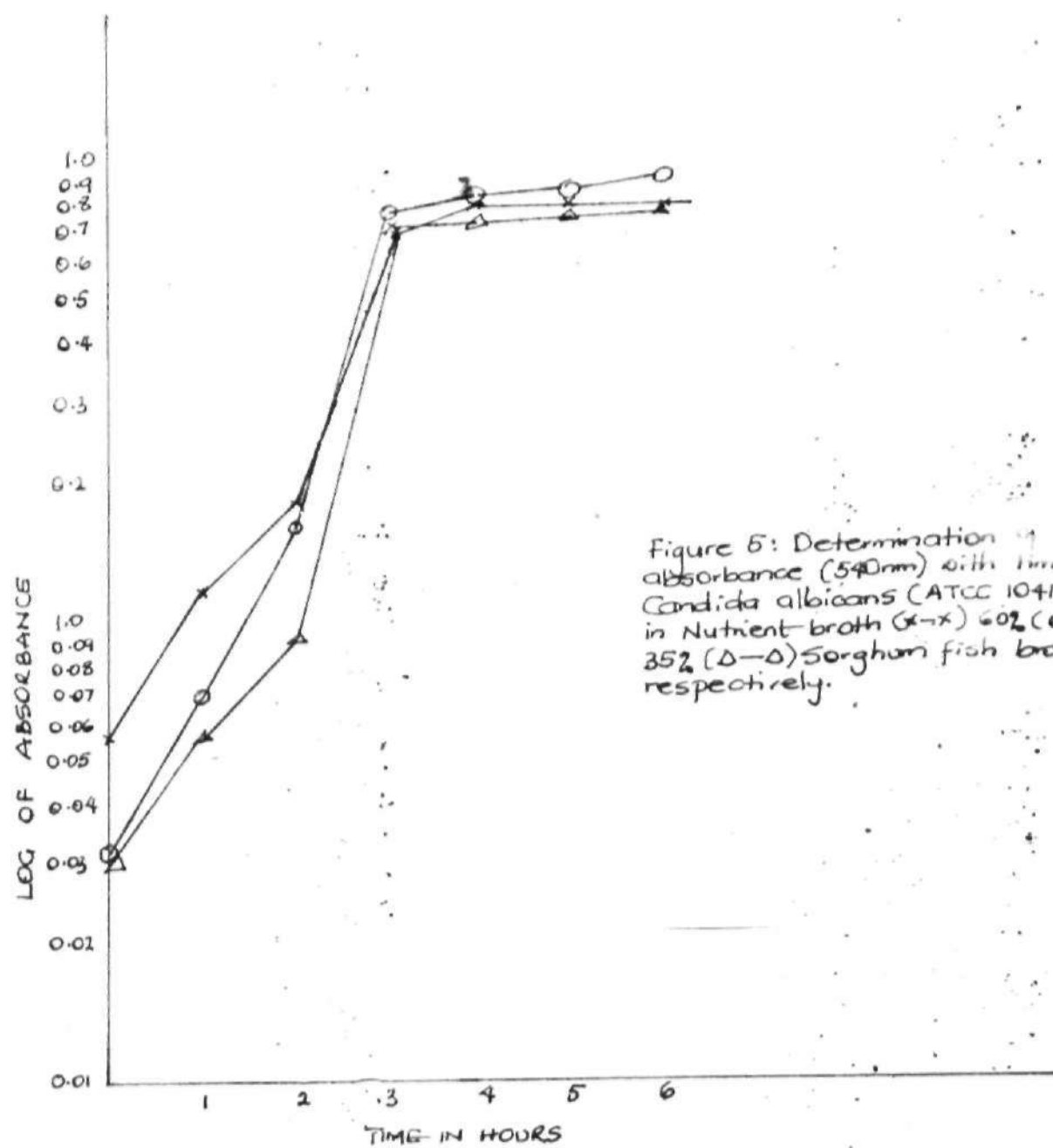


Figure 3: Determination of changes in absorbance (540nm) with time of *Pseudomonas aeruginosa* (NCTC 6750) growing in Nutrient broth (x-x) 60% (o-o) and 35% (Δ-Δ) sorghum fish broths respectively.





LOG CELLS COUNT / ML

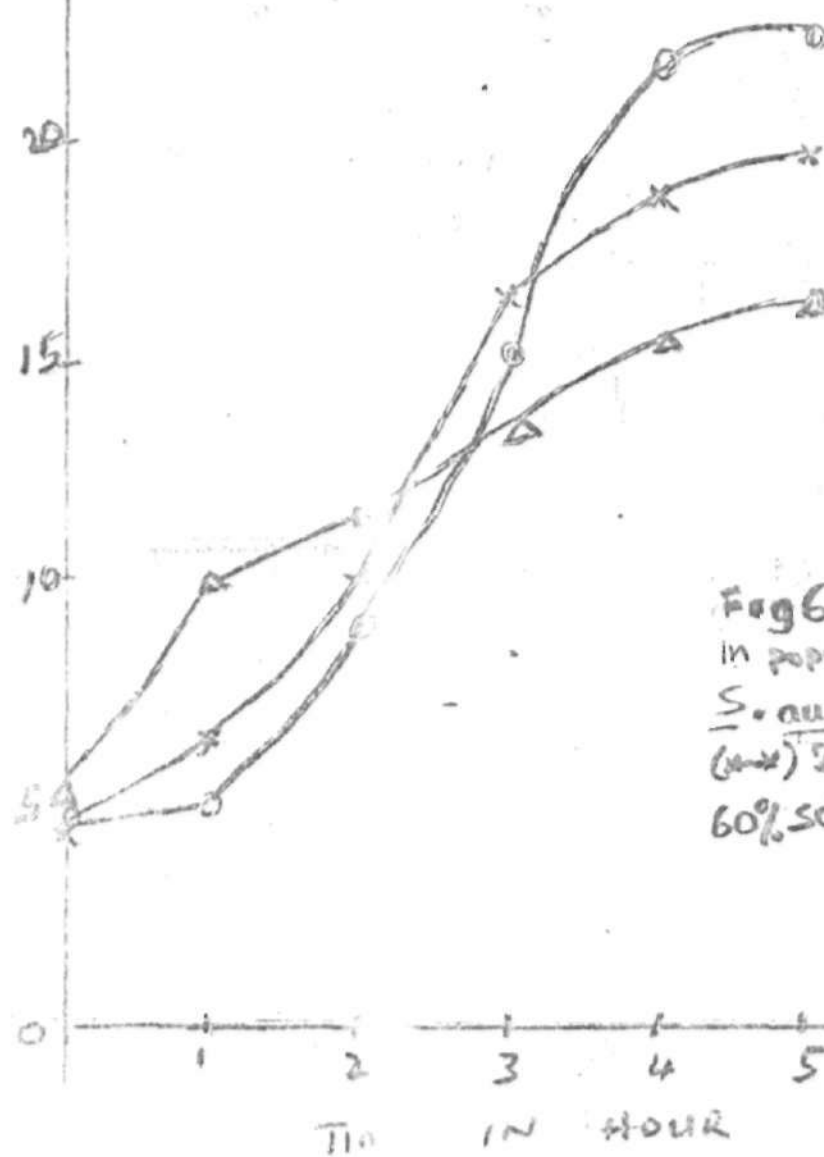
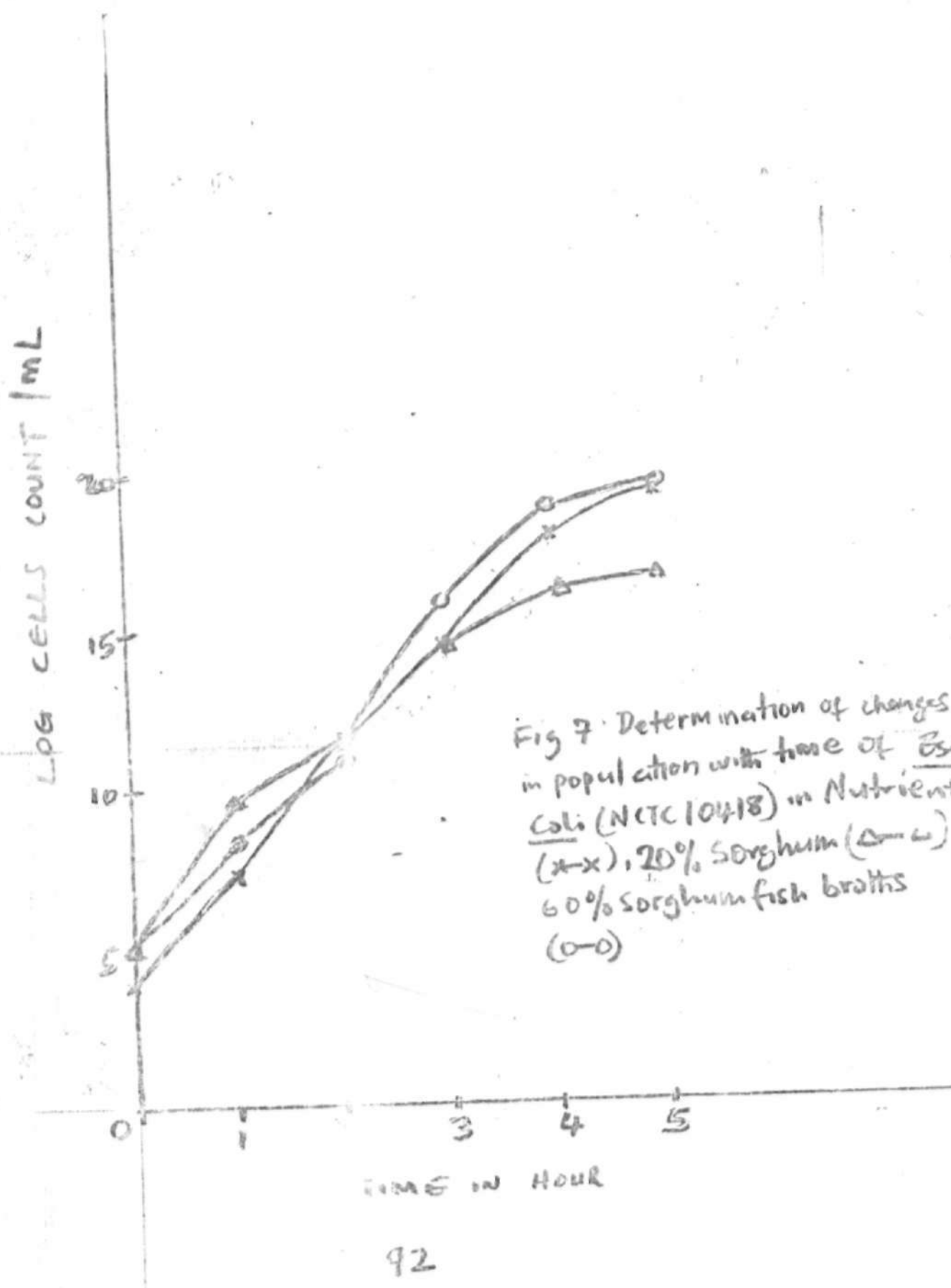


Fig 6: Determination of changes in population with time of *S. aureus* (NCTC 6571) in Nutrient (x) 20% Sorghum (Δ) and 60% Sorghum fish broths (o-o).



CHAPTER FIVE

5.0 DISCUSSION

The growth density of micro-organisms can be determined by use of spectrophotometer. The result can be used to estimate the population density of bacterial and fungal cultures. Many theories have been put forward to support this spectrometric determination of microbial population. Power and Stoward (1962) believed that ideally the absorbed intensity of light should be negligible. In this case, the fraction of light transmitted by a suspension (of microbial culture) could be expressed as e^{-X} , where X is the extinction coefficient associated with absorbing, that is the turbidity and 't' is the optical path length in the sample. The turbidity of a bacterial culture defined in this way is directly proportional to the population density provided other factors such as average cell size and refractive index of the suspending fluid are not altered; provided the orientation of the non-spherical organisms example Escherichia coli, Bacillus subtilis, etc is not biased by the movement of the suspension. Ricia (1970) explained that the relative intensity of light absorbed in different directions (in a colorimeter for example) is dependent upon its wavelength, the size and shape of the organism. In the case of non-spherical organism (example Escherichia coli) its orientation with respect to the

directions of incident and absorbed light are very negligible.

The use of absorbance measurement is a widely accepted means of determining quantity of bacterial cultures. So long as the culture is growing, viable cell counts of such a culture correlate well with the absorbance values. The main advantage of this photometric method is that of gain in speed (or time) taken to obtain reasonably reliable results.

The growth patterns of the test micro-organisms showed three(3) phases of growth viz lag, log and decline or stationary phases. The lag phase observed in some formulations and standard media with the test micro-organisms used represent a period during which the cells depleted of metabolites and enzymes and the result of unfavourable conditions which was obtained at the end of their previous culture history. During the period of lag phase, enzymes and some useful intermediate substances are formed which accumulate until they are present in concentrations which permit growth to resume. This phase may be longer if the nutrient in the medium is minimal, the medium has to warm up to optimum growth temperature or if toxic substances are present usually there may be increase in cell size but little or no increase in cell number.

In the log phase, also called exponential phase, growth of the microbe is believed to have actively commenced in the media via binary fission at a fixed interval of time. This usually occur after the inoculum has adapted itself to the new

environment, the increase is exponential or logarithmic. The disruption of this phase occurs when either the nutrients in the medium become exhausted or toxic materials (metabolic products) accumulated and inhibit growth. For aerobic organisms, oxygen constitutes a limiting factor when absent or reduced in the medium.

The decline phase occurs usually as a result of the exhaustion of essential nutrients and toxic waste accumulations leading to death of organisms.

From the growth pattern of each micro-organism on the formulated media and Nutrient broth, Candida albicans grew better in all the formulated media than Nutrient broth. This observation could be explained by the diversified nutritional requirements of Candida albicans towards culture media. Candida albicans is not nutritionally demanding and has been reported by Baker, (1980) to grow well in both simple and complex media. However, grows poorly in Nutrient broth.

Escherichia coli and Staphylococcus aureus were rated the same in their ability to thrive and multiply very well in all the formulated media. The fact that Staphylococcus aureus could grow responsibly well in the formulated media showed that the sorghum broth and sorghum broth enriched with fish, bean husk and soymilk digest could support the growth of some fastidious micro-organisms, such as Streptococcus pneumonia, Neisseria species, Brucella species. However, Pseudomonas aeruginosa and Bacillus subtilis did not grow well

supplemented media. Oloke et al (1991) showed that Bacillus subtilis did not grow well enough in yellow maize supplemented media. The inability to perform well in shaking formulated cultures could be due to length generation (Stanier, 1980).

Sorghum media alone compared reasonably well with sorghum media enriched with supplements. This was not surprising, since sorghum was found to be rich in utilizable aminoacids, sugars and mineral salt (Miller, 1958; Kramar et al, 1969; Eka 1971; Axtel et al, 1972). It may be assumed that it was the knowledge of these nutritive potentials that led Olurinola et al (1986, 1989, 1990) to the development of this raw material for routine culture media.

The amount of sugars present in Sorghum broth tend to regulate the growth potentials of the media on the test micro-organisms. It was observed that when the concentration of sorghum starch suspension (digest) was increased from five to twenty percent, there was better growth. At higher concentration, the growth of all the test micro-organisms except Staphylococcus aureus declined. Sugars, such as glucose, maltose and Lactose may be partially decomposed to form organic acids on autoclaving (Cruickshank et al, 1980). The organic acids which may act as inhibitory substances thus resulting in poor growth at higher concentrations.

Sorghum media enriched with supplements such as yeast, fish, bean husk and soymilk all gave better growth than plain sorghum media. However, sorghum broth supplemented with yeast

digest perform poorest. The poor growth supporting ability of yeast supplement may be due to the fact that the spent yeast used (although washed several times) still contain high alcoholic and inhibitory substances which can impede the growth of the test micro-organisms.

Sorghum media enriched with fish and milk digests may provide alternative media to Nutrient broth in view of their good performance. This is not surprising as some workers such as Shackle 1975 and Ricardo, 1985 have reported the rich amount of amino acids. (Appendix IV).

Although soymilk was found rich in amino acids (Shackle, 1975) and as such a good potential supplement for culture medium formulation, when it was used as supplement. They perform poorly when used as supplement in the Sorghum Medium and relatively high inoculum of the organisms used. Rather than the known inhibitory substances inherent in soymilk such as antitryptic (Kakade 1969) and antipeptic enzymes (Kusano, 1983). Again most valuable nutrients could be lost through preparation of soymilk and the sterilization of complete media.

Sorghum broth enriched with bean husk digest was found to be poorer medium than NB. The bean husk was obtained as waste with little beans particles and a lot of testa (seed coat). The process of subjecting the bean husk to dryness by air-dry method could result to high fermentation rate which would lead to formation of Lactic acid, alcohols and some neutral

compounds (Buschell and Slater, 1981). These substances would result to inhibition of some metabolic pathways, thus reducing its microbial growth supporting ability.

Sorghum broth enriched with fish digest performed best, when compared with NB and other formulations in growing the test micro-organisms. This is not surprising especially in view of the rich amount of essential amino acids present in fish (Shacklady, 1975).

The formulated media solidified very well on addition of one percent agar. This solidification was as good as the standard laboratory solid media that normally contain about 1.2 to 1.5 percent agar. The slight reduction in needed agar may be due to the gelling property of carbohydrate sources, such as sorghum.

5.1 CONCLUSION

This work has revealed that sorghum media supplemented with forty-eight (48) (0.504, /100N) and sixty (60) (0.63, /100mL) percent of fish digests have the ability to support bacterial and fungal growth better than the conventional NB. The growth supporting ability of these formulations when inoculated with standardized cultures of Echerichia coli (NCTC 10418), Staphylococcus aureus (NCTC 657), Pseudomonas aeruginosa (NCTC 6750), Bacillus subtilis (NCTC 8241) and Candida albicans (ATCC 10231) compared very favourably with NB. This is evidenced by the results obtained from absorbance values after 18 hours static cultures, growth culture determinations and viable count experiments.

Similarly, the sorghum media with twenty(20) percent concentration of sorghum starch suspension (digest) appears to be another alternative to NB and sorghum fish broth for a routine culture medium, since the growth of test micro-organisms in twenty(20) percent sorghum broth was reasonably good. These formulations when solidified with one percent agar were equally comparable with NA.

Thus 20% Sorghum broth, and Sorghum broth medium enriched with 35% and above of fish digest can serve as substitutes for the expensive imported NB and NA, with subsequent national economic benefits.

Summary of the formulation

TABLE 24: COMPOSITION OF 60% and 35% SORGHUM FISH BROTH

<u>Constituent</u>	<u>Quantity present</u>	<u>g/l</u>
1. Sorghum starch digest	0.026	
Sorghum protein digest	1.05	
Fish protein digest	6.30	
Distilled water - to -	<u>1L</u>	
	7.376	
2. Sorghum starch digest	-	0.026
Sorghum protein digest	-	1.80
Fish protein digest	-	3.67
Distilled water	-	<u>1L</u>
		5.499g/l

5.2 SUGGESTIONS FOR FURTHER RESEARCH

The potential of sorghum as an alternative source of medium formulations will not be an overemphasis. This work when pursued vigorously has the potential to revert our dependence on industrialized nations for expensive commercially prepared culture media. It is advice that this work should be rigorously continued until Sorghum media is made practical in our Laboratory.

The following suggestions may be useful

1. To identification the various mineral salts present in sorghum formulated media.
2. Development of a suitable mycological media from sorghum grain

- extract.
3. Development of a selective/indicator media from sorghum broth.
 4. Development of sorghum peptone powder using acid and enzyme hydrolysis.
 5. Development sorghum starch suspension concentrates or powder form.

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APPENDIX II : ABSORBANCE VALUES FOR ESCHERICHIA COLI
STAPHYLOCOCCUS AUREUS, PSEUDONOSAS AERUGINOSA,
BACILLUS SUBTILIS AND CANDIDA ALBICANS IN NUTRIENT
AND SORGHUM BROTHS AT 37°C IN 60MINUTES INTERVAL AT
540NM.

MEDIA	TEST	MICRO-ORGANISMS	ABSORBANCE VALUES AT TIME (MINUTE)						
			0	60	120	180	240	300	360
Nutrient broth		Escherichia coli	0.167	0.167	.243	.306	.362	.457	.803
		Staphylococcus aureus	0.195	.239	.284	.326	.431	.648	.659
		Pseudomonas aeruginosa	0.274	.368	.524	.484	.561	.813	.850
		Bacillus subtilis	0.172	.258	.305	.375	.442	.64	.658
		Candida albicans	0.146	0.146	.244	.296	.339	.439	.508
15% Sorghum broth		Escherichia coli	0.169	0.169	.180	.238	.23	.299	.476
		Staphylococcus aureus	0.161	.216	.257	.271	.309	.495	.466
		Pseudomonas aeruginosa	0.202	.209	.249	.238	.245	.316	.238
		Bacillus subtilis	0.171	.245	.313	.300	.282	.399	.344
		Candida albicans	0.154	0.154	.194	.269	.370	0.410	.514
20% Sorghum broth		Escherichia coli	0.165	0.165	.212	.302	.337	.459	.667
		Staphylococcus aureus	0.184	.230	.340	.377	.469	.660	.637
		Pseudomonas aeruginosa	0.208	.227	.284	.296	.336	.467	.391
		Bacillus albicans	0.161	.205	.257	.342	.381	.550	.506
		Candida albicans	0.133	0.133	.171	.280	.355	.435	.738

APPENDIX III : ABSORBANCE VALUES FOR ESCHERICHIA COLI
STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA,
BACILLUS SUBTILIS AND CANDIDA ALBICANS IN NUTRIENT
AND SORGHUM BROTHS AT 37°C IN 60MINUTES INTERVAL AT
540NM.

MEDIA	MICRO-ORGANISMS	ABSORBANCE VALUES IN TIME (MINUTE)						
		0	60	120	180	240	300	360
Nutrient broth	Escherichia coli	0.169	.243	.306	.362	.457	.803	.762
	Staphylococcus aureus	0.195	.239	.284	.326	.431	.648	.762
	Pseudomonas aeruginosa	0.274	.368	.524	.484	.561	.813	.850
	Bacillus subtilis	0.172	.258	.305	.375	.442	.640	.658
	Candida albicans	0.146	.244	.296	.339	.439	.508	.437
20% Sorghum broth	Escherichia coli	.163	.212	.302	.337	.459	.667	.654
	Staphylococcus aureus	.184	.230	.34	.377	.469	.660	.637
	Pseudomonas aeruginosa	.208	.227	.284	.296	.336	.467	.391
	Bacillus subtilis	.161	.205	.257	.342	.381	.550	.506
	Candida albicans	.133	.171	.280	.355	.436	.738	.600
15% Sorghum bean broth	Escherichia coli	.169	.180	.238	.230	.299	.476	.465
	Staphylococcus aureus	.161	.216	.57	.271	.309	.495	.466
	Pseudomonas aeruginosa	.202	.209	.249	.238	.245	.316	.238
	Bacillus subtilis	.171	.245	.313	.300	.282	.399	.344
	Candida albicans	.154	.194	.269	.370	.310	.514	.524
5% Sorghum bean broth	Escherichia coli	0.062	.138	.200	.283	.342	.521	.528
	Staphylococcus aureus	.111	.112	.144	.166	.245	.389	.403
	Pseudomonas aeruginosa	.140	.199	.224	.166	.203	.239	.205
	Bacillus subtilis	.033	.056	.095	.130	.175	.255	.175
	Candida albicans	.105	.128	.190	.260	.289	.611	.560
10% Sorghum bean broth	Escherichia coli	.089	.045	.157	.175	.240	.445	.410
	Staphylococcus aureus	.096	.089	.133	.139	.193	.416	.357
	Pseudomonas aeruginosa	.173	.184	.215	.191	.21	.296	.218
	Bacillus subtilis	0.047	.094	.150	.157	.182	.502	.247
	Candida albicans	.084	.137	.189	.199	.211	.487	.255
15% SBB	Escherichia coli	.103	.126	.178	.189	.245	.394	.41
	Staphylococcus aureus	.103	.195	.234	.197	.258	.425	.366
	Pseudomonas aeruginosa	.093	.198	.223	.186	.216	.318	.208
	Bacillus subtilis	.039	.053	.109	.129	.175	.325	.215
	Candida albicans	0.099	.139	.205	.282	.340	.639	.687
20% SBB	Escherichia coli	.108	.126	.196	.175	.199	.386	.399
	Staphylococcus aureus	.056	.108	.168	.155	.144	.398	.319
	Pseudomonas aeruginosa	.131	.190	.210	.174	.162	.217	.187
	Bacillus subtilis	.011	.068	.123	.106	.034	.498	.212
	Candida albicans	.100	.12	.185	.286	.22	.720	.570

30% SBB	Escherichia coli	.059	.077	.116	.131	.139	.333	.359
	Staphylococcus aureus	.056	.063	.087	.102	.144	.351	.330
	Pseudomonas aeruginosa	.030	.143	.161	.158	.162	.191	.133
	Bacillus subtilis	.011	.013	.0124	.003	.034	.252	.108
	Candida albicans	.021	.052	.088	.140	.22	.77	.816
5% Sorghum milk broth	Escherichia coli	.052	.073	.157	.168	.221	.386	.446
	Staphylococcus aureus	.073	.108	.185	.212	.295	.471	.521
	Pseudomonas aeruginosa	.112	.113	.191	.257	.297	.322	.252
	Bacillus subtilis	.079	.145	.249	.295	.325	.560	.468
	Candida albicans	.053	.083	.185	.265	.318	.609	.499
10% SMB	Escherichia coli	.079	.089	.144	.192	.270	.473	.516
	Staphylococcus aureus	.085	.102	.162	.205	.287	.471	.521
	Pseudomonas aeruginosa	.079	.113	.191	.226	.253	.246	.246
	Bacillus subtilis	.019	.024	.040	.120	.186	.407	.302
	Candida albicans	.067	.083	.121	.198	.276	.661	.736
15% SMB	Escherichia coli	0.073	.088	.135	.167	.216	.352	.401
	Staphylococcus aureus	.057	.084	.135	.194	.240	.356	.400
	Pseudomonas aeruginosa	.105	.111	.162	.244	.263	.213	.216
	Bacillus subtilis	.012	.024	.104	.169	.241	.343	.398
	Candida albicans	.044	0.073	.138	.217	.311	.534	.643
20% SMB	Escherichia coli	.055	.059	.096	.114	.144	.236	.274
	Staphylococcus aureus	.076	.116	.140	.160	.194	.273	.306
	Pseudomonas aeruginosa	.045	.085	.130	.183	.218	.223	.204
	Bacillus albicans	.057	.065	.112	.237	.331	.476	.539
30% SMB	Escherichia coli	.035	.068	.103	.139	.193	.239	.246
	Staphylococcus aureus	.086	.084	.211	.165	.208	.335	.325
	Pseudomonas aeruginosa	.042	.070	.134	.227	.25	.289	.235
	Bacillus subtilis	.008	.020	.085	.168	.201	.211	.238
	Candida albicans	.028	.051	.097	.170	.265	.488	.596
5% Fish broth	Escherichia coli	.313	.428	.578	.581	.712	.871	.863
	Staphylococcus aureus	.220	.371	.532	.530	.668	.85	.828
	Pseudomonas aeruginosa	.066	.155	.239	.273	.309	.341	.296
	Bacillus subtilis	.026	0.08	.148	.297	.356	.483	.462
	Candida albicans	.177	.316	.493	.582	.679	.815	.853
10% SFB	Escherichia coli	.148	.159	.180	.164	.189	.258	.284
	Staphylococcus aureus	.116	.131	.140	.133	.179	.295	.252
	Pseudomonas aeruginosa	.029	.027	.032	.003	0.039	.115	0.053
	Bacillus subtilis	.040	.042	.047	.016	.074	.088	0.085
	Candida albicans	.103	.097	.107	.147	.198	.622	.795

15% SFB	E. Coli	.234	.349	.563	.560	.705	.825	.834
	Staph.	.259	.339	.533	.537	.658	.853	.820
	Pseud.	0.032	.075	.141	.215	.251	.338	.256
	B. subtilis	.029	.065	.144	.202	.288	.422	.411
	C. albicans	.120	.21	.382	.505	.631	.771	.818
20% SFB	E. coli	.194	.253	.396	.472	.559	.699	.74
	Staph.	.135	.198	.306	.359	.463	.654	.682
	Pseud.	.013	.012	.029	.078	.185	.256	.200
	B. subtilis	0.013	.009	.021	.031	.089	.228	.208
	C. albicans	.081	.116	.193	.289	.384	.642	.701
30% SFB	E. coli	.217	.286	.450	.470	.555	.580	.635
	Staph.	.194	.298	.448	.487	.483	.652	.675
	Pseud.	.016	.024	.04	.08	.148	.231	.207
	B. subtilis	.022	.187	.327	.402	.511	.685	.742
	C. albicans	.105	.191	.342	.436	.536	.658	.680

APPENDIX IV ABSORBANCE VALUES FOR ESCHERICHIA COLI,
STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA,
BACILLUS SUBTILIS AND CANDIDA ALBICANS IN NUTRIENT
AND ENRICHED SORGHUM BROTHS AT 37°C IN 60 MINUTE
INTERVAL AT 540NM.

MEDIA	MICRO-ORGANISMS	ABSORBANCE VALUES IN TIME (MINUTE)						
		0	60	120	180	240	300	360
Nutrient broth	E. coli	0.092	.16	.318	.639	.70	.703	.684
	Staph.	0.069	.131	.227	.596	.680	.700	.657
	Pseud.	0.06	.11	.195	.702	.740	.750	.672
	B. subtilis	0.042	.09	.232	.469	.503	.508	.499
	C. albicans	0.056	.12	.182	.754	.796	.800	.789
35% SDB	E. coli	.136	.174	.243	.470	.427	.498	.516
	Staph.	.124	.136	.181	.450	.377	.427	.449
	Pseud.	.089	.090	.138	.320	.334	.343	.382
	B. subtilis	.161	.20	.251	.435	.438	.454	.497
	C. albicans	.070	.120	.200	.872	.822	.835	.868
28% SDB	E. coli	.106	.136	.200	.400	.386	.404	.381
	Staph.	.052	.086	.130	.320	.322	.336	.323
	Pseud.	.062	.075	.135	.210	.246	.256	.260
	B. subtilis	.091	.143	.203	.465	.51	.525	.544
	C. albicans	.124	.181	.281	.933	.960	.986	.991
60% SDB	E. coli	.013	.055	.133	.335	.384	.408	.443
	Staph.	0.055	.065	.112	.387	.395	.411	.437
	Pseud.	0.019	.024	.086	.267	.256	.255	.252
	B. subtilis	.062	.106	.167	.304	.302	.315	.344
	C. albicans	.034	.101	.186	.892	.815	.931	.957
35% SDB	E. coli	0.076	.092	.147	.521	.405	.485	.522
	Staph.	.056	.054	.090	.509	.407	.465	.501
	Pseud.	.013	.032	.086	.341	.240	.218	.179
	B. subtilis	.075	.126	.205	.375	.385	.388	.386
	C. albicans	.173	.235	.410	1.178	1.162	1.187	1.305
28% SDB	E. coli	.087	.09	.150	.478	.421	.497	.519
	Staph.	.052	.051	.085	.502	.416	.460	.483
	Pseud.	.017	.031	.083	.366	.239	.225	.163
	B. subtilis	.041	.083	.155	.453	.453	.467	.492
	C. albicans	.026	.029	.098	.663	.657	.682	.728
60% SDB	E. coli	.062	.071	.128	.437	.408	.489	.510
	Staph.	.055	.052	.089	.479	.368	.417	.454
	Pseud.	.014	.033	.084	.363	.232	.210	.177
	B. subtilis	.021	.063	.151	.407	.409	.414	.425
	C. albicans	.023	.040	.094	.663	.651	.67	.695

35% SFB	E. coli	0.06	.121	.207	.691	.710	.736	.766
	Staph.	.04	.13	.232	.682	.700	.716	.767
	Pseud.	.03	.042	.105	.400	.440	.460	.454
	B. subtilis	.032	.056	.127	.681	.685	.702	.709
	C. albicans	0.03	.056	.094	.719	.741	.755	.778
28% SFB	E. coli	.134	.167	.291	.762	.682	.785	.776
	Staph.	.12	.125	.218	.715	.613	.725	.738
	Pseud.	.032	.042	.108	.327	.248	.258	.301
	B. subtilis	.034	.057	.133	.426	.343	.35	.377
	C. albicans	.036	.064	.13	.733	.740	.759	.796
60% SFB	E. coli	.070	.11	.341	.839	.890	.893	.858
	Staph.	.060	.133	.236	.818	.840	.860	.822
	Pseud.	.04	.07	.128	.551	.597	.603	.602
	B. subtilis	.038	.071	.173	.458	.497	.500	.481
	C. albicans	.031	.070	.164	.793	.827	.842	.871

APPENDIX V: AMINO ACID COMPOSITION g/16g N

Amino acid	Fish	Soybean
Isoleucine	4.6	5.4
Leucine	7.3	7.7
Phenylalanine	4.0	5.1
Tyrosine	2.9	2.7
Threonine	4.2	4.0
Tryptophan	1.2	1.5
Valine	5.2	5.0
Arginine	5.0	7.7
Histidine	2.3	2.4
Lysine	7.0	6.5
Cystine	1.0	1.4
Methionine	2.6	1.4
Total sulphur containing acids.	3.6	2.8

Reported by ShackLady, 1975.

APPENDIX IVb: ESSENTIAL AMINO ACID CONTENT OF COWPEA (Mg/gN)

Amino acid	Average
Arginine	500
Histidine	213
Isoleucine	318
Leucine	484
Lysine	486
Methionine	79
Cystine	32
Phenylalanine	263
Tyrosine	124
Threonine	251
Tryptophan	68
Valine	314

Reported by Ricardo, 1985