PRODUCTION AND NUTRIENT ANALYSIS OF SINGLE CELL PROTEIN FROM AGROS-WASTE USING SACCHAROMYCESCEREVISIAE

BY

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ABSTRACT

This study was carried out to produce single cell protein (SCP) from pineapple peels (pp) and watermelon peels (wp) and a combination of the two samples (50/50 pp/wp) using *Saccharomyces cerevisiae* under submerged fermentation. The proximate, amino acid mineral and microbiological analyses were done using standard methods. The peels were collected, washed and dried in the oven at 50^{o}C for 24 hours, ground and sieved using 0.05mm sieve. Forty grams of the pineapple and water melon peels each were separately hydrolysed with 1% HCl and sterilized by autoclaving at 121^{o}C for 15min and filtered to get the substrates which were then added with mineral salt medium. The pH of the substrates was adjusted to 5.5 and the medium was inoculated with 2mL ($5.0x10^{3}$ cells) of *Sacchromyces cerevisiae* and incubated at 28^{0}C for 7days. The substrate was filtered and `the residue was dried in the oven at 50^{0}C for 24hours. The results of the proximate composition revealed that the crude protein (59%) and ash (7.2%) of the SCP (50/50 pp/wp) were significantly ($p<0.05$) higher than those of pp SCP and wp SCP while carbohydrate had the lowest value (20.11%). SCP (50/50 pp/wp) had significantly ($p<0.05$) high Na (2282 mg/100g), Ca (60172 mg/100g) and Mg (27312 mg/100g) contents while SCP (pp) had higher K (6000 mg/100g) and Fe (48 mg/100g) contents. The SCP from (pp) had comparative higher values for both essential and non- essential amino acids than either SCP (wp) or SCP (pp/wp). It is recommended that the use of SCP as food or in animal feed should be encouraged because of its high amount of protein, mineral and amino acids. Furthermore, utilization of these agro-wastes will help in addressing environmental pollution caused by these wastes.

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LIST OF ABBREVATIONS

PP: Pineapple Peels

WP: Watermelon Peels

SCP:Single Cell Protein

MC: Moisture Content

CP: Crude Protein

CF: CrudeFibre

CHO: Carbohydrate

CFU: Colony forming unit

B1-B6: Yeast isolate identification code

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The increasing world population generates the challenge of providing necessary food sources. In particular, proteins supply poses a problem since essential amino acids cannot be replaced. Protein is a nutrient needed for normal growth and maintenance of human body. Proteins (made up of amino acids) are important dietary constituents because they are required for the structure, function, and regulation of the body's major tissues and organs. Protein deficiency in food may pose a problem because essential amino acids, which cannot be synthesized by the body itself, are not replenished (Nassera *et al*., 2011). The increasing world deficiency of protein is becoming a major problem for humankind and animals alike. Single cell protein can be a potential alternative to solve the global protein deficiency problem (Bacha *et al*., 2011).

Since early fifties, efforts have been made to explore new, alternate and unconventional protein. For this reason, in 1996, new sources mainly bacteria, yeasts, fungi and algae were used to produce protein biomass named Single Cell Protein (SCP). The term was coined in 1966 by Caroll Wilson at Massachusetts Institute of Technology (MIT) United States of America (Srividya *et al.,* 2013). Single cell protein are dried cells of microorganisms, which are used as protein supplement in human foods or animal feeds. Besides high protein content (about 60-82%of dry cell weight), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals (Nassera *et al*., 2011). Microorganisms like bacteria, yeasts, fungi and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids. Conventional substrates such as starch, molasses, fruit and

vegetable wastes have been used for SCP production as well as unconventional one such as petroleum by-products, natural gas, ethanol, methanol and lingo cellulosic biomass (Dharumaduria *et al*., 2011, Srividya *et al.,* 2013). The worldwide, large-scale development of SCP processes has contributed greatly to the advancement of present-day biotechnology. Research and development of SCP processes has involved work in the fields of Microbiology, Biochemistry, Genetics, Chemical and Process Engineering, Food Technology, Agriculture, Animal Nutrition, Ecology, Toxicology, Medicine Veterinary Science and Economics. In developing SCP processes new technical solutions for other related technologies in waste water treatment, production of alcohol, enzyme technology and nutritional science also improve. Research on Single cell Protein Technology started a century ago when Max Delbruck and his colleagues found out the high value of surplus brewer's yeast as a feeding supplement for animals (Srividya *et al.,* 2013). During the First World War (1914-1918), first Single Cell Protein Technology proved to be more than useful as Germany used it to replace more than half of its imported protein sources by yeast. In 1919, Sak in Denmark and Hay duck in Germany invented a method named Zulaufverfahren in which sugar solution was fed to an aerated suspension of yeast instead of adding yeast to diluted sugar solution. Moreover, *Candila arborea* and *C. utilis* were used during the Second World War (1939-1945) and about 60 percent of the country replaced the food input (Dharumaduria *et al*., 2011).

In microbial protein production, several natural products have been tested. The use of natural cheap substrates and waste industrial products for cultivating microorganisms appear to be general trend. Haider and EL-Hassy (2000) tested date extract supplemented with nitrogen source as a suitable substrate. Several investigations were carried out using

cellulose and hemicellulose waste as a suitable substrate for increasing single cell protein production (Anneli *et al*., 2016; Asiri *et al*., 2021). Further in many cases, the raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use. A variety of SCP processes have been developed with a view to producing food and feed from alternative or waste carbon sources such as carbohydrates, hydrocarbons and their derivatives. Technology has ranged from relatively simple open lagoons or solid-substrate fermentations to large-scale aseptic continuous cultures in fermenter. Various processes have adopted different fermenter designs with respect to process requirements. The choice of substrates has determined the design and strategy of SCP processes.

Various hydrocarbons, nitrogenous compounds, polysaccharides and agricultural wastes such as hemicelluloses and cellulose waste (Zubi, 2005) from plants and fibrous proteins such as horn, feather, nail, and hair from animals have thus been used for the production of SCP (Ashok *et al.,* 2000). These waste products have been converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms and are rich in some growth factors required by microorganisms.

The future of SCP will be heavily dependent on reducing production costs and improving quality by fermentation, downstream processing and improvement in the producer organisms as a result of conventional applied genetics together with recombinant DNA technology. Single cell proteins have application in animal nutrition as fattening calves, poultry, pigs and fish breeding in the foodstuffs area as: aroma carriers, vitamin carrier, emulsifying aids and to improve the nutritive value of baked products, in soups, in ready-to-serve meals, in diet recipes and in the technical field as paper processing, leather processing and as foam stabilizers.

1.2 Statement of the Research Problem

The high demand for protein rich food over the years has been a problem due to world population explosion and the low purchasing power of the poor to afford animal and other traditional source of protein in diet has led to protein deficiency. Protein deficiency is a state of malnutrition that results from eating a diet in which proteins are not enough or present in an inadequate amount**.** It is a major factor responsible for a variety of ailments including mental retardation, kwashiorkor and even death, most especially in developing countries including Nigeria (WHO, 2017), this development could affect the reproduction, growth and also the productivity of a population. Another problem is the environmental pollution caused by the wastes which are littered around due to improper disposal. These agro-waste cause soil pollution by affecting soil bio-diversity and air pollution as a result of the irritating smell produced by heap of these wastes during decomposition (Isaac and Olufemi, 2020). They could also cause water pollution when introduced into water body by run-offs thereby causing turbidity and oxygen depletion in water body.

1.3 Aim and Objectives of the Study

The aim of this research was to produce single cell protein from pineapple and water melon peels using *Saccharomyces cerevisiae.*

1.4 Objectives of the Study

The objectives of the study were to:

i. isolate and identify yeasts from *Burukutu* (Local alcoholic drink)
ii. determine the microbial population and physicochemical properties of pineapple peels and water melon peels (substrates)
iii. produce single cell protein from pineapple and water melon peels
iv. determine the proximate, mineral and amino acid composition of the single cell protein

1.5 Justification for the Study

Due to the illnesses caused by taking low protein in diet which arise as a result of inadequate or high cost of animal or other traditional protein, it has become imperative to undertake this study to produce low-cost protein using waste materials like pineapple peels and water melon peels. Single Cell Protein can alleviate protein deficiency of masses thereby improving the wellbeing of the people and animals alike, while considerably reducing environmental pollution caused by the Agro-wastes. Pineapple and water melon peels are biodegradable and are easy to obtain. Besides, *Saccharomyces cerevisiae* is easy to obtain and cheap, as well.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 What is Single Cell Protein (SCP)?

Single cell protein (SCP) is the term used to describe microbial cells which are grown and harvested primarily for use as human or animal feed (Srividya, 2013). Most of the microorganisms which are used produce growth as single cell rather than as complex multicellular organisms; they can be grown on a variety of materials ranging from agricultural materials and waste (pineapple peels, pawpaw peels, water melon peels, sugar cane bagasse) to hydrocarbons, alcohols and human or animal excreta (Srividya, 2013). SCP contains nucleic acids, carbohydrate cell wall material, lipids, minerals and vitamins. It is a good source of protein, crude fibre and aroma enhancers in food (Srividya, 2013).

2.2 Microorganisms Used for SCP Production

2.2.1 Bacteria

Characteristics that make bacteria suitable for SCP production include rapid growth, short generation time and can double their cell mass in 20 minutes to 2 hours. They are also capable of growing on a variety of raw materials that range from carbohydrates such as starch and sugars to gaseous and liquid hydrocarbons which include methane and petroleum fractions (Bamberg, 2000) to petrochemicals such as methanol and ethanol, Nitrogen sources which are useful for bacterial growth include ammonia, ammonium salts, urea, nitrates, and the organic nitrogen in wastes. It is suggested to add mineral nutrients supplement to the bacterial culture medium to fulfil deficiency of nutrients in concentration sufficient to support growth.

Potential phototrophic bacterial strains are recommended for single cell protein production. Some researchers also suggest use of methanotrophic and other bacterial species for single

cell protein production. Generation time of *Methylophilus methylitropous* is about 2 hours is used in animal feed and in general produces a more favourable protein composition than yeast or fungi. Therefore, the large quantities of single cell protein for animal feed can be produced using bacteria like *Brevibacterium* (Adedayo *et al.,* 2011) *Methylophilus methylitropous, Acromobacter delvaevate, Acinetobacter calcoaceticus, Aeromonas hydrophilla, Bacillus megaterium, Bacillus subtilis* (Gomashe *et al.,* 2014) *Lactobacillus* species, *Cellulomonas* species, *Methylomonas methylotrophu*s (Piper, 2004), *Pseudomonas fluorescens, Rhodopseudomonas capsulate, Flavo bacterium* species and *Thermomonos porafusca* (Dhanasekaran *et al.,* 2011).

2.2.2 Algae

Since ancient times, *Spirulina* was cultivated by people near Lake Chad in Africa and the Aztecs near Texaco in Mexico. They used it as food after drying it. *Spirulina* is the most widely used alga so much that even astronauts take it to space during their space travel. Similarly, biomass obtained from *Chlorella* and *Senedessmus* has been harvested and used as source of food by tribal communities in certain parts of the world. Alga is used as food in many different ways and its advantages include simple cultivation, effective utilization of solar energy, faster growth and high protein content. The alga *Spirulina* has been considered for use as a supplementary protein (Raja *et al.,* 2008). It is a blue green alga having strong antioxidant activity and provokes a free radical scavenging enzyme system.

A diet enriched with *Spirulina* and other nutraceuticals may help protect the stem progenitor cells. *Spirulina maxima* prevents fatty liver development induced by carbon tetrachloride (CCl_4). It is advocated that the use of *Spirulina* should be encouraged in patients suffering from malnutrition, immune suppression, hepatic and neural compromise,

etc. Although further investigations on the antiviral effects of this alga and its clinical implications are strongly needed. Single cell protein (SCP)production by five strains of *Chlorella* species (M109, M121, M122, M138, andM150), isolated from different habitats, and was studied under the influence of eight environmental factors and advocated that temperature, light, nitrate, ammonia, phosphorus, iron, carbonate had positive effect on SCP production except for sodium chloride that had negative effect on the strain M150 (Mahasneh, 2005).

2.2.3 Fungi

Many fungal species are used as a source of protein rich food (Bhalla *et al.*, 2007). Many other filamentous species are also used as source of single cell protein. In 1973, in second International Conference convened at Massachusetts Institute of Technology, USA it was reported that *Actinomycetes* and filamentous fungi produced protein from various substrates. (Dhanasekaran *et al*., 2011). During the World War II, trials were made to utilize the cultures of *Fusarium* and *Rhizopus* (Yousuf, 2012) grown in fermentation as a source of protein food. The inoculums of *Aspergillus oryzae* or *Rhizopus arrhizus* were selected because of their nontoxic nature. Saprophytic fungi grow on complex organic compounds and convert them into simple structures. High amount of fungal biomass is produced as a result of growth. Mycelial yield varies greatly which depends upon organisms and substrates.

There are some species of moulds, for example, *Aspergillus niger* (Yabaya and Ado, 2008). *A. fumigatus*, *Fusarium graminearum* which are pathogenic to human, therefore, such fungi, must not be used or toxicological evaluations should be done before recommending to use as SCP. It has been reported that SCP technology has employedfungal species for

bioconversion of lignocellulosic wastes (Lanihan *et al.*, 2010).The filamentous fungi that have been used include *Chaetomium celluloliticum,Fusarium graminearum* (Zubi, 2005), *Aspergillus fumigatus, A. niger, A .oryzae, Cephalosporium cichorniae, Penicillium cyclopium, Rhizopus chinensis, Scytalidum aciduphlium, Tricoderma viridae, Tricoderma alba* and *Paecilomyces varioti* (Jaganmohan *et al.*, 2013).

In recent times, scientists in Nigeria have undertaken research on the production of single cell protein using different substrates including agricultural wastes with the view of checkmating environmental pollution caused by these wastes (Kelechi and Ukeagbu, 2016).The utilization of banana peels to produce single cell protein using *Aspergillus niger* as the fermenting organism has been studied where media that was supplemented with banana peels had the highest yield of *A. niger* biomass (3.5g/L) when compared to the rest medium that were not supplemented with banana peels with a yield of (2.04 g/L) for protein at 8 days of fermentation.

2.2.4 Yeasts

Yeast single-cell protein (SCP) is a high nutrient feed substitute (Burgents *et al.*, 2004). Among these, most popular are yeast species *Candida* (Bozakouk, 2002), *Hansenula, Pitchia, Torulopsis* and *Saccharomyces*. The production of single cell protein using *Saccharomyces cerevisiae* grown on various fruit wastes has been reported (Tanveer, 2010). The typical oily yeasts genera include *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon* and *Lipomyces*. Cucumber and orange peels were evaluated for the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation where appreciable increase in protein content was achieved with cucumber generating higher amount of protein (53.4%) than orange peels (30.5%)

2.2.4.1 Physiology of Yeasts

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom (Swennen *et al*., 2014). The first yeast originated hundreds of millions of years ago, and at least 1,500 species are currently recognized. They are estimated to constitute 1% of all described fungal species. Yeasts are unicellular organisms that evolved from multicellular ancestors, with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudo hyphae or false hypha. Yeast sizes vary greatly, depending on species and environment, typically measuring 3-4 micro in diameter, although some yeasts can grow to 40 microns in size. Most yeasts reproduce sexually by mitosis, and many do so by the asymmetric division process known as budding. With their single-celled growth habit, yeasts can be contrasted with molds, which grow hyphae. Fungal species that can take both forms (depending on temperature or other conditions) are called dimorphic fungi. The yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols in a process known as fermentation (Gombert and Van Maris, 2015). The products of this reaction have been used in baking and the production of alcoholic beverages for thousands of years. *S. cerevisiae* is also an important model organism in modern cell biology research, and is one of the most thoroughly studied eukaryotic microorganisms (Barbosa *et al.*, 2016). Researchers have cultured it in order to understand the biology of the eukaryotic cell and ultimately human biology in great detail. Other, species of yeasts, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans. Yeasts have been used to generate electricity in microbial fuel cells and to produce ethanol for the biofuel industry (Dos Santos *et al.*, 2016).

2.2.4.2 Genome structure of Yeast

On April 24, 1996, the complete yeast genome sequence was available to the public. The genome contains 12,068 kilobases contained in sixteen linear chromosomes (Drozdova *et al.,* 2016). Unlike prokaryotes, DNA is concentrated in the nucleus, and are grouped into chromosomes during DNA replication. Seventy percent (70 %) of the genome contains open reading frames (ORF's), DNA sequences that would code for a protein, and the average ORF length is about 1450 bp long. Relative to more complex eukaryotes like nematodes (6kb) and humans (30kb), the yeast genome is more compact (Benjamin *et al.*, 2017). In the genome, 5,885 genes code for proteins, 275 code for tRNA, 40 code for snRNA's, and 140 genes on chromosome 12 code for ribosomal RNA. 4% of the genome comprises introns, which are pieces of mRNA cut by snRNA-protein complexes prior to translation (Gang *et al.*, 2019). Out of all the genes that code for proteins, 11% of the protenome is devoted to metabolism, 3% to energy production and storage, 3% to DNA replication, 7% to transcription and 6% to translation. Nearly 430 proteins are involved in intracellular trafficking, and 250 proteins have structural roles.

Protein-coding genes have been documented in the genome, but so far a few of those genes have been identified. Furthermore, the genome also shows signs of two or more copies of a gene in different locations. The genes that code for citrate synthase, an enzyme that converts acetyl CoA and oxaloacetate to citrate, is located in three different chromosomes. Chromosome 3 encodes the enzyme in the peroxidase, chromosome 12 encodes the enzyme in the mitochondria and another copy of the gene is located in chromosome 16 (Peris *et al.*, 2018). One reason for the redundancy in the genome could be that multiple copies of a yeast gene are required in order for it to survive in its natural habitat (Gang *et al.*, 2019).

Using the completed genome, scientists have reconstructed the metabolic network of *S. cerevisiae*. 708 ORF's were identified to take part in metabolism, with the possibility to conduct 1035 metabolic reactions (Peris *et al.,* 2018). More than 85% of these reactions involved transport in and out of the cytoplasmic or mitochondrial membrane, and the other reactions were mainly involved in the metabolism of amino acids, nucleotides and vitamins. Additionally, ORF's involved in metabolism have been classified based on the pathway they are most involved with. Most ORF's take part in the electron transport chain and chemiosmosis, the final steps of aerobic respiration, followed by the breakdown of bigger carbohydrates (Peris *et al.,* 2018).

The mitochondrial DNA sequence has been attempted, but it is incomplete and contains many errors. The mitochondrial genome is about 85,000 base pairs long and contains seven hypothetical ORF's. Further experiments will determine if any of the seven ORF's are expressed in the mitochondria. In addition to the ORF's, the genome contains genes for three subunits of complex IV used in the electron transport chain, and three subunits of ATP synthase.

S. cerevisiae strain *A364A* also contains a circle plasmid. It is 6,318 base pairs long and constitutes 3% of the yeast genome (AnaPaula *et al.,* 2021). Although the sequence contains coding regions for three proteins, the exact identity or function of the proteins is unknown. Like nuclear chromosomes, the plasmid is comprised of chromatin and histones, and can condense itself during mitosis. Unlike bacterial plasmids, which replicate independently of the bacterial chromosome, it replicates only once during the S phase of the cell cycle, and is regulated by the same genes that regulate nuclear DNA replication (AnaPaula *et al.,* 2021). While there is no evidence that it can integrate into chromosomal

DNA, the yeast plasmid is capable of acting as vector in yeast transformation (AnaPaula *et al.,* 2021). Foreign DNA extracted from eukaryotes could now be inserted directly into a eukaryote.

2.3 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is a unicellular fungus that has existed more than 4000 BC. It is responsible for alcohol production. Some of the oldest known uses of *S. cerevisiae* date back to 4,000 BCE in Ancient Egypt, where ancient Egyptians would use yeast to make bread. It has been cultured by humans for thousands of years, as it is the organism known for producing a variety of alcoholic beverages such as beers and wines as well as baked goods such as bread. It is able to carry out this action through a biochemical process called fermentation which generally involves the breakdown of sugar into ethanol, CO_2, and water. *S. cerevisiae* naturally grows on fruits such as grapes and dates as well as grains such as wheat and barley. Its primary form of reproduction is budding, where daughter cell sprout directly from the mother cell.

2.3.1 Morphology of *Saccharomyces cerevisiae*

Colonies of *Saccharomyces* grow rapidly and mature in three days. They are flat, smooth, moist, glistening or dull, and cream in colour. The inability to use nitrate and ability to ferment various carbohydrates are typical characteristics of *Saccharomyces* (Maksin and Mathias, 2018).

2.3.2 Cell structure and metabolism of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae (Fig.2.1) can exist in two different forms: haploid or diploid. It is usually found in the diploid form. The diploid form is ellipsoid-shaped with a diameter of 5-6um, while the haploid form is more spherical with a diameter of 4um (Sherman *et al.*, 2014).

Figure 2.1: Structure and Organelles of Yeast (*Saccharomyces cerevisiae*)

Source: Alicia (2020)

In exponential phase, haploid cells reproduce more than diploid cells. Haploid and diploid cells can reproduce asexually in a process called budding, where the daughter cell protrudes off a parent cell. The buds of haploid cells are adjacent to each other, while the buds of diploid cells are located in opposite poles (Sherman *et al.*, 2014). Additionally, diploid cells can exhibit pseudo hyphal growth if it is growing on a poor carbon source, exposed to heat or high osmolarity. Activated by cAMP, newly developed cells remain attached to the parent cell through a septum (Zaragoza and Gancedo, 2011).

In addition to budding, diploid cells can undergo a meiotic process called sporulation to produce four haploid spores. Haploid spores can be one of two mating type a or α. These spores can also undergo budding to produce more haploid cells. a and α cells can also mate and fuse together, producing a diploid cell. *S. cerevisiae* strains are further distinguished by differences in the haploid stage. In heterothallic strains, the spores resulting from sporulation cannot undergo budding, and their mating type cannot be changed. However, in

homothallic strains, the presence of a *HO* gene allows the spores to change mating type as they grow. Sporulation can be induced if the yeast is exposed to either a poor carbon or nitrogen source or lack of a nitrogen source. Spores also have a higher tolerance to conditions such as high temperature.

As a eukaryote, *S. cerevisiae* contains membrane-bound organelles. Its chromosomes are located in the nucleus, and it uses mitochondria to conduct cellular respiration. Like all other fungi, the cell's shape is based on its cell wall. The cell wall protects the cell from its environment as well as from any changes in osmotic pressure. The inner cell wall has a high concentration of β-glucans, while the outer cell wall has a high concentration of mannoprotein. Chitin is usually located in the septum (Cabib *et al.,* 2015).

S cerevisiae can live in both aerobic as well as anaerobic conditions. In the presence of oxygen, yeasts can undergo aerobic respiration, where glucose is broken to CO_2 and ATP is produced by protons falling down their gradient to an ATPase. When oxygen is lacking, yeasts only get their energy from glycolysis and the sugar is instead converted into ethanol, a less efficient process than aerobic respiration. The main source of carbon and energy is glucose, and when glucose concentrations are high enough, gene expression of enzymes used in respiration are repressed and fermentation takes over respiration (Landy *et al.,* 2017). However, yeasts can also use other sugars as a carbon source. Sucrose can be converted into glucose and fructose by using an enzyme called invertase, and maltose can be converted into two molecules of glucose by using the enzyme mannose (Landy *et al.,* 2017).

2.3.3 Ecology of *Saccharomyces cerevisiae*

It has been difficult to observe and collect *Saccharomyces cerevisiae* outside areas of human contact, so not much research has been done on its interactions in natural environments. Because it is rarely associated with any other environments other than areas that are close to sites of fermentation, people have wondered whether the yeast could ever be found in the wild (Selmecki *et al.*, 2017). So far, most interactions with its environment have been limited to fermentation. In 1871, Louis Pasteur discovered that grapes had to be crushed in order for fermentation to occur (Branco *et al.*, 2015). The grape itself has been an ideal habitat for yeast due to its high sugar concentration and low pH, precluding the growth of rival species (Campbell *et al.*, 2016). Despite this, not many intact grapes contain *S. cerevisiae* at any one time. In an estimate, only one intact grape berry has the yeast on its surface (Campbell *et al.*, 2016).

While intact grapes have little to no yeast present on the skin, damaged grapes are more likely to contain the yeast as well as other organisms. Berries were damaged due to the weather, mould infections or birds feeding on the grapes. Additionally, insects may also appear more often if the berry is already damaged (Wang *et al.*, 2018) These insects would harbour the yeasts in their bodies and deposit them unknowingly while feeding, and the yeast would divide upon exposure to the grape. While it is known that insects harbour microorganisms inside their bodies, it is unknown how yeast is introduced into the insect (Rivero *et al.*, 2015).

2.3.4 Pathology of *Saccharomyces cerevisiae*

Saccharoyces cerevisiae is not normally considered to be a pathogen. In healthy people, disease resulting from *S. cerevisiae* colonizing a particular area are very rare, but have been

reported. While yeast that normally colonize in the GI tract are not the direct cause of any disease, hypersensitivity to antibodies produced against could prove an irritant for people with Crohn's disease, an autoimmune disorder (Perez-Torrado *et al*., 2015). 1% of all vaginal yeast infections occur due to *S. cerevisiae* in the vagina, but symptoms associated with it are identical to the symptoms due to another organism more commonly associated with yeast infections, *Candida albicans* (John, 2016). The only people susceptible to serious problems are immune suppressed individuals, followed by those who have taken *S. cerevisiae* as a probiotic for diarrhoea. For these individuals, the prevailing condition is fungemia (Munoz *et al.*, 2015) caused by the presence of yeasts in the blood, its symptoms have been described as "flu-like".

2.3.5 Scientific classification of *S. cerevisiae*

The ranking shows the various strater of fungi from Kingdom to Genus level of classification: Saccharomyces cerevisiae belongs to Kingdom: Fungi, Division: Ascomycota, Class: Saccharomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae and Genus: Saccharomyces species: S cerevisiae

2.4 General Advantages of Using Microorganisms for SCP Production

According to Srividya *et al*. (2013), the following are advantages of using microorganisms for SCP production:

i. Microorganisms grow at a very rapid rate under optimal culture conditions. Some microbes double their mass in less than 30 minutes.

ii. The quality and quantity of protein content in microorganisms (45-60%) is better when compared to higher plants (22-30%) and animals (15-35%).

iii. A wide range of raw materials, which are otherwise wasted, can be fruitfully used for SCP production.

iv. The culture conditions and the fermentation processes are very simple.

v. Microorganisms can be easily handled, and subjected to genetic manipulations.

Table2.1: Shows the average composition of the different micro-organisms used in SPC production

Composition	Fungi	Algae	Yeast	Bacteria
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1-3
Ash	9-14	8-10	5-10	3-7
Nucleic Acid	7-10	3-8	6-12	8-12

Source: Nassera *et al.* (2011)

2.5 Some factors Affecting SCP Production

Many raw materials have been considered as substrates for SCP production (Nassera *et al.*, 2011). Conventional substrates such as starch, molasses, fruit and vegetable wastes have been used for SCP production, as well as unconventional ones such as petroleum by-products, natural gas, ethanol, methanol and lignocellulosic biomass (Spalvins *et al.*, 2018). Carbohydrate substrates are the most widely used for SCP production due to the fact that carbohydrates are natural microbial substrates and also because carbohydrates constitute a renewable feedstock. The availability of the substrate and its proximity to the production plant are the major factors that determine the design and strategy of SCP production process. Other factors that could affect SCP production include temperature, pH, inoculum size, etc. The temperature requirement of microorganism to carry out fermentation varies from each other, for example, the most common temperature used for the incubation of different microorganisms was room temperature, 25–27°C. However, for some fungi such as *K. fragilis* and *C. utilis*, a temperature between 33 and 35°C was reported as the

optimum (Ghaly *et al*., 2010, Zhoa *et al*., 2014). For bacteria such as *Bacillus* spp. and *E. coli* Kurbanoglu *et al.* (2012*)* used a fixed temperature, 30°C. Gomashe *et al*. (2014) used a temperature of 37°C for *B. subtilis*, and used a temperature of 30°C for the growth of *S. cerevisiae*. However, the optimum temperature for SCP production was 25°C and 30°C when using oil-rich salad oil manufacturing wastewater and cactus pear fruit as carbon sources, respectively (Zheng *et al*., 2015). *F. moniliforme* also showed optimum growth at 28°C with different culture media containing different types of simple carbohydrates. Similarly, Hosseini *et al*. (2011) observed that 28°C was the optimum temperature for the growth of *F. venenatum* when using date sugar as the substrate. *Rhizopus* sp. exhibited different growth morphologies during cultivation at different temperatures. It grew as small mycelial clumps at 30, 35, and 40°C, while it grew as small mycelial pellets at 25 and 45°C pH also effect the production and yield of SCP as most organisms are pH specific, for example, the pH requirement to yeast is between 5.0 and 6.0. Molasses, the residual liquid obtained after crystallization of sugar from the concentrated sugar solution obtained from the milling of sugar cane or sugar beet, contains 45-55 % sugars, namely sucrose, glucose, fructose, raffinose, melibiose and galactose. It is estimated that for every100 kg of cane milled for sugar production, some3.5 to 4.5 kg of molasses is obtained. The use of molasses for the production of SCP is determined by its availability and low cost, its composition and absence of toxic substances and fermentation inhibitors (Bekatorou *et al.,* 2006). Though molasses is a suitable carbon feedstock for SCP production, it requires supplementation with ammonia salts and phosphorus salts. Cassava is a tropical root crop produced in more than 80countries and it is a rich source of starch for SCP production (Bacha *et al.,* 2011)

2.5.2 What are agro-wastes ?

Agricultural wastes are defined as the residues from the growing and processing of raw agricultural products such as fruits, vegetables, meat, poultry, dairy products, and crops. They are the non-product outputs of production and processing of agricultural products that may contain material that can benefit man but whose economic values are less than the cost of collection, transportation, and processing for beneficial use (Obi *et al*., 2016). Their composition will depend on the system and type of agricultural activities and they can be in the form of liquids, slurries, or solids. Agricultural waste otherwise called agro-waste comprise animal waste (manure, animal carcasses), food processing waste (only 20% of maize is canned and 80% is waste), crop waste (corn stalks, sugarcane bagasse, drops and culls from fruits and vegetables, pruning's) and hazardous and toxic agricultural waste (pesticides, insecticides and herbicides, etc.). Estimates of agricultural waste are rare, but they are generally thought of as contributing a significant proportion of the total waste matter in both developing and developed countries. However, despite the challenges caused by these wastes as environmental pollutants, they can be used industrially as substrate in the production of valuable product for man and animal which include biofuels, enzymes, vitamins, antioxidants, animal feed, antibiotics, and other chemicals through solid state fermentation (SSF) using microorganisms such as *Saccharomyces cerevisiae, Aspergillus Niger and Spurilina* (Somadi *et al*., 2016).

2.5.2 Pineapple peels

Pineapple peels are usually the back of pineapple fruit that is peeled off when preparing the fruit for consumption. They can be eaten directly by man or animal without side effects. It

is fibrous in nature and with a dark green/golden mixed colour while the inside is usually light-gold colour. Pineapple peels are rich in several minerals which include calcium (Ca), magnesium (Mg), iron (Fe), phosphorus (P), sodium (Na). It is also rich in vitamins such as vitamin C, sugar and fibre (Feumba *et al*., 2016) which make it a suitable tool for SCP production. Some of its health benefits include ant-inflammatory because of high content of Bromelain, a powerful enzyme found in high concentrations in pineapple skin and stems, helps to cut down inflammation in the body, it aids digestion, immune booster, arthritis and joint pain fighter, vision protector, dental and bone strengthened, anti-cancer and aid good blood circulation.

2.6.2 Water melon peels

Watermelon peels are the rind of water melon fruit, it is green in colour and fibrous in nature. It also has a healthy amount of vitamin A and C, Potassium, Magnesium, sugar, fibre and other important nutrients (Feumba *et al*., 2016) giving it advantage to be adopted for the production of SCP. The rind is completely edible and has no side effect to man and animal alike. it has some many health benefits which include: reduction in blood pressure, improve athletic durability, rich in fibre, increase in sexual libido because of the presence of an amino acid called citrulline.

2.6 Methods of Cultivation of SCP

The production of single cell protein takes place in a fermentation process (Suman *et al.*, 2017). This is done by selected strains of microorganisms which are multiplied on suitable raw materials in technical cultivation process directed to the growth of the culture and the cell mass followed by separation processes. Process development begins with microbial screening, in which suitable production strains are obtained from samples of soil, water, air

or from swabs of inorganic or biological materials and are subsequently optimized by selection, mutation, or other genetic methods. Then the technical conditions of cultivation for the optimized strains are done and all metabolic pathways and cell structures are determined (Ardestani and Alishahi., 2015).

Besides, process engineering and apparatus technology adapt the technical performance of the process in order to make the production ready for use on the large technical scale. Here is where the economic factors (energy, cost) come into play. Safety demands and environmental protection is also considered in the production of SCP in relation both to the process and to the product. Finally, safety and the protection of innovation throw up legal and controlled aspects, namely operating licenses, product authorizations for particular applications and the legal protection of new process and strains of microorganisms (Nassera *et al*., 2011).

2.6.1 Submerged fermentation

In submerged process, the substrate used for fermentation is always in liquid state which contains the nutrients needed for growth. The fermentor which contains the substrate is operated continuously and the product biomass is continuously harvested from the fermentor by using different techniques. The product is filtered or centrifuged and dried. Aeration is an important operation in the cultivation, heat is generated during cultivation and it is removed by using a cooling device. The microbial biomass can be harvested by various methods. Single cell organisms like yeasts and bacteria are recovered by centrifugation while filamentous fungi are recovered by filtration. Other methods like precipitation and the use of semi-permeable or cheese cloth can be employed (Kargi *et al.,*

2005, Suman *et al.,* 2017). It is important to recover as much water as possible prior to final drying done under clean and hygienic conditions.

production of single cell protein using submerged fermentation is shown in figure 2.2 where a substrate is being held in a fermenter and with the addition of nutrient (ammonium sulphate, magnesium sulphate, sodium chloride, calcium chloride) and is allowed to stay between 4and 7 days in an aerated condition (Ageitos *et al*., 2011). The resulting product is being filtered or centrifuged to purify the microbial cells from the substrate and then dried at 80^{o}C and packaged.

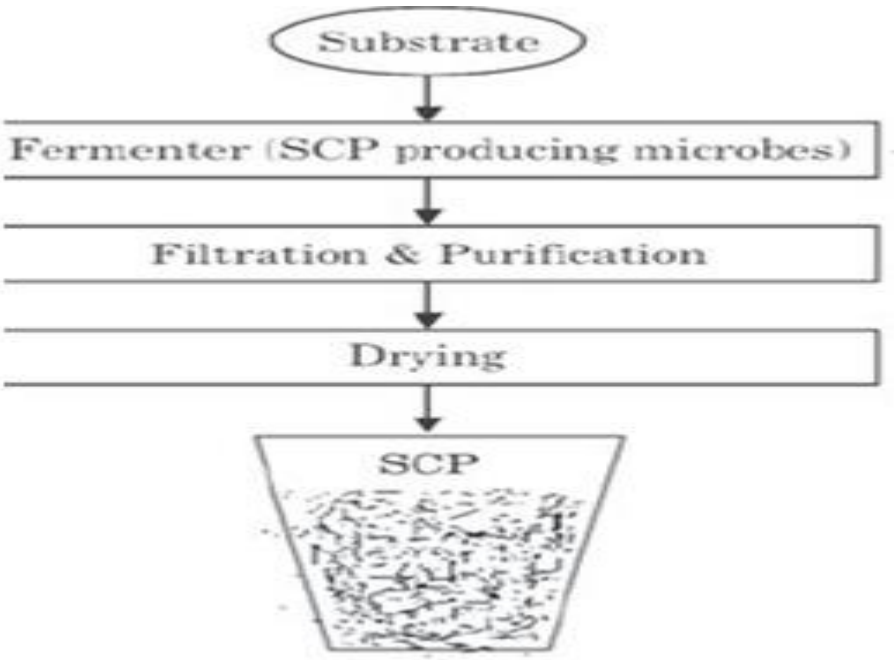

Fig. 2.2: Flow chat for the production of single cell protein

Source: modified from Ageitos *et al*. (2011)

2.6.2 Semisolid fermentation

In semisolid fermentation, the insoluble solid substrate is a solid porous matrix, which absorbs water with a relatively high-water activity and also contains available carbohydrates mineral nutrients and nitrogen sources (Adedayo *et al.,* 2011). The attraction for this type of culturing method comes from its similarity to the natural way of life for several microorganisms and usage of starchy agricultural wastes makes the whole process more economical (Bajpal *et al*., 2017).

2.6.3 Solid state fermentation

Solid state fermentation (SSF) has been extensively studied with thousands of publications describing various types of bioreactor designs, process conditions and microorganisms for the production of various value-added products like SCP, feeds, enzymes, ethanol, organic acids, Bcomplex vitamins, pigments, flavours, (Singhania *et al.,* 2009; Somadi *et al*., 2016). This process consists of depositing a solid culture substrate, such as rice or wheat bran, on flatbeds after seeding it with microorganisms; the substrate is then left in a temperature-controlled room for several day until the fermentation is achieved and thereafter the SCP is harvested using various methods. In recent times, Bratosin *et al*., (2021) used orange pulp, sugar cane bagasse and molasses as substrate to produce SCP using solid state fermentation. Asiri *et al*. (2021) carried out similar production of SCP using fruit wastes as substrate by adopting solid state fermentation process with a reasonable yield when *Saccharomyces cerevisiae* was used as the fermenting organism.

2.7 Nutritional Advantages of SCP

Single cell protein basically comprises proteins, fats, carbohydrates, ash, water, and other elements such as phosphorus and potassium. Aside from the nutritional benefits of single cell protein, another benefit is the fact that SCP can be produced throughout the year. Also,

it plays its role in waste management as waste materials are used as substrate. Small area of land is required and SCP is made in less time (Suman *et al*., 2017). To access nutritional value of single cell protein, many factors must be considered which include nutrient composition, amino acid profile, vitamin and Nucleic acid (NA) content as well as allergies and gastrointestinal effects.

To assess toxicological and carcinogenic affects, long-term feeding trials are also required. A process of drying, harvesting and processing has an effect on the nutritive values of the finished products. The composition of SCP depends on the nature of substrate and also on organism used. Proteins not only provide nutritional value but also perform number of other functions. Single cell protein from yeast and fungi has 50-55% protein, it has high protein carbohydrates ratio (Mchoi and Park, 2003). It contains more lysine, less amount of methionine and cysteine. It also has good balance of amino acids and it has high B-complex vitamins and more suitable as poultry feed. Single cell proteins produced by using bacteria contain more than 80% protein although they have small amount of Sulphur containing amino acids and high in nucleic acid content (Attia *et al.,* 2003).

The method of harvesting, drying and processing has an effect on the nutritive value of the finished product (Bhalla *et al.,* 2007, Suman *et al*., 2017). Proteins not only provide a nutritional component in a food system but also perform a number of other functions with excellent nutrient profiles and capacity to produce mass economically, SCP have been added to aquaculture diets as partial replacement for fishmeal (Olvera-Novoa *et al.,* 2002) and for fortification of rotifer and *Artemia*. Yeast single-cell proteins (SCPs) are playing a greater role in the evolution of aquaculture diets (Gao *et al.,* 2008). Some yeast strains with probiotic properties, such as *Saccharomyces cerevisiae* (Oliva-Teles and Goncalves, 2001)

and *Debaryomyces hansenii* (Tovar *et al.,* 2002) boost larval survival either by colonizing the gut of fish larvae, thus triggering the early maturation of the pancreas, or via the immune stimulating glucans derived from the yeast cell wall (Campa-Córdova *et al.,* 2002; Burgents *et al.,* 2004, Pooramini *et al.,* 2009).

2.8 Drawbacks of SCP Technology

Application of agro-industrial residues (Bacha *et al.*,2011) in bioprocesses such as cultivation of SCP on the one hand provides alternative substrates, and on the other hand, helps in solving pollution problems, which their disposal may otherwise cause some problems (Ashok *et al.,* 2000). Research on SCP has been stimulated by a concern over the eventual food crisis or food shortage that will occur if the world's population is not controlled. The high nucleic acid in SCP could be removed or reduced with one or all of the following treatments: chemical treatment with sodium hydroxide, treatment of cells with 10% sodium chloride, activation of endogenous nucleases during final stage of microbial biomass production and thermal shock (Santin *et al.,* 2003). These methods are aimed at reducing the ribonucleic acid content from about 71% which is considered to be within the acceptable level. Bankra *et al*. (2009) developed a simple method for reduction of nucleic acid in *Brevibacterium* by incubation of non-proliferating cells at pH 10.3 and 55°Cfor 3 hours. These problems of high concentration of nucleic acids are usually about 6-10% elevates serumuric acid levels and becomes cause of kidney stone formation (Bankra *et al.,* 2009).

About 70-80% of total nitrogen is present in amino acids while rest occurs in nucleic acids and this concentration of nucleic acids is higher than conventional protein which is characteristic of all fast growing organisms (Esabi, 2001; Nassera *et al.,* 2011). Another

problem is presence of cell wall which is non digestible, in case of algae and yeast, there may be unacceptable colour and flavours, cells of organisms must be killed before consumption, There is a chance of skin reaction from taking foreign proteins and gastrointestinal reactions may occur resulting in nausea and vomiting (Adedayo *et al.*, 2011). SCP obtained from bacteria also has high nucleic acid content, high risk of contamination during the production process and cell recovery also causes many problems. SCP from bacteria has also been found to be associated with these pitfalls which include: high ribonucleic acid content, high risk of contamination during the production process and recovering the cells is a bit problematic. All these detrimental factors affect the acceptability of SCP as global food.

2.9. Economic Aspect of SCP Production

The need for accurate cost estimations is very relevant, since in the majority of cases the product is competing against protein sources of plant origin, and the profit margins are predictably low. In other cases, such as that of Quorn myco-protein process, fungal protein is competing against meat as a meat substitute, but an added economic effort is required to promote the product against such an established competitor, and the added cost must be compensated for in the production economy. Thus, in all cases, product cost estimation is a central element in the food and feed market industry (Pihlajaniemi *et al*., 2019).

2.9.1 Parameters affecting economic viability of SCP

There are several parameters used in the estimation of economic viability. Raw material costs play a big role in the economic estimation and selecting the raw material for bioprocess the following should be considered price, availability, composition,

oxidative form of carbon. Manufacturing cost and general expenses contribute in the product cost. The product cost normally is divided by the annual production in order to estimate product's unit cost. The former includes all aspects directly related to production, such as direct operating costs, labour, supervision and utilities. Empirical formulae which relate the unknown values of some parameters to other obtainable ones are used in order to build an approximate estimation (McEniry *et al*., 2011).

All of the funds required to build, start and test the production facility before the product is put to the market are included. This investment parameter may be further subdivided into fixed capital, or capital invested in hardware, land and equipment, and working capital, which includes inventory of raw materials, products and supplies, receivable and payable accounts. Profitability is calculated from the return on the investment as a percentage (Pihlajaniemi *et al*., 2019).

Despite the elaborate skills with which cost estimation may be carried out, it is still vulnerable to deviations which are sometimes strong, due to the appearance of unaccounted variables. One such variable of technical nature mentioned already can be the appearance of highly branched colony mutants in the myco-protein production process. Other very important variables are more conventional, but they can make or break a business venture, in the same way as they influence private family economies. Labour costs, fuel prices or interest rates are but a few variables which can unpredictably change as a consequence of local or global developments (McEniry *et al*., 2011).

2.9.2 Advantages and constraints of SCP as a market product

The variability in the market price of other products against which SCP is competing, clearly determines the market price and hence the profitability. One direct competitor for SCP in western countries was brewer's yeast. Identical in almost every feature, brewer's yeast had a bitter taste which carried through to feed formulations, as the only differing characteristic from SCP yeast. However, this competitor was a by-product, the production of which was independent from the market strategy of the producers that led to policies of high turnover, low stock age of the by-product and consequently low market prices. Another competitor was excess baker's yeast. Thus, yeast and fungal SCP had to fall in the by-product market (Ghasem and Najafpour, 2007). One common feature of SCP processes was that they often eliminate waste products, thus covering the function of expensive waste treatment installations. This led to the logic that the substrate may not only be provided at low prices or free, but received with payments by SCP producers. In the case of public wastes, an environmental quota could be paid to SCP producing companies. Such payments would add to those for the final product, with important repercussions on profitability (Nassera *et al.,* 2011).

Though these reasoning made some sense, market reality proved to be very different. Since a profit was expected to materialise from SCP production, the wastes which the process consumed passed on to become substrates, and little interest was paid on their potential environmental hazard once consumed. The use of wastes, in addition brought additional problems in cases where the interest in waste treatment prevailed (Laura, 2020). The production volumes were not determined by the market demand of the product, but by the need to eliminate the waste. In those instances, waste treatment was the product and SCP was a true by-product, which accumulated until buyers could

negotiate bargain sales which liberated stock capacity for the producer. Processes using whey and sulfite liquor were examples vulnerable to these constraints. While SCP protein coexisted with its competitors in the 70's and early 80's, mainly due to the limitations in the availability of brewer's yeast, the emergence of cheap protein from Soya bean and maize in the late 80's and 90's tilted the balance against SCP processes in most countries. Soya bean protein was available at prices which were 50% lower than SCP, with no restrictions on dosage due to high nucleic acid content. The incidence of the price of competitor protein clearly determined the out marketing of SCP (Laura, 2020).

2.10 Future Prospects of SCP

SCP has a proven record as a source of protein which may be obtained with large productivities in compact installations using various materials and products (Suman *et al*., 2017). In the nearest feature, SCP will grow to get more acceptability in the eye of the public and for livestock farmers because of its high amount of protein although there is a little concern about its nucleic acid which can be overcome by chemical or thermal treatment of the cells.SCP in no distance time will occupy its space in the food market due to the fact that the materials for its production are cheap and available (Srividya *et al*., 2013) and also the processes involved in the production are being modified time to time to reduce complexity and time involved in the production process. Apart from the end product of the process being very rich in protein, most of the raw materials in most cases constitute pollution in the environment but by their utilization (raw materials) in SCP production it has helped in cleaning or reducing hazards caused by these pollutants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Bosso is a Local Government Area in Niger State, Nigeria. Its headquarters is Maikunkele. It has an area of 1,592 km^2 and a population of 208,212 as projected in 2019 using the National Population Census figures of 2006 with 2.5% annual growth rate. It lies within the latitude $9^{*}39^{'}11^{*}$ N and longitude of $6^{*}30^{'}57^{*}$ E. (Yahaya *et al*., 2020)

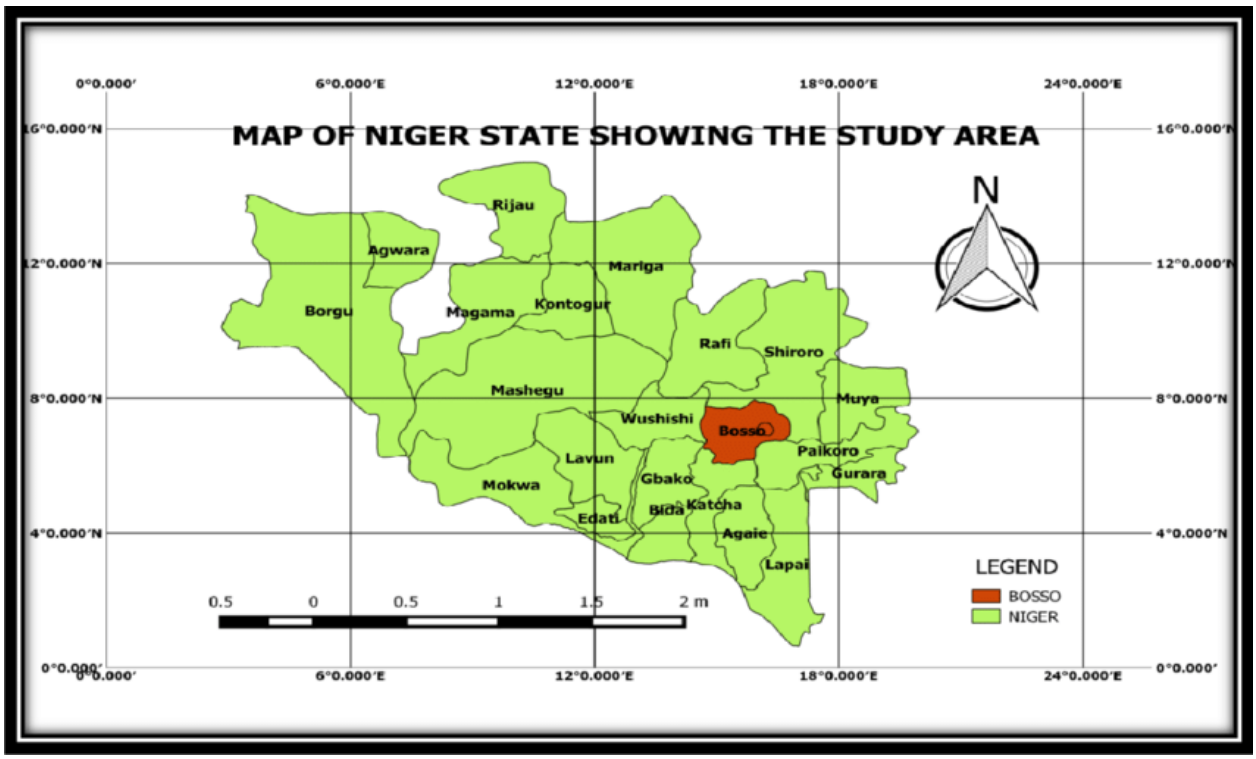

Figure 2.3: Map of Niger State showing the study area (Bosso)
Source: Yahaya *et al*. (2020)

3.2 Collection and Processing of Samples

Pineapple and water melon peels were collected from Kure Ultra-Modern Market, Minna, Niger State, Nigeria, in clean polythene bags and were transported the laboratory of Department of Microbiology, Federal University of Technology, Minna, Niger State,

Nigeria. The pineapple and water melon peels were washed separately with sterile water to remove sand and other impurities that might have been attached to the peels. After washing, the peels were dried in the oven at 60^{o}C for 24 hours. The dried samples were ground into fine powder and sieved through a 0.05mm sieve and stored in the polythene bags at ambient temperature until ready for use. Local alcoholic drink (Burukutu) was bought from Angwan Kaje in Minna, Niger State in a clean plastic container and transported to Microbiology Departmental Research Laboratory of Federal University of Technology, Minna and kept in the refrigerator until ready for use.

3.2.1 Hydrolysis of pineapple and watermelon peels

The hydrolysis of the pineapple and water melon peels was carried out separately according to the method described by Umesh *et al.* (2019), fourty grams (40 g) each of the two agro-waste were separately hydrolysed with one litre of one percent hydrochloric acid and autoclaved and 121^{o}C for 15 minutes allowed to cool and then filtered. To the filtrates were added 0.5-gram $(NH_2)_2SO_4$; 1.0 gram of $KH_2\ PO_4$; 0.5g of $MgSO_4.7H_2O$; 0.1g NaCl and 0.1g of $CaCl_2$ to each of the substrate. The pH of the substrates were adjusted to 5.5 and sterilized before use.

3.3 Isolation and Identification of Yeasts

Local alcoholic drink (burukutu) was serially diluted and plated on Sabouraud dextrose agar (SDA) and incubated at 28^{o}C for 48hours. The colonies that developed were subcultured repeatedly on SDA to obtain pure cultures. The pure cultures were maintained on agar slants for further characterization and identification. The characteristics of the isolates were compared with those of known taxa using the scheme of Bernett *et al*. (1979) and Davenport *et al*. (1980).

3.3.1 Morphological appearance of isolates

The colonies of the Yeast isolates in the plates were observed for their physical appearances including colour, consistency, shape, size and arrangement of the cells.

3.3.2 Microscopy

A wet preparation of the cultures was prepared by taking a pitch of the culture using a wire loop and emulsifying it on a slide and viewed under the microscope using x10 objective for the shape and arrangement of the cells.

3.3.3 Gram's staining

Gram staining was done as described by Fawole and Oso (2007). A speck of yeast growth was taken from 24 hours growth culture to make a thin smear. The smear was allowed to air dry and the slide was flooded with crystal violet for 60 seconds. This was washed with distilled water and thereafter flooded with Gram's iodine for 60 seconds. The slide was washed with distilled water, and 70 % ethanol was added for decolourization and washed after 30 seconds. The smear was counter stained with safranin for 30 seconds, washed with water, and air-dried. The ascospores of the yeast stained red while vegetative cells appeared purple when viewed under oil immersion objective lens of the microscope.

3.3.4 Sugar fermentation test

Sugar fermentation test was carried out as described by Fawole and Oso (2007). A speck of the isolate was inoculated into the medium containing sugars (lactose, maltose, glucose, and sucrose) and incubated at 37^{o}C for 24 hours. A yellow color indicated a positive test, while the red color denoted negative results

3.4 Microbial Analysis of Substrates

3.4.1 Enumeration of bacteria and fungi

Pour plate method was adopted for the microbial analysis. One gram (1g) of the dried substrate was weighed into sterilized 9ml of distilled water in a test tube and serially diluted. One mL of the sample after dilution was introduced into an already sterilized Petri-dish and 20 mL of Nutrient agar (NA) and Sabouroud dextrose agar (SDA) was poured into Petri-dishes separately and rocked until homogeneity was achieved for the enumeration of bacteria and fungi, respectively. The media allowed to solidify before incubating at it 37^{o}C for 24 hours for the enumeration of bacteria and at 28^{o}C for 3-5days for the enumeration of fungi.

3.5 Determination of Physicochemical Properties of Substrates

3.5.1 Determination of pH

One gram of sample was weighed and 9ml of distilled water was added and allowed to stand for10min after which a pH meter was used to determine the pH of the sample by inserting the pH electrode into the sample and noting the value (AOAC, 2012). The pH was standardized using pH buffer 4, 7 and 9 by inserting the electrode into the buffer each at a time to calibrate the pH meter.

3.5.2 Determination of Brix/Sugar

This was carried out to determine the level of sugar utilization by the yeast during the fermentation period. One millilitre of hydrolysed substrate was introduced using Pasteur pipette onto the glass side of the Erman Hand Refractometer and then viewed using the eye piece region and the reading taken in percentage (AOAC, 2015).

3.6 Determination of Proximate Composition of water melon and pineapple peels

3.6.1 Determination of crude protein

Micro-Kjeldal method was used where 0.5 g of water melon and pineapple peels were weighed separately and transferred into the digestion tube and 20ml of concentrated sulphuric acid and one selenium tablet (catalyst) were added. The content in the tube was heated at a temperature of 350^{o}C for 6 hours until a clear digest was achieved (Onwuka, 2005). This solution was poured into a standard flask and made up to 100 mL. Ten milliliters (10 mL) of 2% boric acid was introduced into a conical flask and three drops of mixed indicator (Bromocresol green and Methyl red) was added and the colour changed from colourless to pink which was then placed under the collecting spot. Ten milliliters (10 mL) of the digested sample was introduced into the open chamber of the Makhamps apparatus followed by addition of 10ml of 40% NaOH. The mixture was boiled by the steam produced by the boiling water in the flat bottom flask. As the mixture boiled, gas (ammonia) evolved and condensed in the condenser of the apparatus which was collected inform of liquid into the boric acid. As the ammonia was collected in the boric acid, the solution turned blue. The distillate collected was titrated with0.1M HCl until an end point was reached as the colour of the distillate changed to pink which was the initial colour of the boric acid and the mixed indicator.

% Crude protein was calculated as:

$$\%CP=\frac{Tv\times 0.014\times ma\times df}{wt\ of\ s}\times 100 \qquad (1)$$

Where; TV=titre value; 0.014=nitrogen standard; MA=molarity of acid; Df =dilution factor;

Wt of S=weight of sample.

3.6.2 Determination of crude ash

Two grams of water melon and pineapple peels were weighed separately into a crucible and ashed in a murfle furnace at 550^{o}C for three hours (Ibitoye, 2005). The crucible with the content was removed, cooled in the desiccator and weighed the differences in weight was recorded.

% Ash was calculated as:

$$\% \text{ Ash} = \frac{weight\ of\ ash}{initial\ weight\ of\ sample} \times 100 \qquad (2)$$

3.6.3 Determination of crude fat

The method used was adopted from Food Science Laboratory Manual (Aliyu *et al*., 2010). Five grams of water melon and pineapple peels were introduced into the thimble and corked with cotton wool. Two hundred (200ml) of petroleum ether was added into the round bottom flask of the Soxhlet apparatus and corked with Soxhlet chamber. The sample in the thimble was introduced into the Soxhlet chamber and heated at 60^{o}C for three hours after which the sample was removed and weighed.

% Fats was calculated as:

$$\% \text{ Fat} = \frac{weight\ of\ fresh\ sample - weight\ of\ extracted\ sample}{weight\ of\ fresh\ sample} \times 100 \qquad (3)$$

3.6.4 Determination of moisture content

Five grams of water melon and pineapple peels were separately weighed into a dry Petri-dish using a spatula. The weighed sample were loaded into the oven and dried at 105^{o}C until a constant weight was achieved. The sample was removed, cooled in a desiccators and weighed (Onwuka, 2005).

% Moisture content was calculated as:

$$\% \text{ MC} = \frac{initial\ weight\ of\ sample - weight\ of\ oven\ dry\ sample}{initial\ weight\ os\ sample} \times 100 \qquad (4)$$

3.6.5 Determination of crude fibre

Five grams (5 g) of water melon and pineapple peels were weighed separately into a two hundred and fifty milliliter (250 mL) conical flask and 100mL of 1.25% sulphuric acid (H_2SO_4) was added and boiled on a hot plate for 30minutes and then filtered. The residue was collected back into the conical flask and one hundred milliliters of 1.25 % sodium hydroxide (NaOH) was added and boiled for another 30minutes. The peels were filtered and the residue were transferred into a different crucible and dried in the oven at 105^{o}C until fully dried and the weight recorded. The dry peels in the crucible were ashed at 550^{o}C in the furnace for three hours after which they were removed, cooled and weighed (Onwuka, 2005).

% Crude fibre was calculated as:

$$\% \text{ CF} = \frac{weight\ of\ dry\ sample - weight\ of\ ashed\ sample}{initial\ weight\ of\ sample} \times 100 \qquad (5)$$

The carbohydrate was calculated by adding the values of all the parameters and subtracting it from 100: (100-MC+ASH+CF+CP+FAT).

3.7 Determination of Mineral Content

Mineral content of the water melon and pineapple peels were determined according to the method of AOAC (2010) using wet digestion. Two grams of the peels were weighed differently and transferred into a 100ml conical flask in which 20mL of perchloric acid mixture (1:1, that is perchloric acid and nitric acid) was added. The mixture was heated at 200^{o}C for 2hours until a clear solution was achieved. The digested peels were poured into a 100ml standard volumetric flask and were made up with distilled water to 100ml mark. Sodium and Potassium (Na and K) were analysed using flame photometer.

Determination of Sodium and Potassium (Na and K)

After digestion of the sample, 10 mL was introduced into a 20 mL beaker and then introduced into the Nebulizer of the Flame photometer that had already been on and calibrated, then aspiration took place through the capillary of the Nebulizer and excision of the metals occurred and the result was shown on the screen (AOAC, 2012).

Determination of Calcium, Magnesium and Iron (Ca, Mg and Fe)

The determinations of Ca, Mg and Fe were carried out using the Atomic Absorption Spectrophotometer (AAS)(AOAC, 2012). Twenty millilitres of the digested sample was introduced into a 50mL beaker which was inserted into the aspirator of the AAS after insertion of the cathode lamp based on the desired metal to analyse and the excision took place while the result was displayed on the screen.

3.8 Production of Single Cell Protein

3.8.1 Optimization of production parameters

The optimization was carried out using the method described by Umesh *et al.* (2019). One hundred millilitres each of the hydrolysed water melon and pineapple peel substrate were used for each optimization. All experiments were carried out in duplicates. The SCP production was maintained at various pH levels (5.0, 5.5, 6.0) and incubation time (5, 7 and 9 days) where the wet and dry weights were determined (Umesh *et al.*, 2019). The yield was estimated by the difference in weight of the wet and dry biomass. The parameters (pH 5.5 and incubation period of 7 days) were used for further studies.

3.8.2 Production of SCP by submerged fermentation

Submerged type of fermentation was carried out in Erlenmeyer flasks (250mL capacity) containing 100 mL of the media (substrate) and plugged with cotton wool. The substrates were aseptically inoculated with 2ml ($5.0x10^3$ cells) of inoculum (*Saccharomyces cerevisiae*) and incubated at 28^oC for 7 days until the fermentation was achieved (Lanihan *et al*., 2010).

3.8.3 Harvesting and drying of the cells

After 7 days of fermentation, the substrate was filtered using muslin cloth and the residue which is the wet cells was scooped out into a Petri-dish and weighed to get the fresh weight of the cells. The cells were then dried in the oven at 50^oC for 24 hours after which it was weighed. The dry cells were stored in a sterile bottle for further analysis (Lanihan *et al*., 2010).

3.9 Determination of Proximate Composition of Single Cell Protein (SCP)

The crude protein, ash, fat, moisture, fibre and mineral content of the SCP were determined according to the methods of Onwuka (2005), Ibitoye (2005), Aliyu *et al*. (2010), AOAC(2010), and AOAC (2012), as described in Section 3.5 (3.5.1-3.5.5) of the present study.

3.10 Determination of Amino Acid Contents of SCP

The amino acid determination was carried out according to the method described by Lestari *et.al*. (2022).

3.10.1 Sample preparation

The SCP (30mg) was hydrolysed with 6N HCI at 110°C for 24hours. Sample pretreatment was performed automatically in the autosampler, where the probe was combined with borate buffer solution and the AminoTag® reagent before injection.

3.10 .2 Preparation of standard amino acid solutions

Standard solutions of the amino acids (essential and non-essential) were prepared. The solutions were prepared by adding 0.1 M HCl to 500mL of distilled water in a beaker. The concentrations of the standard solutions were serially diluted to give 25, 20, 15 and 10nM each. They were stored in a freezer at 4°C until required. The mixed standard solution contained 25 mmol of each amino acid derivative.

3.10.3 Derivatization of the Samples

The filtered sample (10 μL) after hydrolysis was transferred to a full recovery sampler, to which 70 μL sodium hexane sulphonic acid buffers was added; the solution was vortexed for 5minutes and then 20 μL of reconstituted buffer was added, and the solution was mixed by vortexing for 5minutes.It was then heated on a heating block at 55°C for 10 min.

3.10.4 Chromatographic analysis of samples

Chromatographic separation of prepared samples was carried out on a Buck scientific BLC10/11 – model HPLC (Norwalk, CT, United States) equipped with UV 338nm detector. A C18, 2.5 x 200 mm, 5um column and a mobile phase of 1:2:2 (100 mL sodium phosphate, pH 7.2: Acetonitrile: methanol v/v/v) at a flow rate of 0.45 mL/minute and an operating temperature of 40^{o}C were used. Standard solutions were analysed in a similar manner. In terms of retention time, the composition of each peak was confirmed and using peak area of each amino acid the concentrations were determined in accordance with the external standard method (by extra polating from the calibration curve, prepared by plotting a graph of peak area versus concentration of each amino acid standard solution).

3.11 Data Analysis

All the data generated were subjected to one-wayAnalyses of Variance (ANOVA) using SPSS 19.0. The means obtained were separated using Duncan Multiple Range Test (DMRT) and the significant difference was obtained at 5% level of significance (Steel and Torrie, 1980).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Identification of yeast in Burukutu

Six isolates were obtained from Burukutu. The colonies appeared whitish, creamy and mucoid. The cells were mostly oval. The isolates fermented glucose and maltose and only one isolate fermented lactose while three isolates were able to ferment sucrose (Table 4.1). The isolates were identified as *Sacchromyces cerevisiae, Scaccharomyces boulardi, Deberomyces castelli, Saccharomyces kluverii, Candida torulopsis* and *Candida albicans* (Table 4.1). Species of *Sacchromyces* constitute 50 % of the total isolates obtained.

Table 4.1: Characterization and identification of yeast isolates

Isolates	Shape	Colour	Consistency	L	G	S	M	GR	Yeasts
B1	Oval	Creamy	Mucoid	-	+	-	+	+	*S. cerevisiae*
B2	Oval	Creamy	Mucoid	-	+	+	+	+	*S. boulardii*
B3	Oval	White	Mucoid	+	+	-	+	+	*D. castelli*
B4	Round	Creamy	Mucoid	-	+	-	-	+	*S. kluverii*
B5	Oval	White	Smooth	-	-	+	-	+	*C. torulopsis*
B6	Oval	White	Smooth	-	+	+	+	+	*C. albicans*

Key: +: Positive, -: Negative, L: Lactose, G: Glucose, S: Sucrose, M: Maltose, GR: Grams reaction, S: *Saccharomyces, C: Candida,* D*: Deberomyces,* B1-B6: Isolate code

Table 4.1.2 Microbial population of pineapple and watermelon peels

Microbial counts of the pineapple and water melon substrates are shown in Table 4.2. The pineapple peels (pp) had higher fungal counts ($1.02x10^8$cfu/g) than either watermelon peels (wp) or the combined samples (pp/wp) However, there was not much difference in the fungal counts of the two substrates ($9.3x10^7$ and $9.0x10^7$cfu/g) respectively. The bacterial counts from the combined substrates showed a higher count ($1.2x10^8$ cfu/g) followed by water melon substrate ($6.3x10^7$cfu/g) and pineapple substrate ($4.0x10^7$cfu) respectively, (Table 4.2).

Table 4.2 Microbiological counts of pineapple and water melon peels

Sample	Fungal counts (CFU/g)	Bacterial counts (CFU/g)
Pineapple peels	$1.02x10^8$	$4.0x10^7$
Water melon peels	$9.3x10^7$	$6.3x10^7$
Pineapple/water Melon peels (50/50)	$9.0x10^7$	$1.2x10^7$

Microbial counts were obtained before sterilizing the substrates.

4.1.3 Physicochemical properties of the pineapple and watermelon peels

4.1.3.1 pH and Sugar content of the pineapple and watermelon peels

Table 4.3 shows the physical and chemical parameters of the substrates. The agro-wastes were at pH 5.5 at the start of the experiment but at the end of the fermentation process, there was no observable change in pH ($p>0.05$) for the agro-wastes except for the sample blend that was significantly different from the two agro-wastes and had the lowest pH value (4.00). Similarly, the agro-waste blend showed a significant difference ($p<0.05$) for sugar content before fermentation when compared to remaining two samples and the level of sugar utilization was significantly higher ($p<0.05$) at the end of the fermentation period with a reduction from 5.8% to 2.0% while reduction from 4.0% to 3.0% and 3.2% to 2.8% were recorded for pineapple and water melon peels, respectively (Table 4.3).

Table 4.3: pH and sugar content of pineapple and watermelon peels used

Sample	pH	pH	Sugar	Sugar

	before fermentation	after fermentation	before fermentation	after fermentation
PP	5.5±0.0[a]	4.3±0.1[a]	4.00±0.0[b]	3.00±0.0[a]
WP	5.5±0.0[a]	4.29±0.0[a]	3.20±0.0[c]	2.80±0.0[a]
PP/WP	5.5±0.0[a]	4.00±0.0[b]	5.80±0.0[a]	2.00±0.0[b]

Key: PP:Pineapple peels, WP: Watermelon peels, PP/WP: pineapple and watermelon peels
Mean on the same column with the same superscript are not significantly different (P>0.05)

4.1.3.2 Proximate composition of pineapple and watermelon peels

The proximate composition of agro-wastes and their combination on dry matter basis are shown in Table 4.4. The results revealed that the agro-waste were significantly (P<0.05) different in the proximate parameters. The moisture content of the pineapple peels (97.22 %) was significantly different (p< 0.05) from the moisture content of either watermelon peels (96.01 %) or the combination of the two substrates (96.19 %). The combination of the two agro-wastes gave a higher ash content (4.88%) while lower ash content was observed for water melon peels (3.5%) than the pineapple peels (3.99 %). For crude protein, pineapple peels had the highest value (8.75%) while water melon peels had the lowest value (7.35%) The combined agro-wastes had the highest crude fibre (6.44%), followed by pineapple peels (5.3%) while water melon peels had the least value (2.08%). The fat content of the individual agro-waste and their blend were not significantly (P>0.05) different from each other (Table 4.4). Water melon peels had the highest carbohydrate content (81.03%), this was followed by pineapple peels (77.53%) while their combination (50/50 pp/wp) had the lowest value (74.33%). Statistical analysis of variance (ANOVA) generally revealed that significant differences (p<0.05) existed among the various peels in regards to crude protein, crude fat and carbohydrate (Table 4.4).

Table 4.4: Proximate composition of pineapple and water melon peels (% dry matter)

Sample	MC	ASH	CP	CF	FAT	CHO
PP	97.88±0.02^{a}	3.99±0.01^{b}	8.75±0.15^{a}	5.31±0.19^{b}	2.28±0.22^{a}	77.53±0.28^{b}
WP	96.01±0.01^{b}	3.50±0.00^{c}	7.35±0.00^{b}	2.08±0.03^{c}	2.06±0.12^{a}	81.03±0.05^{a}
PP/WP	96.19±0.07^{b}	4.88±0.13^{a}	8.50±0.50^{ab}	6.14±0.24^{a}	2.00±0.00^{a}	74.33±0.05^{c}

Key: PP:Pineapple, WP:Water melon, 50/50:Pineapple and water melon(w/w)
Mean in thesame columns with the same superscript are not significantly different (p>0.05)

4.1.3.3 Mineral composition of watermelon and pineapple peels

Table 4.5 shows the mineral composition of pineapple and water melon peels. Watermelon peels had a considerable higher sodium (Na) than pineapple peels. However, a combination of the two substrates had the highest sodium (Na) content. The values were significantly different (p<0.05). The potassium (K) content of the combined peels (50/50 pp/wp) had higher value (3967 mg/100g) than the pineapple and watermelon peels, this value was also significantly different at (p<0.05). The calcium (Ca) content of the pineapple peels was significantly lower as compared to that of watermelon (97.71 mg/100g) and combined peels (9709 mg/100g). The combined peels (50/50 pp/wp) had the highest amount of Magnesium which was significantly different at (p<0.05). However, the Iron (Fe) content of the pineapple peels was higher than that of watermelon and the combined peels, the values were not significant different (p>0.05).

Table 4.5: Mineral composition of pineapple and water melon peels (mg/100g)

Sample	Na	K	Ca	Mg	Fe

PP	7.40±0.20^{c}	1418±16.00^{c}	4271±9.90^{b}	10775±0.15^{c}	6.55±0.55^{a}
WP	27.26±0.11^{b}	3721±12.00^{b}	9771±0.91^{a}	26920±0.91^{b}	4.20±0.00^{b}
PP/WP	29.92±0.19^{a}	3967±53.4^{a}	9709±8.39^{a}	29206±8.39^{a}	4.17±0.19^{b}

Key: PP:pineapple, WP:water melon, 50/50:pineapple and water melon(pp/wp)

Mean with different super script at thesame columns shows a significant difference ($p<0.05$)

4.1.4 Single cell protein produced

4.1.4.1 Wet and Dry single cell protein

The wet and dry cells of SCP produced are shown in Table 4.6. The wet cell yield was higher when the combined peels was used compared to pineapple and watermelon peels used individually. However, there was no significant difference ($p>0.05$) between the amount of SCP generated from pineapple and watermelon peels. A similar pattern was observed when the cells were dried with the combined peels supporting higher amount of SCP than the pineapple and watermelon peels (Table 4.6).

Table 4.6: Single cell protein produced from pineapple and water melon peals

Sample	Wet cells	Dry cells
PP	45.94±0.62^{b}	6.26±0.02^{b}
WP	43.69±0.48^{b}	6.04±0.03^{b}
PP/WP	57.66±0.45^{a}	13.89±0.22^{a}

Key: PP:pineapple, WP:water melon, PP/WP: Pineapple and water melon substrate (50/50)

Mean with different super script in the same columns shows a significant difference ($p<0.05$)

4.1.4.2 Proximate composition of single cell protein

Table 4.7 shows the proximate composition of the SCP produced from the different agro-wastes. The moisture contents of the SCP were not different from each other at (p>0.05). However, there was a significant difference (p<0.05) between agro-waste blend SCP for ash with the highest value of 7.20% while the lowest value was observed for pineapple peels SCP (2.97%). The crude protein of the SCP shows a significant difference (p<0.05) between the sample blend (59.77%) when compared with the pineapple and watermelon SCP (49.31% and 48.30%) respectively. The crude fibre of the SCP showed a significant difference (p<0.05) across all the samples with SCP from watermelon peels having the highest value of (2.42%) while the lowest value was observed for SCP from sample blend (1.99%). Fat content also showed significant differences across all the SCP with a higher value for the SCP from the sample blend. However, the carbohydrate content of the SCP produced from the sample blend had the lowest value (20.11 %) compared to the rest two SCP.

Table 4.7: Proximate composition of SCP (% dry matter) produced from pineapple and watermelon peels

Sample	MC	ASH	CP	CF	FAT	CHO
PP	6.84±0.16^{a}	2.97±0.03^{b}	49.31±0.31^{b}	2.26±0.04^{b}	3.88±0.00^{c}	34.75±0.46^{a}
WP	5.19±1.19^{a}	3.56±0.50^{b}	48.3±0.00^{b}	2.42±0.02^{a}	4.06±0.00^{b}	34.74±0.00^{a}
PP/WP	6.55±0.25^{a}	7.20±0.00^{a}	59.77±0.28^{a}	1.99±0.01^{c}	4.21±0.03^{a}	20.11±0.31^{b}

Key: PP:pineapple, WP:water melon, 50/50: pineapple and water melon(w/w),MC:moisture content, CP:crude protein, CF:crudefibre, CHO:carbohydrate

Mean in the same columns with the same super script shows no significant difference (p>0.05)

4.1.4.3 Mineral composition of single cell protein

The mineral composition of SCP produced is shown in Table 4.8. The sodium (Na) content of the SCP from watermelon peels was quite high followed by that of the combined substrates which were not significantly different (p>0.05) from that of the watermelon peels. However, a significant difference (p<0.05) existed between the SCP from watermelon peels and that of the pineapple peels. The potassium (K) content of the SCP from pineapple peels was higher (6000mg/100g) than the potassium content of the SCP from watermelon peels and the sample blend. For the Calcium (Ca) content, the sample blend SCP had the highest value (60172 mg/100g) followed by pineapple peels (44600 mg/100g) and watermelon peels (43897 mg/100g). The Magnesium content of the SCP also showed higher value for pineapple peels and differed significantly (p<0.05) from the rest two samples (Table 4.8). The Iron (Fe) content of the SCP from pineapple peels (48mg/100g) was higher as compared to that of watermelon peels (45mg/100g) and the sample blend (41mg/100g), the value differed from each other significantly (p<0.05) (Table 4.8).

Table 4.8: Mineral composition of SCP (mg/100g) produced from pineapple and watermelon peels

Sample	Na	K	Ca	Mg	Fe
PP	2100.4±0.42^{b}	6000±0.00^{a}	44600±0.00^{b}	10246±3.50^{c}	48±0.00^{a}
WP	2300.0±1.15^{a}	5800±0.00^{b}	43897±0.00^{c}	56714±4.63^{a}	45±1.00^{b}
PP/WP	2282.0±0.60^{a}	2624±0.20^{c}	60172±0.80^{a}	27312±0.20^{b}	41±0.50^{c}

Key: PP:pineapple, WP:water melon, 50/50: pineapple and water melon(w/w)
Mean from the same columns with different super script shows a significant difference at (p<0.05)

4.1.4.4 Amino acid content of single cell protein

Table 4.9 shows the amino acid profile of SCP produced from different agro-wastes. A total of ten (10) essential amino acids were detected in the samples. Tryptophan, Histidine, Leucine, Phenylalanine, Lysine, Valine, Methionine, Threonine, Arginine of the SCP from pineapple peels had higher values and differed significantly ($p<0.05$) from the amino acids in SCP from watermelon and the combined samples. However, Isoleucine showed no significant difference ($p>0.05$) between SCP from watermelon and sample blend but varied significantly ($p< 0.05$) to the SCP from pineapple peels.

Table4.9: Essential amino acid profile (g/100g) of single cell protein (SCP) produced

Sample	TRY	HIS	LEU	ISO	PHE	VAL	LYS	MET	THR	ARG
PP	2.85± 0.05^{a}	3.06± 0.14^{a}	5.53± 0.00^{a}	4.23± 0.01^{a}	1.81± 0.00^{a}	3.97±0 .00^{a}	4.28± 0.01^{a}	5.01± 0.01^{a}	4.27± 0.01^{a}	3.88± 0.00^{a}
WP	2.13± 0.00^{c}	2.76± 0.00^{c}	5.11± 0.01^{c}	3.55± 0.25^{b}	1.24± 0.00^{c}	3.46± 0.00^{c}	3.85± 0.00^{c}	4.72± 0.01^{c}	3.73± 0.00^{c}	3.27± 0.00^{c}
PP/WP	2.64± 0.00^{b}	2.81± 0.01^{b}	5.35± 0.00^{b}	3.94± 0.07^{ab}	1.57± 0.00^{b}	3.52± 0.00^{b}	3.93± 0.00^{b}	4.87± 0.00^{b}	3.94± 0.01^{b}	3.65± 0.00^{b}

Key: TRY: Tryptophan, HIS:Histidine, LEU:Leucine, ISO:Isoleucine, PHE:Phenylalaline,VAL:Valine, LYS:Lysine, MET:Methionine, THR:Threonine, ARG:Arginne

Mean with the same super script in a row shows no significant difference at ($p>0.05$)

A total of eight (8) non-essential amino acids were detected in the SCP whereAsparagine, Alanine, Glutamate, Trpsine, Cystein, Proline and Serine of the SCP from pineapple peels were all higher and varied significantly ($p<0.05$) from those in SCP produced from watermelon peels and the sample blend (Table 4.10). Similarly, all non-essential amino acids detected in the SCP from sample blend differed significantly ($p<0.05$) from those of other substrates. Lower values for non-essential amino acids were observed for the SCP produced from watermelon peels (Table 4.10).

Table 4.10: Non-essential Amino acids profile (g/100g) of single cell protein (SCP) produced

Sample	ASP	ALA	GLU	GLY	TRY	CYS	PRO	SER
PP	7.19 ±0.00[a]	8.93 ±0.00[a]	14.18 ±0.01[a]	4.92 ±0.01[a]	3.06 ±0.00[a]	3.82 ±0.00[a]	3.18 ±0.00[a]	2.82 ±0.00[a]
WP	6.12 ±0.12[c]	8.33 ±0.00[c]	12.64 ±0.00[c]	4.28 ±0.00[c]	2.18 ±0.00[c]	3.43 ±0.00[c]	2.83 ±0.00[c]	2.13 ±0.00[c]
WP/PP)	6.82 ±0.00[b]	8.72 ±0.00[b]	13.42 ±0.00[b]	4.83 ±0.00[b]	2.46 ±0.00[b]	3.74 ±0.00[b]	2.95 ±0.00[b]	2.64 ±0.00[b]

Key: ASP: Asparagine, ALA:Alanine, GLU:Glutamate, GLY:Glycine, CYS:Cysteine, PRO:Proline,SER:Serine TYR: Tryosine

Mean on the same column with the same super script shows no difference at ($p>0.05$)

4.2 Discussion

4.2.1 Identification of yeast in Burukutu

The various yeasts isolated from the local alcoholic drink (Burukutu) caused the fermentation of sugars and their utilization due to enzymes present in the various isolates. These enzymes include zymase, invertase and maltase. The enzymes are known to be responsible for the conversion of sugar into alcohol and other products during fermentation. The yeasts were identified as *Saccharomyces cerevisiae, Saccharomyces boulardii, Debaromyces castelli, Saccharomyces kluverii, Candida torulopsis and Candida albicans*. These yeast isolates have some enzymes in them (maltase, invertase) which help them in the breakdown of sugar (Cripwell *et al*., 2019) as such enhancing fermentation of the peels.

4.2.2 Microbial population of pineapple and watermelon peels

The initial fungal load of pineapple peels was higher than that of watermelon peels and the combined substrates used. This variation could be attributed to the high amount of sugar present in the pineapple peels. The presence of this sugar encourages the proliferation of microorganisms because of its utilization as carbon source for growth (Blagodatskaya *et al.*, 2014). However, the bacterial load was more for the combined substrates than either the pineapple peels or the watermelon peels; this variation in the microbial load could be attributed to the source of the peels (Mahan *et al*., 2018).

4.2.3 pH and sugar content of pineapple and watermelon peels

The physicochemical parameters of the substrates showed an increase in pH and reduction in sugar as the fermentation proceeded. The increase in pH could be attributed to more microbial activities on the sugars and minerals present in the substrates while a reduction in sugar content could be as a result of the composition and utilization of the substrate by

S*accharomyces cerevisiae*. This attribute gave the combined sample a comparative advantage over pineapple peels and watermelon peels as substrates that can be used to grow both acidophilic and neutrophilic microorganisms. These findings are in agreement with the work of Kim *et al.* (2018) and Sharma *et al*. (2020) who reported that factors such as pH, acidity, concentration and temperature affect the rate of fermentation. Qusheng and Mathew (2018) also reported on the influence of pH on the growth of microorganism in the environment.

4.2.4 Proximate composition of pineapple and watermelon peels

The proximate composition of the substrates used showed different level of variations. The moisture content of the pineapple peels (97.88 %) and water melon (96.01 %) peels were higher compared with the findings of Abarshi *et al*. (2017) who reported 79.92 % and 80.50%, respectively. The high moisture content in fruit could increase the microbial activities and fermentation process. The little variation in the moisture content could be attributed to the nature of soil, environment and the age of the fruit from where it was harvested Mahan *et al.* (2018).In the same vein, the crude protein content of the Agro-waste (8.75 % and 8.5 %) for pineapple and combined samples were slightly higher than that of water melon substrate. The reasonable presence of crude protein in the substrate will provide nitrogen to the microorganisms and also help in the formation of cell wall during fermentation. These findings are different from the values obtained (3.5% and 6.4%) by Abarshi *et al*. (2017) for crude protein in pineapple and watermelon peels, respectively. However, Ash content of the sample blend was significantly higher when compared with the two individual samples. This high value (4.88%) can be linked to the high amount of minerals in the sample blend than the individual agro-wastes, The ash content for pineapple

agro-waste (3.99 %) was higher than water melon agro-waste (3.50%) but they were all higher than the values reported by Abarshi *et al.* (2017).The crude fibre content of the substrates was more for the sample blend which gives it a comparative advantage in supporting microbial growth and enhances digestibility (Nassera *et al*., 2011). The carbohydrate content was also appreciably higher in watermelon peels which on hydrolysis provided carbon source to support microbial growth during fermentation (Zhoa *et al.*, 2014).

4.2.5 Mineral composition of watermelon and pineapple peels

Mineral content of the substrates used had different values across all the sample. Higher amount of sodium (Na) was recorded in combined substrates than either the pineapple or watermelon peels, this implies that sodium will be more available in the combined substrates for the microorganisms during fermentation. Although sodium is an obligate mineral required for microbial growth especially in rumen bacteria, it is most needed by non-marine bacteria for growth and metabolism. The substrates were also rich in potassium, with higher value in combined sample than each of the two substrates. The presence of potassium in the substrate makes it a good medium as potassium plays a role in the activity of intracellular enzymes, acts as an intracellular second messenger, and is involved in the maintenance of a constant internal pH and membrane potential in microorganisms. This finding is in agreement with the work of Angelika (2013) who reported an increase in the growth of microorganisms due to the enhancement of the intracellular activities influenced by potassium. High amount of calcium present in the combined substrate showed superiority above others. High amount of calcium was observed in the combined samples (9709 mg/100g) than either watermelon peels (9771

mg/100g) or pineapple peels (4271 mg/100g) and this makes the substrate a good medium to support microbial cell production during fermentation as calcium is involved in the maintenance of cell structure, motility, transport and cell differentiation processes such as sporulation, heterocyst formation and fruiting body development (Marie *et al*., 2014). It also maintains cell shape and integrity during growth and division (Marie *et al*., 2014). Calcium helps in the development of bones in animals. This finding is in line with the work of Mahan *et al*. (2018) who reported an increase in calcium content when orange and lemon peels were combined. The Magnesium and Iron contents of the substrates were also present in appreciable amount, with higher amount in the combined substrates than either pineapple peels or watermelon peels. This variation could be attributed to interaction of the minerals from the two samples combined. The Iron was higher in the SCP from pineapple peels (6.55 mg/100g) than substrates from watermelon and the combined peels. Magnesium and Iron are important as they promote the activities (oxido-reduction and enzyme reaction) of a microbial cell (Mahan *et al*., 2018).

4.2.6 Wet and dry single cell protein

The production of single cell proteins (SCP) from agro-wastes was undertaken in this study, The recovered cells (wet and dried) from fermentation using pineapple and watermelon peels individually were lower than the combined samples. The highest yield of SCP from the combined sample could be attributed to high amount of sugar, mineral and other components present in the substrates which indicates that production of SCP using combination of peels improved the yield of cell. The presence of these components (sugar and minerals) in appreciable amounts had a comparative advantage in the amounts of cell produced because of their importance in metabolism and growth of microorganisms (Gary,

2021). This report is in agreement with the finding of Mahan *et al*. (2018), who reported higher yield with sample combination of orange and lemon peels.

4.2.7 Proximate composition of single cell protein

The proximate composition is one of the most important nutritional analyse that is used to establish the various type of nutrient and their amount. The SCP produced had high values for proximate when compared to the convectional protein like beef, pork, legumes (Anneli *et al*., 2016). The moisture content of the SCP was more in sample blend than the individual samples. However, moisture content in the SCP could encourage food spoilage if not properly managed. This finding is line with the work of Abarshi *et al.* (2017) who reported a higher moisture content for the SCP produced using pineapple peels as against watermelon and sample blend SCP. The ash content (7.20 %) was higher for the sample blend which is an indication of high mineral presence in the SCP. As such, the SCP can be a good source of minerals and can be eaten as food or added in animal feed. Minerals help in cellular functions and development (Anneli *et al*., 2016). This finding is similar to the work of Mahan *et al*. (2018) who reported an increase in ash content from the sample blends of orange peels and lemon peels. The crude protein of the SCP was appreciably high across all the SCP produced. However, the crude protein content of the SCP produced from the sample blend was higher (59 %) than the crude proteins from either of the two SCP. This increase could be as a result of more available sugar and minerals present in the sample blend that promoted more microbial activities. This finding means that the SCP can be incorporated in food and animal feed. Protein are important in human and animals alike in the production of blood, synthesis and replacement of worn out tissues, growth and metabolism (Bratosin *et al*., 2021). The values obtained in this study are in line with the

work of Akalya *et al*. (2017) who reported an increase in the protein values for the SCP produced using sample blends of pineapple, papaya, muskmelon and watermelon respectively. The crude fibre of SCP from the pineapple peels had higher values than watermelon SCP and the blend sample SCP. The fibre content in SCP is valuable as it is involved in digestion of food materials in animal and humans (Abarshi *et al*., 2017). The fat content of the SCP was low probably due to the fermentation process involved in the production of the SCP. However, fat takes part in cellular function as it is used as energy in the cell after being broken down to fatty acids, Fat also serves as signalling molecule in the cell and plays a role of insulating some major organs of the body such as the heart, kidney and liver (Suzanne, 2018). The values obtained are in agreement with the findings reported by Abarshi *et al*. (2017). The carbohydrate value of the SCP was low in all the SCP analysed, this could be attributed to the high amount of protein present in the SCP. This attribute limits the SCP to be used as source of energy but can supply little amount of energy when ingested by man or animals to support cellular function. This finding is similar to the one obtained by Mahan *et al*. (2018) who reported a low carbohydrate content in SCP produced from different agro-wastes.

4.2.8 Mineral composition of single cell protein

The mineral content of the SCP produced show different concentration base on the type of peels used. Then SCP from watermelon had higher value (2300 mg/100g) while pineapple had the lowest value for sodium. The high value recorded for sodium in water melon SCP could be as a result of the chemical constituent present in the substrate. This implies that more sodium will be available during the fermentation process. The potassium content of the SCP analyzed showed a higher value for the SCP from pineapple peels. This high

amount of potassium as compared to other elements could be attributed to chemical components that were harmonized in the pineapple substrate. This gives it a comparative advantage over watermelon SCP. The SCP from the combined samples had higher calcium content (60172 mg/100g) than SCP from either pineapple or watermelon peels probably due to the amount of cell mass yield. This implies that when ingested as food or added to animal feed can help provide more calcium which help in the development of bones (Marie *et al*., 2014). This finding is in line with the report of Akalya *et al*. (2017) who observed high amount of calcium in SCP produced from agro-wastes. The Magnesium content was higher in SCP produced from watermelon (56714 mg/100g) when compared with the two SCP. This high value can be attributed to the chemical components of the substrates used in the production of the SCP.

4.2.9 Essential amino acids content of single cell

A total of ten (10) essential amino acids were obtained in the study. The SCP from the pineapple peels had the highest value (2.85 g/100g) for Tryptophan when compared to the other SCP. The value was higher than the FAO (2011) 1.4 g/100g standard but was low when compared with that of fish. This implies that SCP form the combined substrates can compete favourably with other sources of protein like meat in the supply of Tryptophan. The body uses tryptophan to help make melatonin and serotonin. Melatonin helps regulate the sleep-wake cycle, and serotonin helps regulate appetite, sleep, mood, pain and also the liver uses it to produce niacin that is needed for DNA production (Shivalkar and Prabha, 2018). Similarly, SCP from the pineapple peels had the highest value (3.06 g/100g) for Histidine when compared to the rest of the samples. However, it was slightly lower than FAO standard of 3.18 g/100g. Histidine helps in the synthesis of protein in the body. The

leucine and isoleucine contents were higher in the SCP produced from pineapple peels (5.53 g/100g and 4.23 g/100g), respectively, than the other substrates. This may be attributed to the constituents of the substrate used. The value obtained were higher than the FAO standard of 4.8 g/100g and 4.2 g/100g. Leucine and Isoleucine stimulate protein synthesis and reduce protein breakdown, especially of muscle protein following physical trauma (David, 2019). A similar trend was observed for Phenylalaline, Valine, lysine, Methionine, Threonine and Arginine with all their values higher in the SCP produced from the pineapple peels than other substrates. This could be influenced by the type of nutrient and sugar present in the substrates. Although the values were lower than the FAO standard except for Methionine and Arginine.

4.2.10 Non-essential amino acids content of single cell protein

A total of eight (8) non-essential amino acids were detected in the study. The value for Asparagine was higher for the SCP produced from the pineapple peels substrate (7.19 g/100g) when compared to the rest SCP. The sugar and other components of the substrate could have influenced this difference (Dhanasekeran *et al,* 2011; Umesh *et al*. 2019).The value obtained for Asparagine for watermelon and the combined sample was lower than the FAO standard (7.0 g/100g) while the pineapple SCP had value similarto the FAO standard (7.00 g/100g) This suggests that the SCP from pineapple peels can supply appreciable amount of Asparagine when utilised as an alternative source of protein in food or animal feed. Asparagine is important in the body as it serves as a precursor for the synthesis of DNA, RNA and ATP (Aremu and Ekinede, 2018). The Alanine content of the SCP from the pineapple peels was higher than the rest of the sample. This may be due to variation in the constituents of the agro-wastes. This implies that the SCP from pineapple peels will

provide more Alanine to the body of humans or animals when used as a source of food or feed. Alanine functions in the body in the breakdown of tryptophan and vitamin B6. It is a source of energy for muscles and the central nervous system. It strengthens the immune system and helps the body use sugars (Aremu and Ekinede, 2018).

The Glutamate value obtained from all the SCP showed that the SCP from pineapple peels had the highest value which conforms to the FAO standard of 14.10 g/100g while SCP from watermelon peels and combined samples were slightly lower (12.64 g/100g and 13.42 g/100g respectively) than the FAO standard. This suggests that the SCP from pineapple peels can compete favourably with conventional protein from meat, fish and other sources. Glutamate plays a central role in fundamental brain functions, including synaptic plasticity (important for learning and memory), formation of neural networks during development and repair of the CNS (Meldrum, 2018). Pineapple peels SCP had the highest value (4.92 g/100g) for Gycine content while the SCP from watermelon peels had the least. However, all the values obtained were lower compared to the FAO standard of 6.90 g/100g. This indicates that the supply of Glycine by these SCP will be low when compared with the convectional proteins. The Tryosine content was more in the SCP from pineapple peels than the watermelon peels and the combined samples. However, Tryosine from pineapple peels SCP was same with that of FAO (3.2 g/100g) SCP from pineapple substrate had the highest value (3.82 g/100g) for cysteine while watermelon and the combine substrate SCP were slightly lower. However, the cysteine obtained for the pineapple peels SCP was higher than the FAO standard. Proline and serine in SCP from pineapple peels were both comparatively higher than that of watermelon peels and the combined samples. These results were contrary to the findings of Umesh *et al.* (2019) who reported a lower value for

these amino acids using pineapple peels. Serine helps in plant metabolism, development, cell signalling and biosynthesis of biomolecule (Milosz *et al*, 2018).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION OF RESEARCH TO KNOWLEDGE

5.1 Conclusion

This research work demonstrated the possibility of isolating some yeasts including *Saccharomyces cerevisiae* from Burukutu to ferment agro-waste using submerged fermentation for single cell protein production. The Single Cell Protein (SCP) produced was found to have high amount of nutrients, minerals and Amino acids when compared to other sources of protein. However, utilization of these agro-wastes at a ratio of (50/50 w/w) produced a higher yield of cells and better proximate composition and minerals in the SCP. The amino acid profile was higher for the SCP produced from pineapple peels than those of watermelon peels and a combination of both substrates.

5.2 Recommendations

It is recommended that:

i. Pineapple and watermelon peels should be employed in SCP production because of their high amount of sugar and minerals
ii. The use of agro-waste can help in producing cheap protein when compared to animal and plant protein, thereby bridging the gap of protein deficiency.
iii. The use of these agro-waste in SCP Production can help in converting waste to wealth.
iv. The use of these agro-waste (pineapple and watermelon peels) will help in controlling environmental pollution.

5.3 Contribution of Research to Knowledge

The research has indeed revealed that SCP has a comparative higher protein (49 % - 59 %), fat (3.88 % - 4.2 %), ash, (22.97 % - 7.20 %) crude fibre (1.99 % - 2.46 %) and carbohydrate (20.11 % - 34.75 %), mineral Na (2100 mg/100 – 2282 mg/100g) K (2624 mg/100g – 6000mg/100g) Ca (44600 mg/100g – 60172 mg/100g) Mg (10246 mg/100g – 27312 mg/100g) and Fe (41 mg/100g – 48 mg/100g) and amino acids than the convectional protein source (Animal and plant protein sources).

This study has also contributed eminently to knowledge especially in the field of sciences, in exposing the importance of Agro-waste (pineapple and watermelon peels) as a substrate for the production of cheap protein using yeast (*Saccharomyces cerevisiae*). The use of these agro-waste has further opened the door in converting waste to wealth and also help in mitigating the menace caused by these agro-waste to the environment.

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APPENDIX A

Preparation of media

i. Preparation of Sugar Medium

Ten grams of Lactose, Glucose, Sucrose and Maltose were weighed individually into one litre conical flask and made up to 1000 ml with distilled water. Ten millilitres of the medium was dispensed into test tubes and sterilized by autoclaving at 121^{o}C for 15minutes. The sterilized medium was removed and allowed to cool.

ii. Preparation of sabouroud dextrose agar (SDA)

Fourty six grams (46 g) of Sabouroud dextrose agar powder was weighed and dissolved in one thousand millilitre of distilled water in a conical flask and pre-heated on a hot plate in order to fully dissolve the media. After dissolution, the media was sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C.

iii. Preparation of Nutrient agar (NA)

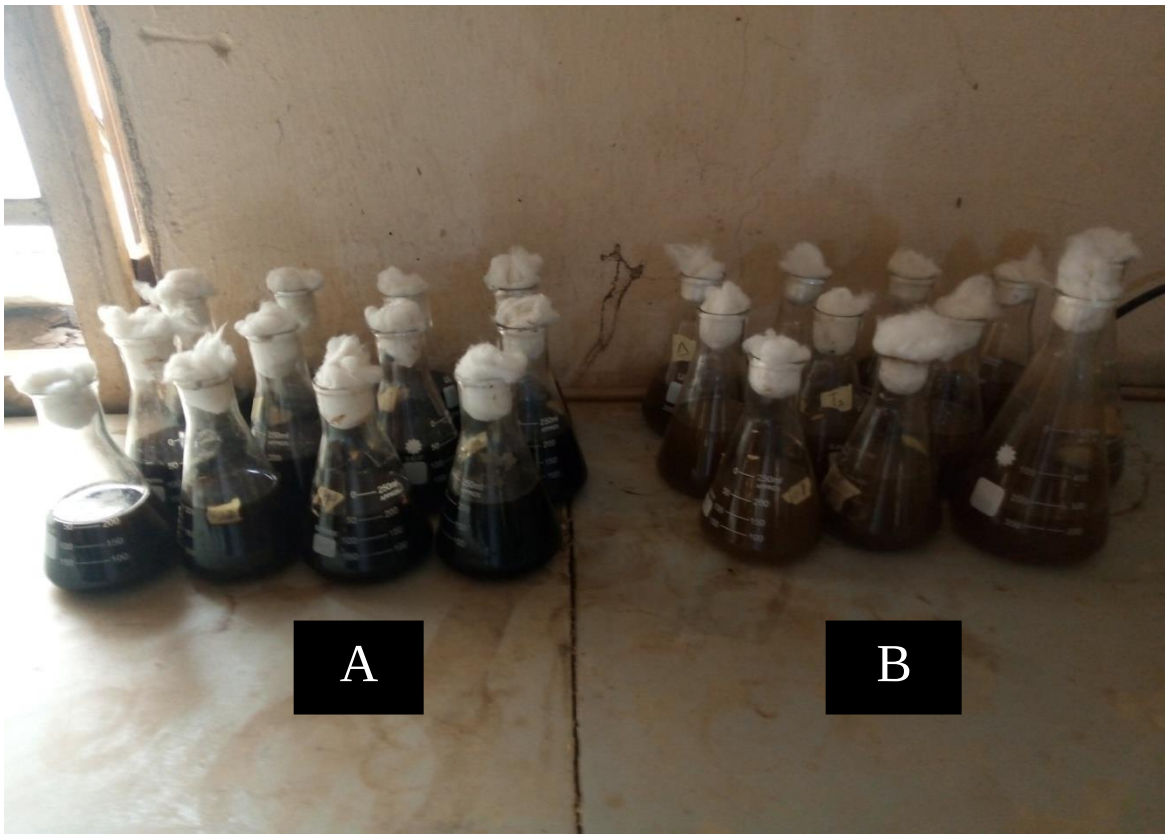
Twenty-eight grams (28 g) of Nutrient Agar powder was weighed and dissolved in one thousand millilitres of distilled water in a conical flask and pre-heated on a hot plate in order to fully dissolve the media. After dissolution, the media was sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C.

APPENDIX B

Substrates for SCP production

(i) Substrates before fermentation (Pineapple peels)

(ii) **Substrate after fermentation (Pineapple peels)**



A= Watermelon/ Pineapple peels (substrate) **B= Watermelon peels(substrate)**

Filtration and Harvesting of Yeast cells

APPENDIX C

Statistical Analysis (ANOVA)

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TRY	1	2	2.8550	.00707	.00500	2.7915	2.9185	2.85	2.86
	2	2	2.1250	.00707	.00500	2.0615	2.1885	2.12	2.13
	3	2	2.6350	.00707	.00500	2.5715	2.6985	2.63	2.64
	Total	6	2.5383	.33499	.13676	2.1868	2.8899	2.12	2.86
HIS	1	2	3.0600	.01414	.01000	2.9329	3.1871	3.05	3.07
	2	2	2.7650	.00707	.00500	2.7015	2.8285	2.76	2.77
	3	2	2.8100	.01414	.01000	2.6829	2.9371	2.80	2.82
	Total	6	2.8783	.14247	.05816	2.7288	3.0278	2.76	3.07
LEU	1	2	5.5250	.00707	.00500	5.4615	5.5885	5.52	5.53
	2	2	5.1100	.01414	.01000	4.9829	5.2371	5.10	5.12
	3	2	5.3450	.00707	.00500	5.2815	5.4085	5.34	5.35
	Total	6	5.3267	.18630	.07606	5.1312	5.5222	5.10	5.53
ISO	1	2	4.2300	.01414	.01000	4.1029	4.3571	4.22	4.24
	2	2	3.5450	.24749	.17500	1.3214	5.7686	3.37	3.72
	3	2	3.9450	.00707	.00500	3.8815	4.0085	3.94	3.95
	Total	6	3.9067	.32715	.13356	3.5633	4.2500	3.37	4.24
PHE	1	2	1.8050	.00707	.00500	1.7415	1.8685	1.80	1.81
	2	2	1.2400	.00000	.00000	1.2400	1.2400	1.24	1.24
	3	2	1.5650	.00707	.00500	1.5015	1.6285	1.56	1.57
	Total	6	1.5367	.25367	.10356	1.2705	1.8029	1.24	1.81
VAL	1	2	3.7850	.00707	.00500	3.7215	3.8485	3.78	3.79
	2	2	3.4550	.00707	.00500	3.3915	3.5185	3.45	3.46
	3	2	3.5150	.00707	.00500	3.4515	3.5785	3.51	3.52
	Total	6	3.5850	.15732	.06423	3.4199	3.7501	3.45	3.79
LYS	1	2	4.2800	.01414	.01000	4.1529	4.4071	4.27	4.29
	2	2	3.8450	.00707	.00500	3.7815	3.9085	3.84	3.85
	3	2	3.9250	.00707	.00500	3.8615	3.9885	3.92	3.93

	Total	6	4.0167	.20724	.08460	3.7992	4.2341	3.84	4.29
MET	1	2	5.0500	.01414	.01000	4.9229	5.1771	5.04	5.06
	2	2	4.7150	.00707	.00500	4.6515	4.7785	4.71	4.72
	3	2	4.8650	.00707	.00500	4.8015	4.9285	4.86	4.87
	Total	6	4.8767	.15029	.06136	4.7189	5.0344	4.71	5.06
THR	1	2	4.2700	.01414	.01000	4.1429	4.3971	4.26	4.28
	2	2	3.7300	.00000	.00000	3.7300	3.7300	3.73	3.73
	3	2	3.9400	.01414	.01000	3.8129	4.0671	3.93	3.95
	Total	6	3.9800	.24364	.09947	3.7243	4.2357	3.73	4.28
ASP	1	2	7.1850	.00707	.00500	7.1215	7.2485	7.18	7.19
	2	2	6.1200	.16971	.12000	4.5953	7.6447	6.00	6.24
	3	2	6.8150	.00707	.00500	6.7515	6.8785	6.81	6.82
	Total	6	6.7067	.48956	.19986	6.1929	7.2204	6.00	7.19
ARG	1	2	3.8750	.00707	.00500	3.8115	3.9385	3.87	3.88
	2	2	3.2750	.00707	.00500	3.2115	3.3385	3.27	3.28
	3	2	3.6450	.00707	.00500	3.5815	3.7085	3.64	3.65
	Total	6	3.5983	.27081	.11056	3.3141	3.8825	3.27	3.88
ALA	1	2	8.9450	.00707	.00500	8.8815	9.0085	8.94	8.95
	2	2	8.3350	.00707	.00500	8.2715	8.3985	8.33	8.34
	3	2	8.7150	.00707	.00500	8.6515	8.7785	8.71	8.72
	Total	6	8.6650	.27559	.11251	8.3758	8.9542	8.33	8.95
GLU	1	2	14.1800	.01414	.01000	14.0529	14.3071	14.17	14.19
	2	2	12.6350	.00707	.00500	12.5715	12.6985	12.63	12.64
	3	2	13.4650	.00707	.00500	13.4015	13.5285	13.46	13.47
	Total	6	13.4267	.69163	.28236	12.7008	14.1525	12.63	14.19
GLY	1	2	4.9200	.01414	.01000	4.7929	5.0471	4.91	4.93
	2	2	4.2750	.00707	.00500	4.2115	4.3385	4.27	4.28
	3	2	4.8300	.00000	.00000	4.8300	4.8300	4.83	4.83
	Total	6	4.6750	.31252	.12759	4.3470	5.0030	4.27	4.93
TYR	1	2	3.0650	.00707	.00500	3.0015	3.1285	3.06	3.07
	2	2	2.1800	.00000	.00000	2.1800	2.1800	2.18	2.18
	3	2	2.4650	.00707	.00500	2.4015	2.5285	2.46	2.47

	Total	6	2.5700	.40408	.16496	2.1459	2.9941	2.18	3.07
CYS	1	2	3.8150	.00707	.00500	3.7515	3.8785	3.81	3.82
	2	2	3.4250	.00707	.00500	3.3615	3.4885	3.42	3.43
	3	2	3.7350	.00707	.00500	3.6715	3.7985	3.73	3.74
	Total	6	3.6583	.18433	.07525	3.4649	3.8518	3.42	3.82
PRO	1	2	3.1750	.00707	.00500	3.1115	3.2385	3.17	3.18
	2	2	2.8350	.00707	.00500	2.7715	2.8985	2.83	2.84
	3	2	2.9450	.00707	.00500	2.8815	3.0085	2.94	2.95
	Total	6	2.9850	.15527	.06339	2.8220	3.1480	2.83	3.18
SER	1	2	2.8550	.00707	.00500	2.7915	2.9185	2.85	2.86
	2	2	2.1250	.00707	.00500	2.0615	2.1885	2.12	2.13
	3	2	2.6350	.00707	.00500	2.5715	2.6985	2.63	2.64
	Total	6	2.5383	.33499	.13676	2.1868	2.8899	2.12	2.86

			Sum of Squares	df	Mean Square	F	Sig.
TRY	Between Groups	(Combined)	.561	2	.280	5.609E3	.000
		I Contrast	.048	1	.048	968.000	.000
		i Deviation					
		r					
		e					
		a					
		r					
			.513	1	.513	1.025E4	.000
		]					
		e					
		r					
		r					
	Within Groups		.000	3	.000		
	Total		.561	5			
HIS	Between Groups	(Combined)	.101	2	.051	336.778	.000
		I Contrast	.062	1	.062	416.667	.000
		i Deviation					
		r					
		e					
		a					
		r					
			.039	1	.039	256.889	.001
		]					
		e					
		r					
		r					
	Within Groups		.000	3	.000		
	Total		.101	5			
LEU	Between Groups	(Combined)	.173	2	.087	866.167	.000
		I Contrast	.032	1	.032	324.000	.000

		i Deviation r e a r]	.141	1	.141	1.408E3	.000
		e r r					
	Within Groups		.000	3	.000		
	Total		.174	5			
ISO	Between Groups	(Combined)	.474	2	.237	11.552	.039
		I Contrast	.081	1	.081	3.962	.141
		i Deviation r e a r]	.392	1	.392	19.142	.022
		e r r					
	Within Groups		.062	3	.021		
	Total		.535	5			
PHE	Between Groups	(Combined)	.322	2	.161	4.824E3	.000
		I Contrast	.058	1	.058	1.728E3	.000
		i Deviation r e a r]	.264	1	.264	7.921E3	.000
		e r r					

	Within Groups		.000	3	.000		
	Total		.322	5			
VAL	Between Groups	(Combined)	.124	2	.062	1.236E3	.000
		I Contrast	.073	1	.073	1.458E3	.000
		i Deviation r e a r	.051	1	.051	1.014E3	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.124	5			
LYS	Between Groups	(Combined)	.214	2	.107	1.072E3	.000
		I Contrast	.126	1	.126	1.260E3	.000
		i Deviation r e a r	.088	1	.088	884.083	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.215	5			
MET	Between Groups	(Combined)	.113	2	.056	563.167	.000
		I Contrast	.034	1	.034	342.250	.000

		i Deviation r e a r	.078	1	.078	784.083	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.113	5			
THR	Between Groups	(Combined)	.296	2	.148	1.111E3	.000
		I Contrast	.109	1	.109	816.750	.000
		i Deviation r e a r	.188	1	.188	1.406E3	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.297	5			
ASP	Between Groups	(Combined)	1.169	2	.585	60.697	.004
		I Contrast	.137	1	.137	14.211	.033
		i Deviation r e a r	1.033	1	1.033	107.183	.002
		] e r r					

	Within Groups		.029	3	.010		
	Total		1.198	5			
ARG	Between Groups	(Combined)	.367	2	.183	3.665E3	.000
		I Contrast	.053	1	.053	1.058E3	.000
		i Deviation					
		r					
		e					
		a					
		r					
			.314	1	.314	6.273E3	.000
		]					
		e					
		r					
		r					
	Within Groups		.000	3	.000		
	Total		.367	5			
ALA	Between Groups	(Combined)	.380	2	.190	3.796E3	.000
		I Contrast	.053	1	.053	1.058E3	.000
		i Deviation					
		r					
		e					
		a					
		r					
			.327	1	.327	6.534E3	.000
		]					
		e					
		r					
		r					
	Within Groups		.000	3	.000		
	Total		.380	5			
GLU	Between Groups	(Combined)	2.391	2	1.196	1.196E4	.000
		I Contrast	.511	1	.511	5.112E3	.000

		i Deviation r e a r	1.880	1	1.880	1.880E4	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		2.392	5			
GLY	Between Groups	(Combined)	.488	2	.244	2.929E3	.000
		I Contrast	.008	1	.008	97.200	.002
		i Deviation r e a r	.480	1	.480	5.760E3	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.488	5			
TYR	Between Groups	(Combined)	.816	2	.408	1.224E4	.000
		I Contrast	.360	1	.360	1.080E4	.000
		i Deviation r e a r	.456	1	.456	1.369E4	.000
		] e r r					

	Within Groups		.000	3	.000		
	Total		.816	5			
CYS	Between Groups	(Combined)	.170	2	.085	1.697E3	.000
		I Contrast	.006	1	.006	128.000	.001
		i Deviation r e a r	.163	1	.163	3.267E3	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.170	5			
PRO	Between Groups	(Combined)	.120	2	.060	1.204E3	.000
		I Contrast	.053	1	.053	1.058E3	.000
		i Deviation r e a r	.068	1	.068	1.350E3	.000
		] e r r					
	Within Groups		.000	3	.000		
	Total		.121	5			
SER	Between Groups	(Combined)	.561	2	.280	5.609E3	.000
		I Contrast	.048	1	.048	968.000	.000

	i Deviation r e a r	.513	1	.513	1.025E4	.000
	] e r r					
Within Groups		.000	3	.000		
Total		.561	5			

TRY

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	2.1250		
3	2		2.6350	
1	2			2.8550
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

HIS

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	2.7650		
3	2		2.8100	
1	2			3.0600
Sig.		1.000	1.000	1.000

Means for groups in homogeny

ISO

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
2	2	3.5450	
3	2	3.9450	3.9450
1	2		4.2300
Sig.		.068	.141

Means for groups in homogeneous subsets are displayed.

LEU

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	5.1100		
3	2		5.3450	
1	2			5.5250
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

PHE

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	1.2400		
3	2		1.5650	
1	2			1.8050
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

VAL

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	3.4550		
3	2		3.5150	
1	2			3.7850
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

LYS

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	3.8450		
3	2		3.9250	
1	2			4.2800
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

MET

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	4.7150		
3	2		4.8650	
1	2			5.0500
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

THR

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	3.7300		
3	2		3.9400	
1	2			4.2700
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ASP

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	6.1200		
3	2		6.8150	
1	2			7.1850
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ALA

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	8.3350		
3	2		8.7150	
1	2			8.9450
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

ARG

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	3.2750		
3	2		3.6450	
1	2			3.8750
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

GLU

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	12.6350		
3	2		13.4650	
1	2			14.1800
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

TYR

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	2.1800		
3	2		2.4650	
1	2			3.0650
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

GLY

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	4.2750		
3	2		4.8300	
1	2			4.9200
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

SER

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	2.1250		
3	2		2.6350	
1	2			2.8550
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

CYS

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	3.4250		
3	2		3.7350	
1	2			3.8150
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

PRO

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
2	2	2.8350		
3	2		2.9450	
1	2			3.1750
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.