JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL ENGINEERING

PRODUCTION AND CHARACTERIZATION OF LIQUID–ORGANIC FERTILIZER BY COMBINING SHEEP WOOL AND BANANA PEEL

By

Gadisa Tesfaye

A Thesis Submitted to Jimma University, Jimma Institute of Technology, School of Chemical Engineering in Partial Fulfillment of the Requirement for the Degree of Masters of Science in Chemical Engineering (Process Engineering)

Advisor: Dr. Edo Begna

Co-Advisor: Mr. Lemma Dame

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Dereje Tadesse (Ass.Professor) __________ 04/01/2022
Internal Examiner Signature Date

Edo Begna (PhD) __________ 04/01/2022
Advisor Signature Date

Yasin Ahmed (MSc) __________ __________
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Gadisa Tesfaye ____________ ___________

Signature Date

Approved by:

Edo Begna (PhD) ______________ ___________

Advisor name Signature Date

Lemma Dame (MSc) ____________ ___________

Co-advisor name Signature Date

Ketema Beyecha (MSc) ____________ ___________

Program coordinators Signature Date

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ABSTRACT

Fertilizer is an important nutrient used for soil conditioning and plant growth. The application of synthetic fertilizer has a significant adverse effect on the soil ecosystem and human health. Valorization of organic matter could evade the use of chemical fertilizer since they are environmentally friends. Sheep wool and banana peels were used to produce liquid organic fertilizer by alkali hydrolysis. Ash and moisture content, and fourier transform infrared radiation analysis of raw materials was done before the experiments. The extraction of keratin protein from sheep wool by alkali was optimized using the response surface method. The effect of different process parameters such as, reaction temperature, the concentration of NaOH, and reaction time were investigated. The optimum percentage of keratin yield was (75.62%) resulting at 63.97℃ of reaction temperature, 0.70N of concentration of NaOH, and 46.86 min of reaction time. The experimental extraction yield (75.15%) agreed with the predicted value (75.62%) indicating the validation of the model. The macro and micronutrient level of the produced fertilizer was investigated. The fertilizer produced from only wool has 11.96%, 0.25%, and 0.10 % of Nitrogen, phosphorus, and potassium respectively, and from only peel has contains 0.71%, 1.72%, and 6.89% of nitrogen, phosphorus, and potassium respectively. The produced fertilizer by combining mass fraction of wools and peels contains primary nutrients, (5.76 -6.87) % of nitrogen, (2.58 – 3.6)% of potassium and (0.76 – 1.54)% of phosphorus, and, they were well fit the boundary limit of Food and agricultural organization, and industrially produced organic fertilizer. The composition of sheep wool and banana peel influences the pH of the produced liquid organic fertilizer. Based on the result of obtained data, liquid-organic fertilizer from sheep wool and banana peel contains a good value of total organic carbon (16.24 -19.24) % and electrical conductivity (8.65 -7.52) dsm^{-1}.

Keywords: Fertilizer, Sheep wool, Optimization, Banana peel, and liquid- organic fertilizers

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ACRONYMS

AAS	Atomic absorption spectrophotometer
AISE	Agricultural input supply enterprise
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
C/N	Carbon to nitrogen ratio
CCD	Central composite design
CSA	Central statistics agency
EC	Electrical conductivity
ECOFI	European consortium of the organic fertilizer industry
EGTOP	Expert group for technical advice of organic production
FAO	Food and agricultural organization
FAOSTAT	Food and agricultural organization corporate statistical database
FTIR	Fourier transform infrared radiation
HPLC	Higher performance liquid chromatography
IFA	International fertilizer association
IPCC	Intergovernmental panel on climate change
ISO	International standard organization
LOF	Liquid organic fertilizer
PLC	Private limited company
RSM	Response surface method
SOC	Soil organic carbon
TOC	Total organic carbon
UV	Ultraviolet

1. INTRODUCTION

1.1 Background of the study

Fertilizer is any substance, solid or liquid, inorganic or organic, natural or synthetic, single or combination of elements, that is used to deliver critical nutrients to plants in soil or plants. It is used to progress the level of available plant nutrients, to sustain the physical and chemical properties of the soil. Besides, it is also important to improve plant growth, yield, and products (Pathamesh Kumar, 2017). Fertilizers materials as a means of complementing the natural food supplies of the soil are of considerable importance among the various factors involved in the economic production of crops as well as proper maintenance of the soil. Plants cannot survive without certain elements such as nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, and some other elements (Ostwald, S et al., 1983).

Depending on the chemical compound composition and origin fertilizers are classified as organic and inorganic fertilizers (H. Sabry, 2015). Inorganic fertilizers are produced from non-renewable resources such as coal, natural and rock mineral, and are also known as mineral or chemical fertilizer (Liyuan L *et al*.,2020). Besides, inorganic fertilizers contain mineral salt which acidifies the soil, repel it, and do not deliver a food source for microorganism, and extremely it has a great impact on human health and the environment (Mohammednur, 2020). Organic fertilizers are one of the most important fertilizers used to evade the use of chemical fertilizers for crop production (Chandini et al., 2019). They are produced from several waste materials and by-products such as animal manure, municipal soil waste, and sewage sludge are present, being used in agriculture crop production (Sudharmaidevi *et al*., 2017). It is also produced from keratin-based material such as human hair, tannery hair, chicken feather, wool, horns, and nails (Donato and Mija, 2020), and the waste banana peel (Hussein *et al*., 2019).

In most parts of the world, the total mass of sheep wool, is considered a useless waste material, and its accumulation in the waste stream causes several environmental contamination problems (Birhanu, 2018). It stays in the dump due to its slow degradation and occupying a large space. Accumulation of waste wool for a long time increases the nitrogen concentration in the aquatic bodies, causing a problem on aquatic life. Besides, open dumps of hair generate hair dust which causes embracement to people around them and gives several respiratory problems (Birhanu, 2018). Currently, the world's fourth most important food crop after rice, wheat, and maize are desert banana (Tadele, 2019). Banana is one of the most important crops for foreign exchange income for many countries, particularly, for Africa, Asian, and Latin American countries (Kahsay, 2018). Desert banana is also the major fruit crop that is widely grown and consumed in Ethiopia (Tadele, 2019). About 18-33% of the whole fruit of banana is peel, and it was contained, potassium, phosphorus, calcium, and other minerals which are essential for plant growth (Hussein et al., 2019). They are disposed of as by-products and improperly thrown on the public road, flood passing ditch. For the development of economic and environmentally safe, it is fairly desirable to develop effective and lucrative methods to use these resources. Besides, tanning industries and slaughterhouses generate waste Wools as a by-product and disposed to the environment. Though the leachate from these wastes is mixed with water and causes water contamination and eliminates the microorganism by increasing the nitrogen concentration. Wool is organic material that contains nitrogen elements used as a primary nutrient for plant growth and other elements that have been used as potential soil amendments. Moreover, the wool contains (10 – 17%) cysteine residue and it was insoluble fibrous protein in a most organic solvents, water, weak acid, and base (Peng and Zhang, 2019).

Nowadays, the fertilizers from keratin materials are produced by chemical (strong acids *(Patil et al.*, 2020; Popko *et al.*, 2015) or alkalis (Asha *et al.*, 2016; Mohammednur, 2020; Nustorova, 2014) and /or hydrothermal treatment (Nurdiawati *et al.*, 2017)**.** Alkali hydrolysis was used to recover tryptophan amino acids and while acid hydrolysis was not (Deban et al., 2018; Mohammednur, 2020) . In addition, keratin based materials were contains low composition of potassium, phosphorus and other nutrients that are essential for plant growth (Möller, 2014). (Möller, 2014) also stated that, keratin materials increase imbalances in nutrient composition of fertilizer. Though, the purpose of the study was to produce and characterize the organic liquid fertilizer by combining banana peel (to improve the level of potassium, phosphorus and other nutrients) and sheep wool. Besides, the study was carried out using NaOH in alkali hydrolysis, to degrade the disulphide bonds of wool keratin and in order to recovery the amino acid such as tryptophan in the wool and banana peel.

1.2 Statement of Problem

Currently, due to the increase in agricultural production, the demand for fertilizer is intensely increasing all over the world. Though, most of the commercial fertilizers are produced from non-renewable resources such as coal and natural gas. Hence, they have skirmished with the environment and physicochemical properties of soil. Besides, to overcome such problems, the researchers are daily finding other biomass feedstocks that are useful for the production of fertilizers. Tanning industries and slaughterhouses, generate a significant amount of sheep wool and, indecorously throw it into the garbage, roadside, drainage system, and municipal waste stream. Due to the wool contains disulfide bond, it stays for longer periods and lodging large volume of space leftover, as well as leachate from these wastes, rises the nitrogen concentration in the water bodies, roots eutrophication and water pollution. The major way to overwhelm such a problem is to develop a way to employ this waste material as a resource. As potential material resource wool waste has a benefit as it is renewable and available in every vicinity. Many researchers have been produced organic fertilizers from keratin-based material such as, Chicken feather, human hair, wool and etc. Keratin materials have high nitrogen and sulfur contents and are extremely low in potassium and phosphorus contents, and it increases imbalances in nutrient composition (Möller, 2014). However, according to food and agricultural organization the recommended values of primary nutrient in organic fertilizer Nitrogen ≥ 1%, phosphorus ≥ 1.5% and potassium ≥ 1.5% (Ayilara et al., 2020). In addition, banana peels are mainly obtained as, waste products from restaurants and juice processing houses in the town. Besides banana peels have a potential sources of potassium, phosphorus, other macro, and micronutrients (Akter et al., 2021; Durgo & Mandura, 2020; Hussein et al., 2019; Mayur Dattatray Khairnar, 2020) which are essential for plant growth.

Therefore, to minimize the environmental pollutions by waste wool, to improve the level of macronutrients in fertilizer such as potassium, phosphorus and other nutrients contents in organic fertilizer by adding banana peel, to meet green environment and gain commercial value of waste wool and banana peel, a great attention must be given on valorization of sheep wool and banana peels for production liquid organic fertilizers.

1.3 Objective

1.3.1 General objective

The general objective of the study was to produce and characterize organic-liquid fertilizer by the combining sheep wool and banana peel.

1.3.2 Specific objective

- To determine the proximate (moisture content and ash content) of waste sheep wool and banana peel
- To determine the optimum process parameter, (temperature, time, and concentration of NaOH solution) for maximum production of protein yield.
- To determine the total concentration of primary nutrients (nitrogen, phosphorus, and potassium), the total concentration of secondary nutrients (calcium, magnesium, and sulfur), and micronutrients (iron, zinc, copper, and manganese) in produced fertilizer.
- To study the effect of mass fraction of wool and peel on pH of produced fertilizer.
- To determine density, EC, C/N ratio, TOC, and functional groups of (wool, peel and produced fertilizer).

1.4 Significance of the study

This study has two core aids that can be applied in practical experiments as guidance and as a contribution for further researches. Its practical benefits include valorization of these two waste materials to produce an improved nutrient level of organic fertilizer from sheep wool and banana peel, and it can be used as a base for scale-up production of organic fertilizers. The other benefit includes using this research as input for another researcher. It can be used as a base for researches focused on ways to improve the nutrient level of organic fertilizers from waste materials.

1.5 The Scope of the study

The study was generally covered lab-scale production of organic liquid fertilizers from sheep wool and banana peel. This includes the collections of sheep wool and the banana peels. The optimum protein yield obtained from sheep wool by alkali hydrolysis was covered. Determining the model equation and the optimum condition such as extraction temperature, time, and concentration of NaOH extraction of protein yield. The research also covers producing fertilizers, using wool sheep and banana peel to improve the nutrient level of the fertilizers. It also covers the characterization of produced organic fertilizers such as FTIR analysis, primary nutrient, secondary nutrient, and micronutrient analysis, total organic carbon, electrical conductivity, and pH analysis.

2. LITERATURE REVIEW

2.1 History and worldwide use of fertilizer

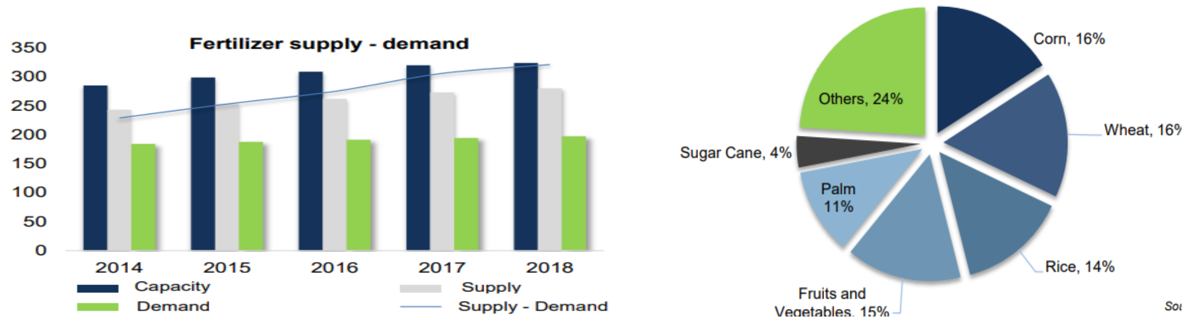
Fertilizer is the chemical composition of several vital minerals and elements that are used for soil amendment and enhancing plant growth. A different scientific researcher has defined fertilizer in different ways. Webster dictionary defines fertilizer as any of various a substance that is added to the soil to enhance the growth of plants (Hazra, 2016). Plants are grown, by extracting the nutrients they need from the soil (Ayilara *et al*., 2020). It was also used for distributing an important nutrient for plant growth and nutritive value (H. Sabry, 2015; Kang, 1995).

The development of fertilizers can be viewed as a natural and synthetic or chemical origin that is useful to soil or to plant tissue as the source of plant nutrients. Historically, natural fertilization came from natural sources such as compost, animal manure, crop rotation, and by-products of human-made industries (fish processing waste or bloodmeal from animal slaughter). The first chemical or synthetic fertilizer was phosphorus-based and it was invented in the early 19th century, which was composed of superphosphate that was made by treating bones with Sulphuric acid (Williams., 2010). In early formulation, the bone materials in phosphorus fertilizer were replaced with phosphate rock and coprolites. The potassium fertilizer was invented in 1861 in Germany and between 1914 to 1919 in Northern America. The use of this fertilizer was expanded when potassium deposits were exposed in 1931 in New Mexico and 1958 in the Saskatchewan territory (Williams., 2010).

In 1903 the first nitrogen fertilizer and calcium nitrate were developed. In 1945 the use of NPK fertilizer has been gradually increased (Williams., 2010). This transition was vital in renovating the worldwide food system and agreeing to larger-scale industrial agriculture with great crop yields (FAO, 2021). However, synthetic fertilizer used in agriculture has given wide attainment environmental consequences (H. Sabry, 2015). The most serious problem with chemical fertilizers is groundwater pollution; while nitrogen is water-soluble and can stay in groundwater for long periods, the addition of more nitrogen over time has an accumulative effect (Hazra, 2016). According to the IPCC report on climate change and land, the production of inorganic fertilizer and the associated land-use applications are significant contributors to global warming (FAO, 2021). This is also true in Ethiopia, where it is believed that chemical fertilizer-based rigorous production has physically deteriorated and biologically deactivated the soil. Crop production specialists, researchers, and especially political leaders are perplexed about the relationship between fertilizer consumption and crop productivity (Legesse et al., 2019). They believe that synthetic fertilizer alone can increase yields and encourage its unwarranted use for poverty alleviation, even though the negative impact of fertilizers on soil and climate is frequently treated as external costs that must be accepted (Reda & Hailu, 2017). Currently, due to its potential use in agricultural sectors, and environmental protection is very important in an industrial world, different researchers and industries have started to produce organic fertilizer from different organic matters.

2.2 Market size of world fertilizers industry

According to IFA, fertilizer demand increased by 3.1 percent from 2013 to 2014, reaching 184 billion tons. It was stated that fertilizer production in 2014 was 243 million tons, accounting for 85 percent of global production capacity. This increase in the number of transactions and consumption boosted factory productivity all over the world. This trend is expected to continue through 2018, when total supply and fertilizer demand will be 197 and 280 billion tons, respectively, up 82 percent from 2014. Figures 2.1 shows the world fertilizer using supply-demand and fertilizer demands by crops (Doan, 2018).



a) b)

Figure 2. 1 world fertilizer a) supply-demand size, and b) fertilizer demand by crops (Doan, 2018).

2.3 Consumption of fertilizer in Ethiopia

Fertilizer was used to supply nutrients for plant growth as well as to improve the soil's condition. The nutrients could be obtained by cycling them through grassland and crop residue decomposition. However, due to the total clearance of leftovers after harvesting for livestock feed, recycling of these waste materials is challenging in Ethiopia. The use of chemical fertilizer increases as crop residue is removed, and the amount of chemical fertilizer consumed in Ethiopia has increased dramatically. Ethiopia was one of the Sub-Sahara African countries that imported the most primary nutrients, according to the FAOSTAT online database (Shahidur Rashid and Nigussie Tefera, 2013). The consumption of fertilizer in Ethiopia was very remarkably increased since the start in 1960 (Reda and Hailu, 2017).

According to AISE, the fertilizer imports and use in Ethiopia were rapidly increased. Fertilizer imports have increased by 124 percent from 346,000mt in 2004/5 to 778,000mt, the average annual imports of 2012 and 2013 which indicates that the higher-level fertilizer imports compared to farmer use (Bachewe et al., 2015). The application rate on average increased from 66kg per hectare in 2003/04 to 104 kg per hectare in 2015/2016 (Legesse et al., 2019). However, nowadays chemical fertilizer-based exhaustive production in Ethiopia is believed to physically decline and biologically deactivate the soil (Reda and Hailu, 2017).

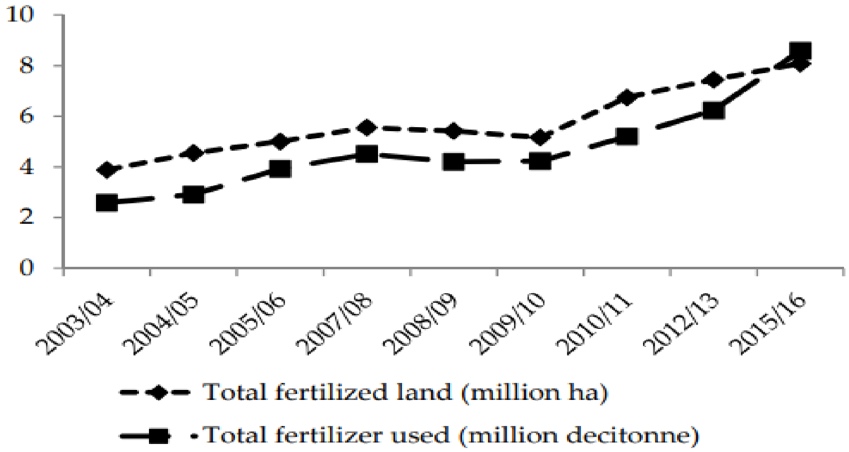


Figure 2. 2 Total fertilizer use and fertilizer land (Legesse et al., 2019).

2.4 Plant Nutrients

Nutrients are a substance that is used to improve the soil fertility and crop quality of plants. The researcher has determined that 17 nutrients are essential elements (Nabwami, 2015). Among 17 essential elements, three of them such as carbon, oxygen, and hydrogen are derived from water and air and are also known as non-mineral nutrients. The remaining 14 mineral nutrients include six macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and Sulphur, and eight micronutrients including, manganese, molybdenum, copper, zinc iron chloride, and boron (Mohammednur, 2020)

2.4.1 Macronutrient

Macronutrient fertilizers are classified as primary macronutrients (nitrogen, potassium, and phosphorus) and secondary macronutrients (calcium, magnesium, and Sulphur) (Hazra, 2016).

I) The function of Primary Macronutrient Elements in Plant Nutrition and Growth

a) Nitrogen (N)

Nitrogen elements are needed by plants in greater amounts than, other primary nutrient elements (such as potassium and phosphorus) (Mohidin et al., 2015). It is important for, promotion rapid growth, increasing leaf sizes, and quality forms an amino acid, which is an essential component in plants and building blocks of protein and enzymes, ensuring the development of fruit and seed, plays a crucial role in the metabolic process and influences the rate of crop growth and quality (Nabwami, 2015).

b) Phosphorus (P)

Phosphorus is the most vital nutrient for the growth of all living species. It is the second most important nutrient after nitrogen, and it is found in numerous organic compounds' such as DNA, RNA, ATP, and phospholipids. Pathogens are important in plant metabolic processes, seedling, and fungal illnesses, soil-borne diseases, virus diseases, and decorative crop plants (Bhaduri and Chakraborty, 2014). Phosphorus also serves as an activating catalyst for amino acid synthesis, decomposes carbohydrates created during photosynthesis, and elaborates other metabolic processes necessary for optimal growth, including photosynthesis, glycolysis, respiration, and fatty acid production. ATP has a high-enzyme activity. For instance, uptake of nutrients and their transport within the plant and their assimilation into different biomolecules are required ATP energy for the plant process. Among the role and qualities of plants on which phosphorus are; increases features of plant physiology, such as respiration and photosynthesis, enhances seed germination and growth, stimulates blooming, root growth, and increases the quality of certain crops (Mohammednur, 2020).

c) Potassium (K)

Potassium is the plant nutrient that is vital for plant growth, physiology, regulate function in biochemical process related to protein synthesis, carbohydrate metabolism, enzyme activation and plays a crucial role in various abiotic stress such as drought, salinity, metal toxicity, and high temperature (Hasanuzzaman *et al*., 2018). The crop yield has been influenced by favorable and unfavorable environmental conditions where Unfavorable conditions would create potentially damaging physiological changes with plants known as stress. Hence potassium has taken place, in physiological function deals, with plant health and resistance to biotic and abiotic stresses such as diseases, pests, drought, salinity, cold, and frost (Nabwami, 2015; Rawat *et al*., 2016).

II) The function of Secondary Nutrients in Plant

Functions of each secondary nutrient in the plant were perceived in Table 2.1

Table 2. 1 The function of secondary nutrients in plant growth. Sources (**Bhaduri and Chakraborty, 2014**).

Secondary nutrients	Function in plant growth
Sulfur (S)	✓ To synthesis sulfur-containing amino acids ✓ To form disulfide bonds between a cysteine residue ✓ To synthesize coenzyme and chlorophyll
Calcium (Ca)	✓ Strengthen the cell wall ✓ Involved in cell elongation and division, membrane permeability ✓ Act as detoxify agent by neutralizing organic acids in plant ✓ Improve soil structure by increasing water penetration and providing a favorable soil environment for the growth of plant roots and soil microorganism
Magnesium (Mg)	✓ Act as an enzyme activator in the synthesis of nucleic acid (DNA and RNA) ✓ Regulate the uptake of the other essential nutrient element as a carrier of phosphate compound throughout the plant.

	✓ Translocated to active growing parts of the plants for chlorophyll formation, enzyme activation for protein synthesis

2.4.2 Micronutrient

Micronutrients are one of the nutrients that are used to sustain the development of plant growth. The function of each micronutrient in plants were discussed in Table 2.2.

Table 2. 2 The function of micronutrients in plant growth. Source (Alloway, 2008).

Micronutrient	Function in plant growth
Manganese (Mn)	➢ Function in part of plant enzyme system, activating several metabolic functions ➢ Activate indole acetic acid oxidase and oxidize indole acetic acid
Molybdenum (Mo)	➢ Is a necessary component of two major enzyme ➢ In plants, nitrate reductase and nitrogenize ➢ Required for some soil microorganisms for nitrogen fixation
Iron (Fe)	➢ Essential in synthesis and maintenance of chlorophyll in plants ➢ Associated with protein metabolism ➢ Important in hem enzyme system of plant metabolism
Zinc (Zn)	➢ Important for the synthesis of tryptophan ➢ Has a role in RNA and protein synthesis

	➢ Used to anhydrase carbonic enzyme
Copper (Cu)	➢ Essential for synthesis and stability of chlorophyll ➢ Takes place in plant enzymes in photosynthesis
Chlorine (Cl)	➢ Increase cell osmotic pressure and water content of plant ➢ Reduce the harshness of fungal disease
Boron (B)	➢ Used to the synthesis of RNA formation and in cellular activities ➢ Used in root growth, pollen germination, and growth of pollen tube

2.5 Types of Fertilizers

Depending on the chemical compound and its origin, fertilizers are classified into synthetic or inorganic and organic fertilizers (H. Sabry, 2015; Jaja and Barber, 2017).

2.5.1 Inorganic Fertilizer

Those fertilizers which are constituted by the inorganic chemical substance are mentioned as inorganic fertilizers, i.e., granular triple superphosphate, potassium chloride, urea, anhydrous ammonia, etc. These fertilizers are typically non-biodegradable (Hazra, 2016). Synthetic nitrogen fertilizers are made from natural gas and petroleum products, as well as phosphorus and potassium fertilizers made from rocks (H. Sabry, 2015). A large number of inorganic fertilizers have been applied to increase the worldwide crop yield (Purbajanti *et al.*, 2019). However, the long-term irrational application of inorganic fertilizers increases a variety of undesirable impacts, on the environment and the physicochemical properties of soil such as degradation of soil organic carbon (SOC)and soil acidification (Gautam, 2019). Besides, environmental pollution, persuaded by the excessive application of organic fertilizers, has awakened extensive attention (Liyuan L *et al.*, 2020).

2.5.2 Organic Fertilizer

Organic fertilizers are those fertilizers that are produced using a biodegradable organic substance. It is a naturally occurring fertilizer and nutrient enhancer of the soil (Hazra, 2016). Organic fertilizers are nutrient-rich fertilizers that are primarily derived from organic materials such as agricultural by-products, plants, and animal husbandry (manure and litter). The physicochemical properties of the soil can be modified by using organic fertilizers. This fertilizer releases nutrients into the soil environment to improve soil properties (Larramendy, 2019). They contain a large amount of organic matter (Indoria et al., 2018), and it is better for the nutrient recycling of soil and the reduction of environmental remediation (Liyuan L et al., 2020). Organic fertilizer is made in numerous forms like solid organic fertilizer and liquid organic fertilizer. Solid organic fertilizer products, such as compost and manure. When, compared with solid organic fertilizer, Liquid organic fertilizers the abundant organic matter and soluble nutrients could maintain soil sustainability and plant health. Specialized horticultural production has fostered the emergence of new liquid organic fertilizers. In addition, the integration of watering and fertilization patterns could improve nutrient use efficiency and decrease the risk of nutrient loss. Moreover, the special compounds in liquid organic fertilizers, such as chitin, humic and fulvic acids, and amino acids, can be Biostimulants to plants (Mohammednur, 2020).

Types of Organic Fertilizers

Organic fertilizers are classified as naturally occurring and manufacturing organic fertilizers (Hazra, 2016). Naturally occurring organic fertilizers are produced from, animal manure, slurry waste, peat, seaweeds, sewage, guano waste, and other bio-degradable, and whereas manufacturing organic fertilizer, those that are manufactured by decomposition of natural waste materials such as compost, blood meal, bone meal and seaweed extract (Hazra, 2016).

Raw Material Used for Production of Organic Fertilizers

Currently, to sustain a green environment and to minimize the use of chemical fertilizers, researchers have started to produce organic fertilizers from different waste organic raw materials. Many organic materials from different animal sources such as manure, keratin materials such as hoof and horn (Choi & Nelson, 1996), poultry feather (Chaitanya et al., 2021; Nurdiawati et al., 2017; Zul et al., 2020), human hair (Kabir, 2016; Pathamesh Kumar, 2017; Patil et al., 2020), sheep wool (Ghosh & Collie, 2014), blood meal (Jeon et al., 2013), bone and combined solid wastes (Cruz et al., 2006) are evaluated as a fertilizer production source. Keratin is derived from the Greek word "Kera," which means "horn," and it is a fibrous protein that is rigid. It is the third most abundant polymer in the ecosystem, following chitin and cellulose (Chaitanya et al., 2021). It is a fibrillar protein family with a compact structure in which a large number of disulfide bonds influence its properties, specifically its mechanical and chemical resistance (S Pawel et at., 2011). They are abundant as a byproduct of the poultry and tanning, processing industries. In comparison to other fibrous proteins such as collagen and fibroin, keratin contains a large amount of cysteine residue with a thiol group, resulting in a fibrous protein (Buciscanu, 2016; Mckittrick *et al*., 2012).

Production and Consumption of Sheep

Sheep is one of the smallest ruminant livestock is highly produced in the world. Ethiopia has also produced around 26.1 million sheep for meat consumption (Shenkute, 2009). Correspondingly, (Birhanu, 2018) states that due to its slow degradation sheep wool stays in the dumps /waste streams, while after a long time its accumulation increases the nitrogen concentration in the water bodies which result in problem on aquatic life. Besides, High waste accumulation of sheep wool from slaughterhouses and industrial areas, is normally incinerated or goes to landfill sites. However, incineration is an expensive disposal method and causes air pollution, and using landfills for a material that has many uses is not economically feasible.

Production and consumption of banana

Banana is also a major fruit crop that is highly produced in the world. The world production of banana peels was around 106,541,709 tons (Tadele, 2019). It is the major fruit crop that is most widely grown and consumed in Ethiopia. It is cultivated in several parts where the growing conditions are favorable. About 18-33% of the whole fruit of the banana was peeled (Hussein et al., 2019). A banana peel, also called banana skin in British English, is the outer covering of the banana fruit. Banana peels have a mineral that are essential macronutrient elements such as calcium, potassium, magnesium, phosphorus, and sulfur, and micronutrient elements such as iron, zinc, iodine, copper fluoride, sodium, and manganese (L Syukrian et al.,2021). Minerals are essential elements for optimal body metabolic function. But, currently in most parts of the world banana peel was not being used for any other purposes and is mostly dumped as solid waste at a large expense (Hussein et al., 2019). With the high demand and increase in daily consumption of bananas, there is a high waste accumulation of banana peel from the household, agricultural areas, and industrial areas, which are normally used as animal feed, incinerated, or go to landfill sites. However, incineration is an expensive disposal method and causes air pollution.

Fertilizer Production Methods from Biomass

a) composting method

Composting is a recycling process in which organic materials are biologically converted into amorphous and stable humus-like substances (under conditions of optimum temperature, moisture, and aeration) that can be handled, stored, and applied to land without environmental effect (Ayilara et al., 2020). It is defined as a decomposable stabilized and mineralized humus transformation process by bacteria, micro-, and higher-level organisms of decomposable organic constituents (agricultural, urban commercial, etc. wastes) in solid wastes and it is not a fertilizer but is used only for the structural improvement of the soil (Indoria et al., 2018). Yet, it is possible to obtain fertilizer of superior quality by adding enough nitrogen, phosphorus, and potassium to the compost (Argun et al., 2017).

The process of composting takes place in phases determined by temperature that results from reactions. At 45°C mesophilic phase that takes 2-8 days starts before thermophilic and hygienization at 60 °C involving bacteria degradation of cellulose and lignin transforming nitrogen to ammonia sets in for a period ranging from weeks to months (Ayilara et al., 2020). Once the carbon and nitrogen are exhausted temperature drops to 40-45°C leading to the cooling phase in which there is continued polymer degradation of cellulose temperature and involves condensation and polymerization occurring to form humic and fulvic acids (Peter, 2020).

b) Hydrothermal Method

Hydrothermal treatment is used to degrade the keratin protein at a steam pressure of (10 – 15 psi) (Karthikeyan and Srinivasan, 2014) and temperature (140 -200)°C (Nurdiawati *et al.*, 2017) in the presence of acid (HCl, H_2SO_4, HCOOH) or alkali (NaOH, KOH, Na_2CO_3, K_2CO_3 particularly from keratin-based materials. The principle for extracting keratin is to break down the sulfur bonds in cysteine into hydrolysate soluble peptide products to process a large number of their chain reaction (Rahmawati and Griyanitasari, 2017). In the hydrothermal method, the disulfide linkage of keratin is cleaved by treatment with acid or alkali at the boiling temperature for over 2 -3 hr, and water-soluble polypeptide, oligopeptide, and amino acids are produced (Tewodors, 2017). The main disadvantage of the hydrothermal treatment process is that it results in the partial or complete destruction of amino acid, which contains peptides, with varying molecular weight and nutritional enhancement (Karthikeyan and Srinivasan, 2014), require high thermal energy and pressure for keratin degradation, yielding a product with poor digestibility and variable nutrient quality (Chaitanya *et al*., 2021).

c) Hydrolysis Method

Hydrolysis is a process, where complex molecules are cleaved by the addition of water molecules. It can be achieved with water, with various acid, alkaline solutions, enzymes, heat, and increased pressure can be used in some cases (EGTOP, 2014).

1) Enzymatic hydrolysis

Enzymes are the biochemical catalyst that used for the degradation and valorization of keratin, under certain environmental conditions like, pH, Temperature and ionic strength. Furthermore, enzymatic hydrolysis of proteins is become a complex process, due to several peptide bonds and their specific availability accessibility to enzymatic reaction. The process of obtaining soluble keratin requires either pure keratinous isolated from microorganisms and run under the mild condition which assisted by a chemical reducing agent to degrade disulfide bond (Staroszczyk and Sinkiewicz, 2017). Furthermore, the enzymatic activity yield of soluble keratin is too low to make the enzymatic process suitable for industrial application, requires a long time for completion of the degradation, and is highly expensive (Chaitanya *et al*., 2021), a slower process that makes its commercial application more difficult (Sharma, 2019).

2) Chemical hydrolysis

The chemical hydrolysis method is common in most biomolecule and polymers hydrolysis processes. Chemical hydrolysis includes acid and base hydrolysis. keratin extraction by chemical hydrolysis requires low pH (below 3) for acids and high pH (above 10) for alkali hydrolysis (Mohammednur, 2020). Acid and alkali hydrolysis keratin protein converting keratin fibers into their amino acids. Degradation of keratin fibers by acid hydrolysis of peptide bonds results in the loss of tensile strength. The degradation of protein under acid conditions involves attacking the water molecule on the protonated amide, and the degree of hydrolysis depends on the concentration of acid and alkali solution. The chemical hydrolysis method is used to reduce the temperature, pressure and shorten the time of the process (Banach *et al*., 2014). Besides keratin extraction by chemical hydrolysis are depends on the time of reaction, reaction temperature, pH, and concentration of reagent (Birhanu, 2018). (Mohammednur, 2020) was studied the effect of KOH concentration on the hydrolysis of human hair. He stated that keratin extraction from the solubility of human hair increases as alkali concentrations were increased (0.25 – 0.75) w/w% due to the activation energy is increase. Alkali hydrolysis was used for the quantitative determination of tryptophan, while acid hydrolysis is not (Debananda S*et al*., 2018). The advantage and disadvantages of each method were discussed in Table 2.3.

Table 2. 3 The advantages and disadvantages of different methods

Methods	Advantage	Disadvantage	Reference
Composting	Used to collect waste materials	✓ Require high period from month to year ✓ Give unpleasant odor ✓ Environmental impacts of pathogen	Peter, 2020.
Hydrolysis: - i. enzymatic	✓ Run under mild condition	✓ Require a long time for completion of the process ✓ Slower process and difficult for industrial application	Staroszczyk and Sinkiewicz, 2017; Sharma, 2019.

i. alkali	✓ Gives Higher yield ✓ Used to reduce pressure, temperature, and processing time ✓ Used to recover tryptophan amino acids	✓ require a strong base for keratin degradation ✓ require high pH (above10)	Mohammednur, 2020; Debananda S et al., 2018.
ii. acid	✓ Gives higher yield ✓ Used to reduce pressure, temperature, and time of extraction process	✓ Require strong acid for keratin extraction ✓ Destruction of tryptophan amino acid ✓ Require low pH (below 3)	Mohammednur, 2020.
Hydrothermal	✓ Used to reduce the time for degradation	✓ Consumes high thermal energy ✓ require high pressure ✓ loss of amino acid during extraction	Karthikeyan and Srinivasan, 2014; Chaitanya et al., 2021.

2.6 Current study on the production of organic- fertilizer from different biomass

Nowadays different researchers and commercial industry, have started to produce organic fertilizers from waste biomass, to mitigate the environmental pollution caused by waste biomass and to reduce the use of synthetic fertilizers. (Patil *et al*., 2020) introduce a process to extract organic fertilizer from human hair. They stated that the extraction of liquid organic fertilizer from human hair requires concentrated hydrochloric acid (0.05N HCl) and a stirrer for 15 minutes. The pH of an extracted solution is adjusted by adding 0.1N of NaOH, the separated solid residue was washed by distilled water and dried. The existence of different amino acids in the hydrolysate liquid was detected by the amino acid test. (Unnikrishnan and Ramasamy, 2020), study the extraction of liquid organic fertilizer from human hair and its efficient application on the growth yield of Abelmoschus esculentus. They argue that extracted liquid fertilizer from human hair has contained, 0.12% of nitrogen, 1.763% of phosphorus, 4.158 % Ca, 2.749% magnesium, 3.144% of iron, 0.085% of manganese, and 0.09% of copper.

(Nurdiawati *et al*., 2017) were extracted liquid organic fertilizer from chicken feathers. Hydrothermal treatment was used to obtain liquid organic fertilizer from chicken feathers. They stated that the extraction of organic fertilizer from chicken feathers requires high temperature (140 -200) ℃ and high pressure (0.36 – 1.53) MPa. But high, temperature consumption is reduced to 180℃ if a lime solution is added to the feather. The result of the study shows 83% yield of protein in liquid solution was obtained at 180℃ and 30 minutes after the lime solution is added. The nitrogen present in an organic account was 84.0 – 93.5%. at the higher temperature, more inorganic N compounds formed due to further breakdown of N compounds. (Popko *et al*., 2015) study the production of liquid organic fertilizer production from a chicken feather in a pot experiment. The feather waste was hydrolyzed by 30% of H_2SO_4 and the process was carried out for four hours at 80℃. The pH of the hydrolysate liquid was adjusted to 6 by magnesium oxide. The result of the study shows different quantities of macronutrients and micronutrients in three products. According to the study, the product was contained, 14.8% of Nitrogen, 0.061% of phosphorus, 0.056 % of potassium, 0.168 % of calcium, 0.034 % of magnesium, 2.35 % of sulfur, and a significant number of amino acids in the protein. (Nustorova, 2014) extract organic fertilizer from waste sheep wool by alkali hydrolysis. The hydrolysis was done using 0.15 MKOH -0.05 M NaOH, at 120℃ for 20 min. The pH of the solution was adjusted by H_3PO_4. The researcher claimed that the protein content product is 78.8%. The summary of the reviewed articles were discussed in Table 2.4.

Table 2. 4 Summary of the reviewed articles

Raw materials	Level of Primary nutrient			Article
	N (%)	P (%)	K (%)	
Human hair	0.12	1.673	-	Unnikrishnan and Ramasamy, 2020
Human hair	-	-	-	Patil et al., 2020
Sheep wool	-	-	-	Nustorova, 2014
Chicken feather	15.29	0.02	0.013	Nurdiawati et al., 2017
Chicken feather	14.8	0.061	0.056	Popko et al., 2015

Appreciation for researchers, for their initiation to produce organic fertilizers from biomass-based materials to sustain our green environments. However, the reviewed articles have gaps that were not filled by the researchers. As revealed from Table 2.4, the composition of potassium and phosphorus elements in produced fertilizers is very low compared to nitrogen elements. The amount of primary nutrients obtained under this article have still deviated from the standard values of primary nutrients in organic fertilizer that, recommended by Food and agricultural organization, and most fertilizer production industries. So, the above gaps initiate the researcher to fill the gaps.

The study was to produce balanced nutrient-based organic fertilizer from combination of sheep wool as a source of nitrogen nutrients, and banana peels to improve the level of potassium, phosphorus, and other mineral elements which are essential for plant growth.

3. METHODOLOGY

3.1 Materials

3.1.1 Raw materials and chemicals

The raw materials used for the experiments were sheep wools and banana peels. The chemicals used included copper sulfate (99.5%), sodium hydroxide, potassium sulfate (99.5%), boric acid, ethanol (97%), methyl red, bromo cresol green, hydrochloric acid, sulphuric acid (98%), hydrogen peroxide (30%), potassium bromide (98.5%), acetone, diphenylamine, and ferrous ammonium sulfate. All the chemicals were analytical-grade, and were produced by Loba chemie PLC (India).

3.1.2 Equipment

The major equipment that used in this study were include, beaker, conical flask electronic weight balance, water bath, measuring cylinder, pycnometer, scissors, drying oven, electric heater, plastic bag, pH meter, EC meter, soxhlet extractor, fourier transfer infrared radiation (FTIR), nitrogen and protein analyzer, atomic absorption spectrophotometer, UV spectrophotometer.

3.2 Experimental procedure

3.2.1 Sample collection and pretreatment

The waste sheep wools were collected from Jimma town’s Abattoir, and banana peels were collected Jimma town Ginjo Guduru kebele. The collected samples were washed and cleaned by using tap water to remove the impurities. The cleaned wool and peel were dried by an oven dryer at 105℃ for 24 hours.

3.2.2 Sample preparation

After collecting the sheep wool and banana peels, the collected samples were washed with tap water to remove the impurities. The dried samples were cut into small segments using scissors (sheep wool) at a size of 3 -6mm and ground (banana peel) using mortar.

Soxhlet extraction

The blood and greasy in wool were removed using soxhlet extraction for six hours by acetone. After residual impurities were removed, the wool was washed using distilled water to make it free from the residue of acetone.

3.2.3 Proximate analysis of sheep wool and banana peel

a. Moisture contents

Three different empty petridish were measured by using digital mass balance. 5g of each sample were measured by digital balance and put on the measured petridish. The petridish with all samples were taken to the oven, dried for three hours at 105^{o}c. The dried samples with Petri dish were measured and their mass was recorded. A measured sample with a Petri dish was returned to the oven at the same temperature for 30 minutes. Finally, the process was continued until the constant weight was reached.

The moisture content of the samples was calculated using equation 3.1.

$$\text{Moisture (\%)} = \frac{w_i - w_f}{w_f} \times 100 \tag{3.1}$$

Where:

$w_{i,}$ - the initial weight of the samples

w_f - final weight of the samples

b. Ash contents

Ash content was determined according to AOAC methods (Abida Sul *et al*., 2017). Three different empty crucibles were prepared and inserted in the furnace and kept at 800 °C for 1 hour, after that the crucible was removed and, placed in desiccators until it cools at 150°C and their initial weights were measured. 5g of each sample were measured by putting on the separate crucible and kept in a furnace at 600°C for 2 hours and the weight of the ash where measure. Finally, the experiment where repeated three times to reduce the error made.

The ash content of the wool was calculated using equation 3.2.

$$\text{Ash (\%)} = \frac{w_f}{w_i} \times 100 \qquad (3.2)$$

Where: $w_{f,}$ - the final weight of the ashes

w_i, - the initial weight of the samples.

3.2.4 Keratin hydrolysis

The soxhlet extracted wool was dried, and, the hydrolysis was carried by considering the concentration of NaOH, the reaction time, and temperature factors. The samples were heated at a temperature of (40, 60 and 80)℃, for the time of (30, 45, and 60) min, and using a concentration of NaOH (0.5, 0.75 and 1) N. Different experiments was carried out by varying reaction temperature, and concentration. The pH of the hydrolysate liquids was measured and HCl was added to the solution until the pH of the hydrolysate liquid were reached 7. The solution of the sample was filtered and centrifuged at 10,000 rpm for 5 minutes, and the solid particles were removed. The percentage of protein was determined from Centrifuged samples by the standard method of AOAC using the Kjeldahl's technique (Mæhre et al., 2018). The experimental setup of protein extraction was mentioned in figure 3.1.

3.2.5 Experimental design

Data analysis was carried by design expert version 11.1.2.0 software tool. The tool was used for the optimization of process parameter and the response of the process were discussed from ANOVA. For hydrolysis, there were three factors such as temperature, NaOH concentration, and time, and their effect was analyzed by response surface method (RSM) using central composite design (CCD). Two levels of parameter were used, the percentage of protein in extraction was considered as a response of variables. The significance of main and interaction factors was determined by a design expert. The CCD contains 2^k factorial runs, k_c center runs, and 2k axial runs.

The total number of experimental runs was calculated using equation 3.3 (Montgomery, 1807).

$$N = 2^k + k_c + 2k \tag{3.3}$$

Where: N - is the total number of runs,

k -is the number of independent variables and

k_c - is the number of center points.

A 20-total number of experimental runs having six center points and six axial runs were designed using CCD to extract keratin protein from sheep wool. For the design of experiments, the lowest (-1), central (0), and the highest (+1) levels are specified in Table 3.1 below.

Table 3. 1 process parameters for protein extraction and their value

Independent variable	Factor coding	units	Coded level		
			-1	0	+1
Temperature	A	℃	40	60	80
NaOH concentration	B	N	0.5	0.75	1
Time	C	min	30	45	60

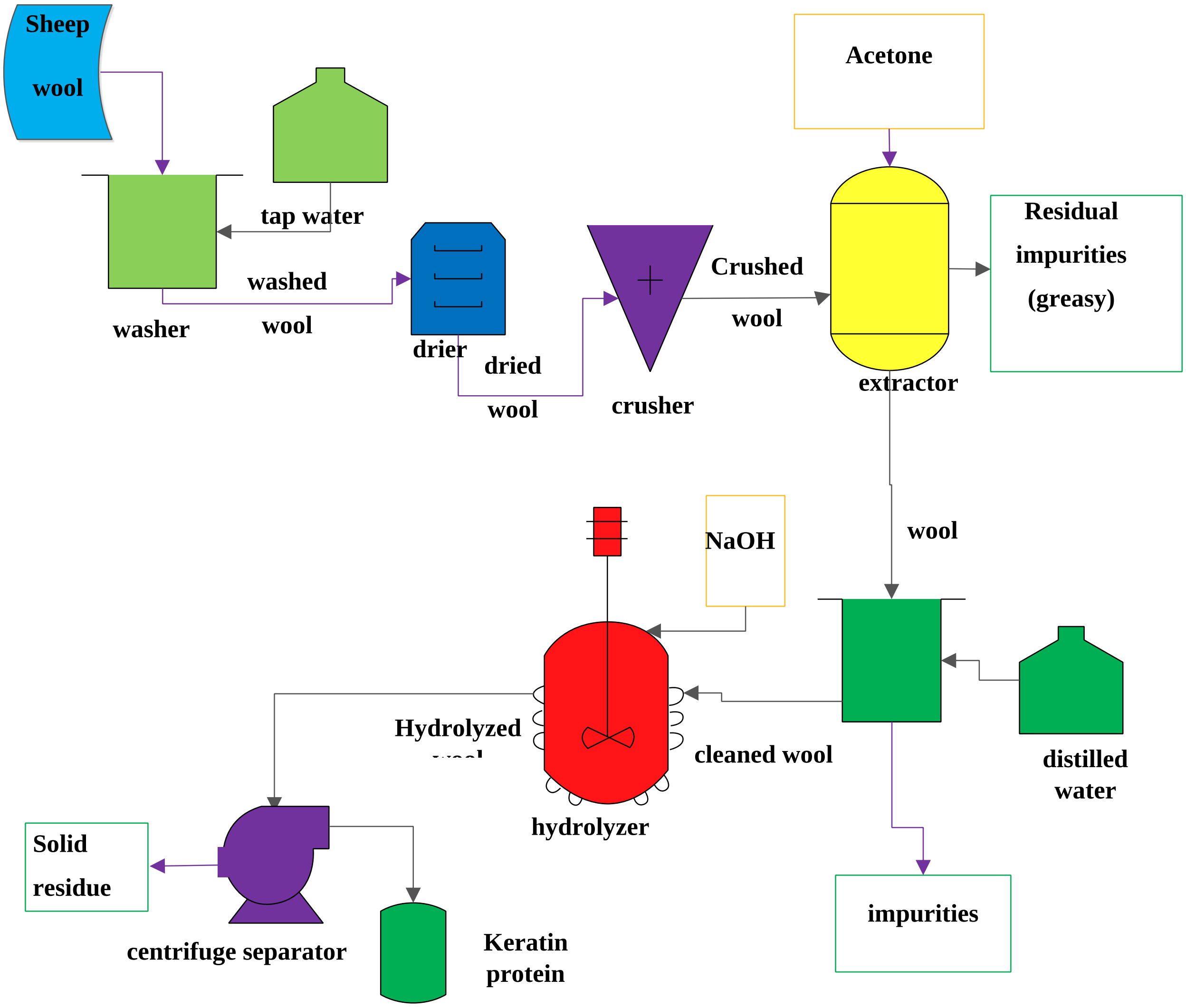


Figure 3. 1 Experimental setup for protein extraction

3.2.6 Preparation of samples for determination of protein content

I. Digestion

The analysis was done by Kjeldahl's method, which evaluates the total nitrogen content of the sample after it has been digested in Sulphuric acid. 10ml of the sample was weighed out into a 500ml Kjedhal flask. 7g potassium sulfate, 0.2 g copper sulfate, and 20 mL concentrated Sulphuric acid were added to measured samples. The sample was digested until a clear green color was obtained. The digest was cooled and diluted with 50ml of distilled water.

II. Distillation

1000ml of Kjedhal flask containing antidumping chips and 400ml of 40% NaOH was added to the flask containing a mixture of 4% boric acid and 3 drops of the mixed indicator were used to trap the ammonia being liberated. The conical flask and the Kjedhal flask were placed on the Kjedhal distillation apparatus with the tubes inserted into the conical flask. The heat was applied to distill out the NH_3 evolved with the distillate collected into the boric acid solution. The distillate was then titrated with 0.12N HCl.

The percentage of nitrogen was calculated using equation 3.4.

$$\text{Percentage N } (\%) = \frac{14 \times M \times Vt \times V}{weight\ of\ sample \times Va} \tag{3.4}$$

Were, M - Actual molarity of the acid

Vt- Volume of HCl used

V- total Volume of diluted sample

Va – aliquot volume distilled

Percentage of protein content determination

The percentage of protein content was calculated using equation 3.5 (Sáez-Plaza et al., 2013).

$$\text{Percentage of protein } (\%) = 6.25 \times percentage\ of\ nitrogen \tag{3.5}$$

Where 6.25 is a factor

Figure 3. 2 Schematic flow chart for protein determination using Kjeldahl’s method

3.3 Production of Liquid-Organic Fertilizer by combining sheep wool and banana peel

Proteins are contained nitrogen and different amino acids which were affected by different process conditions, it was essential to determine the optimum process parameters of protein yield extraction. After the optimum value of process parameters such as reaction temperature, Concentration of NaOH, and reaction time of maximum protein yield extraction was known, the fertilizer was produced by combining banana peel and sheep wool at a different mass ratio, using optimum process parameter at which maximum protein yield was extracted. The mass fraction of sheep wool and banana peel used in fertilizer production was ranged from 0 to 1. This variation of the mass fraction of sheep wool and banana peel at the same range was to fix the total mass of the sample. The produced fertilizer was separated from the solid residue by a vacuum filter and the product was analyzed. The composition of wool and peel used in the experiment were discussed in table 3.2.

Table 3. 2 The level mass fraction of wool and peel in the experiment

	Low level	center	High level
Mass fraction of wool	0	0.5	1
Mass fraction of Peel	0	0.5	1

The experimental setup of fertilizer production was shown in Figure 3.3

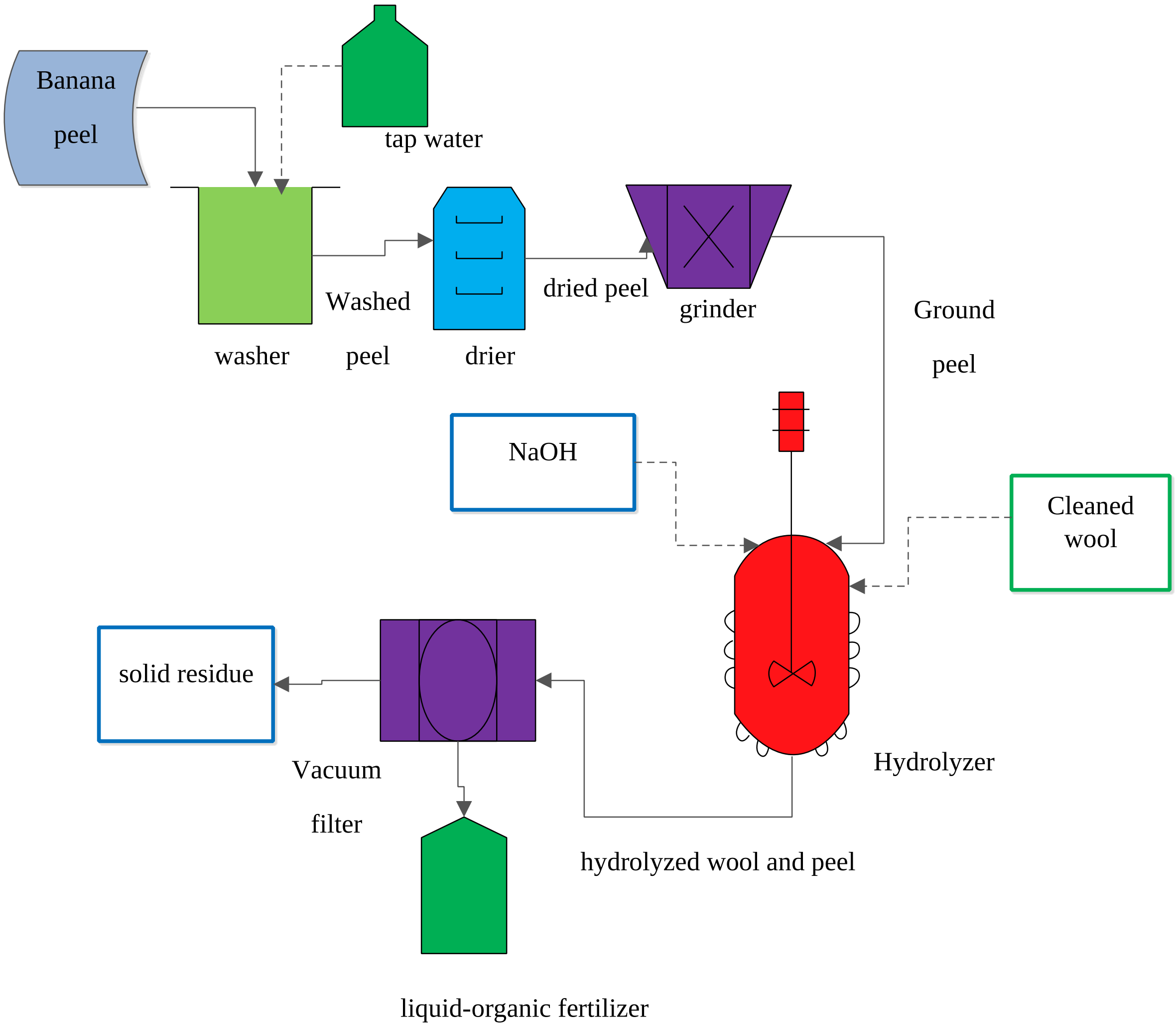


Figure 3. 3 Experimental setup for fertilizer production.

3.3.1 Determination of total concentration of primary, secondary, and micronutrients of the produced Fertilizers

The total concentration of primary, secondary, and micronutrients in the produced fertilizer was analyzed at Holeta Agricultural Research Center. Nitrogen was analyzed using the Kjeldahl's method, Phosphorus, and sulfur using U-V, and potassium, calcium, magnesium, copper, Zinc, Manganese, and iron using AAS.

A. Determination of primary nutrient level in products

i) Determination of total concentration of nitrogen

The total concentration of Nitrogen was determined using procedure 3.2.7 and equation 3.4.

ii) Determination of total concentration of phosphorus

The total concentration of phosphorus in the mixed solution was determined by AOAC using the spectrophotometric method (Yogendra Kumar et al., 2007). 2.5 M of H_2SO_4, 20g of ammonium molybdate in 500ml of water, 0.28g of potassium antimony tartrate in 100ml of water, and 1.76g of ascorbic acid 100ml of water was prepared. 10ml of H_2SO_4, 3ml of ammonium molybdate solution, 1ml of potassium antimony tartrate, and 6ml of ascorbic acid solution were mixed. The standard was prepared from potassium dihydrogen phosphate by varying the concentration as: 2ppm, 4ppm, 6ppm, 8ppm, and 10 ppm and the mixed reagents were added and the Colour development waited for 15min. The absorbance of the sample was recorded using the maximum absorbance of phosphorus solution in standard at 880nm. The standard curve of the standard solution was plotted by taking the concentration of phosphorus in the solution along the x-axis and absorbance at 880nm along the y-axis.

The concentration of phosphorus in the fertilizer was calculated using equation 3.6.

$$Concentration\ of\ phosphorus\ \text{in fertilizer}\ (ppm) = \frac{absorbance\ of\ \text{fertilizer}\ sample - y_{\text{int}\,ercept}}{slope\ of\ thegraph}$$

(3.6)

iii) Determination of total concentration of potassium

The total concentration of the potassium sample was determined by atomic absorption spectrophotometer (ISO 6869:200) (EC, 2012).

B. Determination of secondary nutrient and micronutrient

I) Preparation of samples for determination of macronutrients and micronutrients

Digestion of samples

10ml Samples were digested by addition of 20 mL of (a mixture of HCl and HNO_3, ratio 3:1). The condenser was fitted to the round bottom flask that reflexes for 2 hours at 90 ºC. The round bottom flask wall was washed with distilled water and the sample was filtered out through what man filter № 1 to separate the insoluble solids from the supernatant liquid. The volume was adjusted to 100 mL with distilled water. All samples and blanks were stored in a plastic container. The standard solution of each metal such as K, Ca, Mg, Mn, Cu, Fe, and Zn were prepared for atomic absorption spectroscopy (Agilent Technologies Scientific model 200 series AAS).

a) Determination of total concentration Ca, Mg, Mn, Cu, Zn, and Fe

The digested, sample was connected to AAS to read the concentration of each metal present in the sample. The Ca, Mg, Mn, Cu, Fe, and Zn were analyzed with AAS (Agilent Technologies Scientific model 200 series AA) equipped with deuterium are background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, silt width and wavelength adjustment) were optimized for the minimum signal intensity of the instrument. Hollow cathode lamps operated at the manufacturer recommended conditions were used at their respective primary source line. The concentration of the Ca, Mg, Zn, Cu, Mn, and Fe presented in the sample was determined by reading their absorbance at the wavelength of 422.7nm 285.2nm,213.9nm, 324.8nm, 279.5nm, and 248.3 respectively using AAS.

b) Determination of total concentration of sulfur

100ppm of working standard solution was prepared from potassium sulfate. 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm of the standard solution was prepared from a working standard of 100ppm by diluted using distilled water. The absorbance of the prepared working standard solution was determined using a U-V spectrophotometer at a wavelength of 420nm. The standard curve was plotted by taking the concentration of Sulphur in the solution along the x-axis and absorbance at 420nm along the y-axis.

Then the concentration of Sulphur in the sample was calculated using equation 3.7.

$$Concentration\ of\ \ sulfur\ \text{in fertilizer}(ppm) = \frac{absorbance\ offertilizer\ sample\ sample - y_{\text{int}ercept}}{slope\ of\ \ the\ graph} \tag{3.7}$$

3.3.2 Effect of mass fraction of wool and peel on pH of produced fertilizers

The produced fertilizer was measured using a pH meter, and, the effect of wool and peel composition on the pH of produced fertilizer was studied.

3.3.3 Determination of the density, TOC, EC, C/N ratio, and functional groups of the produced fertilizers

a) Determination of density

The density of fertilizer was determined from specific gravity using a pycnometer. A 100ml of pycnometer bottle was cleaned and weighted. Two pycnometer bottles were filled with fertilizer, and water, and reweighted, and density was determined.

The specific gravity of fertilizer was calculated using equation (3.8).

$$SG = \frac{w_1 - w_0}{w_2 - w_0} \tag{3.8}$$

Where,

SG – specific gravity

w_0 – the weight of pycnometer (g)

w_1 – the weight of pycnometer filled with fertilizer (g)

w_2 – the weight of pycnometer filled with water (g)

The density of the Fertilizer was calculated using equation (3.9).

$$\rho_f = SG \times \rho_w \tag{3.9}$$

Where,

ρ_w - density of water (g/ml)

ρ_f - density of fertilizer (g/ml)

b) Determination of TOC

The total organic carbon was determined by Walkley black method (SLS, 2021**).** 10ml of the produced fertilizer sample was measured and added to 500ml of the beaker. 10ml of 1N potassium dichromate solution and 20ml of H_2SO_4 was added and swirl to mix the suspension and were kept for 30 minutes. 200ml of Distilled water and 10ml of concentrated H_3PO_4 was added using a dispenser and allowed the mixture to cool. 15 drops of diphenylamine indicator were added, and the beaker was placed on a magnetic stirrer and titrated with 0.5M of ferrous ammonium sulfate solution until the color of the solution was changed from violet-blue to green. Two blanks containing all reagent but no fertilizer sample was prepared and treated in the same way.

The total organic carbon of the produced fertilizer was calculated using equation 3.11.

$$\text{Total organic carbon (\%)} = \frac{[v_{blank} - v_{sample}]\ \mathrm{N} \times 0.399}{M} \qquad (3.11)$$

Where, M- the mass of the sample used

v_{blank} –the volume of ferrous ammonium sulfate required to titrate blank

v_{sample}- the volume of ferrous ammonium sulfate required to titrate a sample

N – normality of ferrous sulfate solution

c) Determination of C/N ratio

The C/N ratio of the sample was determined from the TOC of the samples and the total concentration of nitrogen in the sample.

d) Determination of EC

The EC of the produced fertilizer was measured using an electrical conductivity meter and the result was recorded.

e) FTIR analysis.

Functional groups were analyzed using fourier transform infrared ray (Perkins ElmerL1600300), at Jimma Institute of Technology, Material Science and Engineering laboratory. The prepared samples were grinded and mixed with KBr powder in appropriate ratio to make pellet for FTIR spectroscopy analysis. The pelletized sample was analyzed using FTIR spectra, and the spectra were collected in a range of 4000 – 400cm^{-1}.

4. RESULTS AND DISCUSSION

4.1 Proximate analysis of sheep wool and banana peel

A. Moisture content analysis

The moisture content of sheep wool and banana peel was calculated using equations 3.1, and the average value was taken. Moisture is one the most important parameters used for determining the water contents of nutrients. Moisture analysis is used to determine the amount of water vapor and other volatile components present in the sample. In this study, the moisture content of wool was 9.65 ± 0.24 % which is high compared with the value obtained by (Brown et al., 2016). In addition, the moisture contents of the banana peel sample were 10.12 ± 0.16%. The obtained value was less when compared with the value obtained by (Aboul-enein et al., 2016). The deviation of this study from the literature may be caused by geographical location and error of measurement. The moisture content of banana peel and sheep wool indicates that the presence of water-soluble enzymes and coenzymes in banana peel, and can entail the metabolic activities of the plant.

B. Ash content analysis

The ash contents analysis is used for determining the total amounts of minerals present in the sample. The ash contents of the sheep wool in the study were 1.12 ± 0.21% related value was obtained by parallel work (Brown et al., 2016). This low value indicates that keratin-based materials have low mineral contents compared with other organic materials. The ash content of banana peels was 13.01 ± 0.32%. This value is relatively close to the value reported by (Aboul-enein et al., 2016), but higher than the value obtained by (Anhwange et al., 2009). The variation of the results from the literature may be caused by measurement error, environmental conditions, and plant species. Correspondingly, (Aboul-enein et al., 2016) stated that environmental conditions and plant species can affect the proximate analysis value of Banana peel. The moisture content analysis of banana peel and sheep wool indicates banana peel is a more potent source of mineral composition that is essential for plant growth.

4.2 Studies on protein extraction

4.2.1 Statistical Analysis Using Design Expert

Table 4. 1 shows the results of the 20 experimental runs carried out according to response surface methods (RSM). Response surface method is a set of mathematical and statistical approaches for modeling and analysis of problems in which a response interest is influenced by several variables, and it employs a series of design trials to find the best solution. The least-squares regression ANOVA in this investigation was performed with Design-Expert Software 11.1.2.0.

The model equation, interaction effects of the three independent variables (reaction temperature, concentration of NaOH, and reaction time) on the corresponding extraction of keratin protein, and surface plots using the fitted equation obtained from the regression analysis holding one of the independent variables' constants.

Table 4. 1 Experimental and predicted value for extraction of protein yield

Std	run	A: Temperature (℃)	B: NaOH concentration (N)	Time (min)	Actual value (%)	Predicted value (%)
19	1	60	0.75	45	73.00	74.18
11	2	60	0.33	45	48.75	47.82
10	3	93.64	0.75	45	67.23	66.27
14	4	60	0.75	70.23	62.00	61.90
7	5	40	1	60	48.52	48.23
20	6	60	0.75	45	74.12	74.18
17	7	60	0.75	45	75.00	74.18
13	8	60	0.75	19.77	56.24	54.96
5	9	40	0.5	60	31.00	31.55

12	10	60	1.17	45	64.12	63.67
9	11	26.36	0.75	45	18.62	18.20
6	12	80	0.5	60	64.00	64.13
1	13	40	0.5	30	25.00	25.41
4	14	80	1	30	65.00	65.43
8	15	80	1	60	66.97	67.54
2	16	80	0.5	30	62.00	63.27
3	17	40	1	30	40.00	40.84
16	18	60	0.75	45	74.72	74.18
18	19	60	0.75	45	74.20	74.18
15	20	60	0.75	45	73.80	74.18

4.2.2 Model summary statistics for extraction of protein yield

The model summary statistic and suggested design of the study were mentioned in table 4.2.

Table 4. 2 Model summary statistic and suggested design for a percentage of protein extraction

Source	Std. Dev	R^2	Adjusted R^2	Predicted R^2	Press
Linear	12.76	0.5475	0.4626	0.3599	3684.57
2Fl	13.87	0.5653	0.3647	-0.1330	6521.64
Quadratic	**0.9852**	**0.9983**	**0.9968**	**0.9897**	**59.41 Suggested**

Cubic	1.10	0.9987	0.9987	0.8166	1055.82 Aliased

4.2.3 Analysis of variance

The statistical significance of the process component was determined using analysis of variance and revealed in Table 4.3. The F-value in this study is 657.83, which is high. The significance of the models is indicated by the high F value. Due to noise, there is only a 0.01% probability that such a large F- value will occur. Aside from that, the model's p-value was extremely low (0.0001), indicating its relevance. The model terms are significant if the P-value is less than 0.0500. The more significant the model is, the lower the value of p and the higher the value of F.

Table 4. 3 Analysis of variance for quadratic model on the extraction of protein yield

Source	Sum of Square	df	Mean Square	F- Value	p-Value
Model	5746.29	9	638.48	657.83	< 0.0001 significant
A-Temperature	2790.09	1	2790.09	2874.66	< 0.0001
B-Concentration	303.11	1	303.11	312.30	< 0.0001
C-time	58.14	1	58.14	59.90	< 0.0001
AB	88.11	1	88.11	90.78	< 0.0001
AC	13.91	1	13.91	14.33	0.0036
BC	0.7750	1	0.7750	0.7985	0.3925
A^2	1838.27	1	1838.27	1894.00	< 0.0001
B^2	612.19	1	612.19	630.74	< 0.0001

C^2	446.85	1	446.85	460.39	< 0.0001
Residual		10	0.9706		
	9.71				
Lack of fit	7.21	5	1.44	2.89	0.1345 not significant
Pure error	2.50	5	0.4990		
Cor total	5756.00	19			

In this case, A, B, C, AB, AC, A^2, B^2, and C^2 are significant model terms whereas, BC is not significant. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value for the developed empirical model is 2.89. This value implies the Lack of Fit is not significant relative to the pure error. There is a 13.45% probability that a Lack of Fit F-value this large could occur due to some kind of noise. A non-significant lack of fit is good because a non-significant Lack of Fit shows the model is well fitted and is very nearer to the perfect fitness as it was obtained by the model.

4.2.4 Model of adequacy checking

Model adequacy can be deduced considering the regression coefficients of R^2. The regression coefficient (R^2) was used to determine the relationship between the experimental and the predicted responses. As shown from Table 4.4, the response of R^2 was 0.9983 which was mentions that 99.83% of the response variability in extraction capacity can be described by the analyzed process parameters and it could not describe nearly about 0.17% of the variation of the response. It was found that the value of R^2 is close to 1, showing the very correlation between the experimental and predicted value, thus showing the adequacy of the model. In another way, the determinant coefficients of R^2 and adjusted R^2 shown in Table 4.4, specifies the close agreement of experimental and predicted values. The Predicted R^2 of 0.9968 is in reasonable agreement with the Adjusted R^2 of 0.9897; i.e., the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 80.363 indicates an adequate signal. This model can be used to navigate the design space. The low coefficient of variation (1.69%) was obtained, which is the standard deviation divided by the mean indicating the good precision of the experiment.

Table 4. 4 Model statistics of the design

Std. Dev	0.9852
Mean	58.21
C.V%	1.69
R^2	0.9983
Predicted R^2	0.9968
Adjusted R^2	0.9897
Adeq precision	80.3630

4.2.5 Model equation development

The final model equation obtained was used to determine the relationship between responses and independent parameters. The quadratic model fitted the statistics and was selected. The quadratic model that relates the extraction of protein yield with independent process parameters was described by the second-order polynomial equation. The extraction of protein yield was given in equation (4.1) using the function of independent parameters by coded variable.

The final equation in terms of coded factors was given in equation 4.1:

$$Yield = +74.18 + 14.29 \times A + 4.71 \times B + 2.06 \times C - 3.32 \times AB - 1.32 \times AC - 11.29 \times A^2 - 6.52 \times B^2 - 5.57 \times C^2 \quad (4.1)$$

Where, A- reaction temperature, B, Concentration of NaOH and C, reaction time

Equation 4.1 describes the effect of the independent variable. Based on the coefficient in the equation, it was evident that the response yield increases with an increase in reaction temperature

(A), and concentration of NaOH (B) and reaction time (C) which have a positive effect on extraction yield, but reaction temperature has a more positive effect on yield compare to Concentration of NaOH and reaction time. The quadratic terms (A^2, B^2, and C^2) have negative effects on the response yield but the pure quadratic term (A^2) has a substantial effect than the other quadratic terms. The interaction of AB and AC has negative effects on response yield.

4.2.6 Model diagnostic plot

A diagnostic plot indicates a graphical representation of the model that can be used to interpret the chance variation of the values. The normal plot of residuals is given in Figure 4.3. As it was observed from the normal probability plot, the residuals are following a normal distribution, thus the residuals were approximated along a straight line confirming that the normality assumption was satisfied.

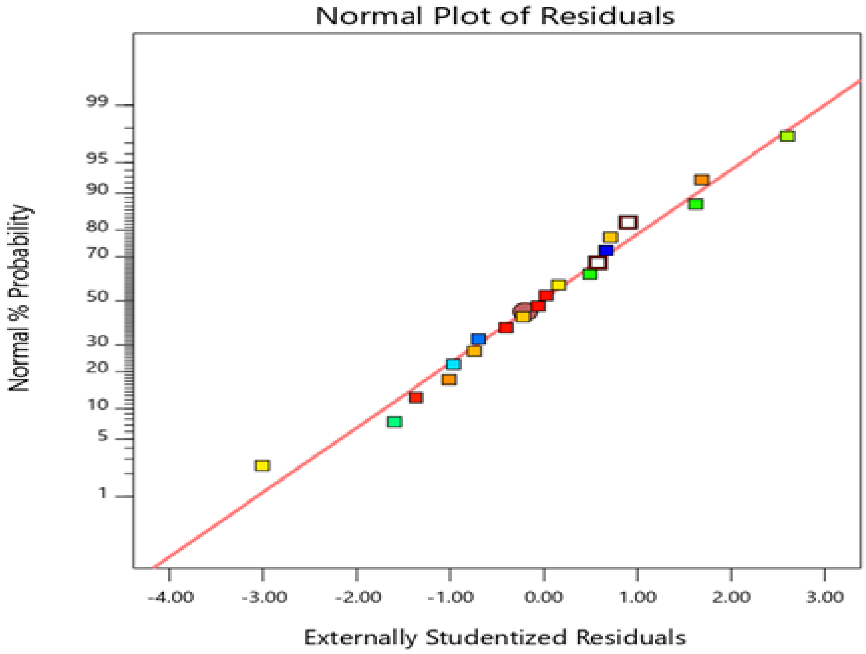


Figure 4. 1 Normal % probability residual plot for keratin protein extraction

Residual versus predicted plot is also one of the most important diagnostic tools for confirming the adequacy of the fitted model to predicting the response by indicating the random scattering of residual. It checks for the assumption of constant variance, thus there are no upward or downward pattern curves observed. Thus, Figure 4.4 indicates that the residuals of the predicted values were randomly scattered on the plot, representing that the fitted values are structureless; therefore, there is no need for adjustment to reduce the personal error for the model.

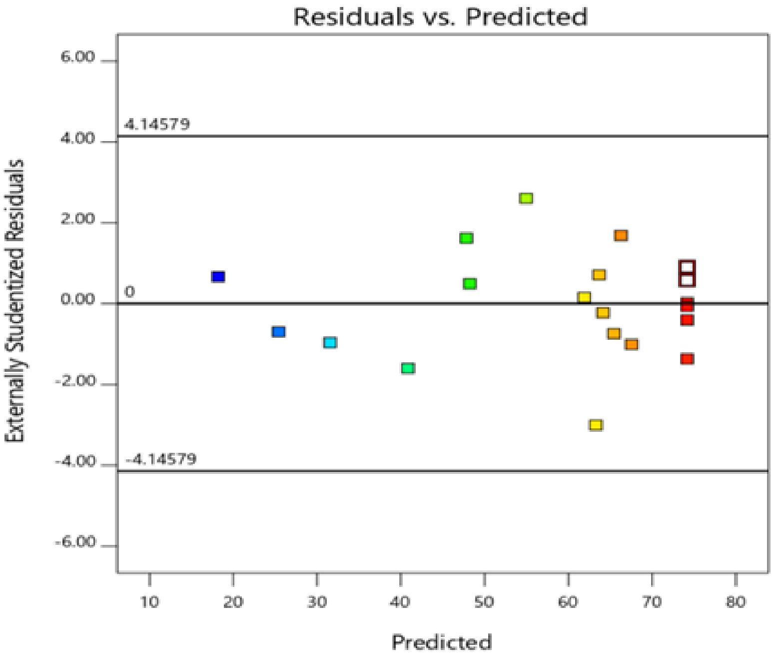


Figure 4. 2 Predicted versus residual plot for keratin protein extraction

The adequacy of the model is further shown from an agreement between predicted versus actual values as shown in figure 4.5. The predicted values obtained were very close to a straight line, indicating a good relationship between experimental and actual values of the response. predicted values obtained by the model were very close to the experimental values and lie reasonably close to the straight-line. Therefore, the result indicates that the actual values were in good agreement with the predicted values as shown in the plot.

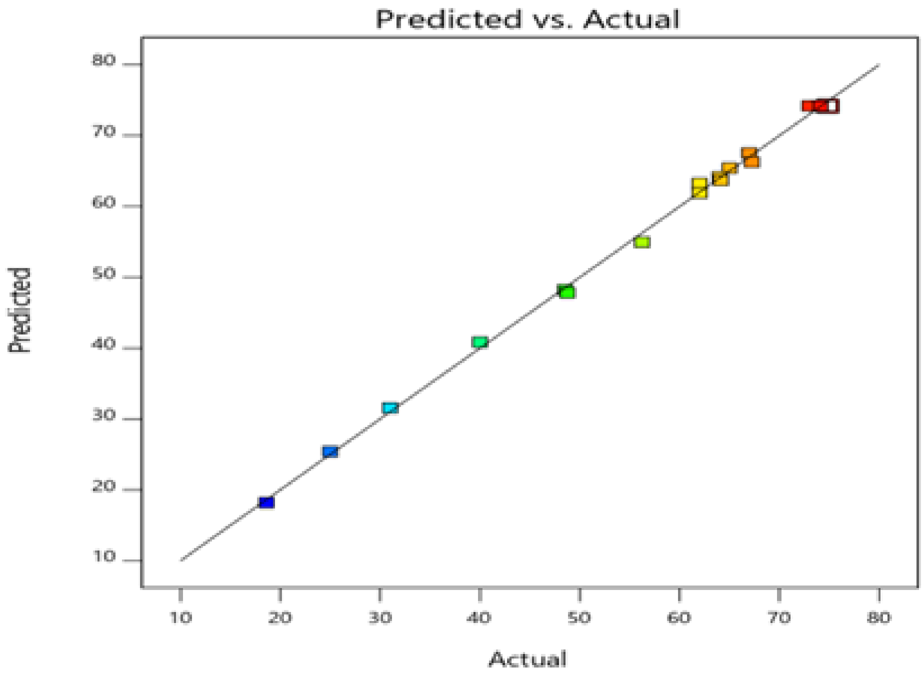


Figure 4. 3 Predicted versus actual plot for keratin protein extraction

4.2.7 Studies of main effects on keratin protein extraction

A- Effect of reaction temperature on extraction keratin protein

Temperature is one of the most important parameters that significantly affect the extraction of keratin protein (Binti et al., 2017; Gindaba et al., 2019; Tewodors, 2017). As shown from figure 4.6 the protein yield was increased as reaction temperature was increased. The increasing reaction temperature can offer much activation energy to accelerate the physical and chemical change of wool sheep and thus promote the increase of protein yield up to optimum condition. However, further increasing of reaction temperature decreases keratin protein yield, this due to at high reaction condition protein will lose its structure. At high temperatures, the natural protein becomes denature (Gindaba et al., 2019).

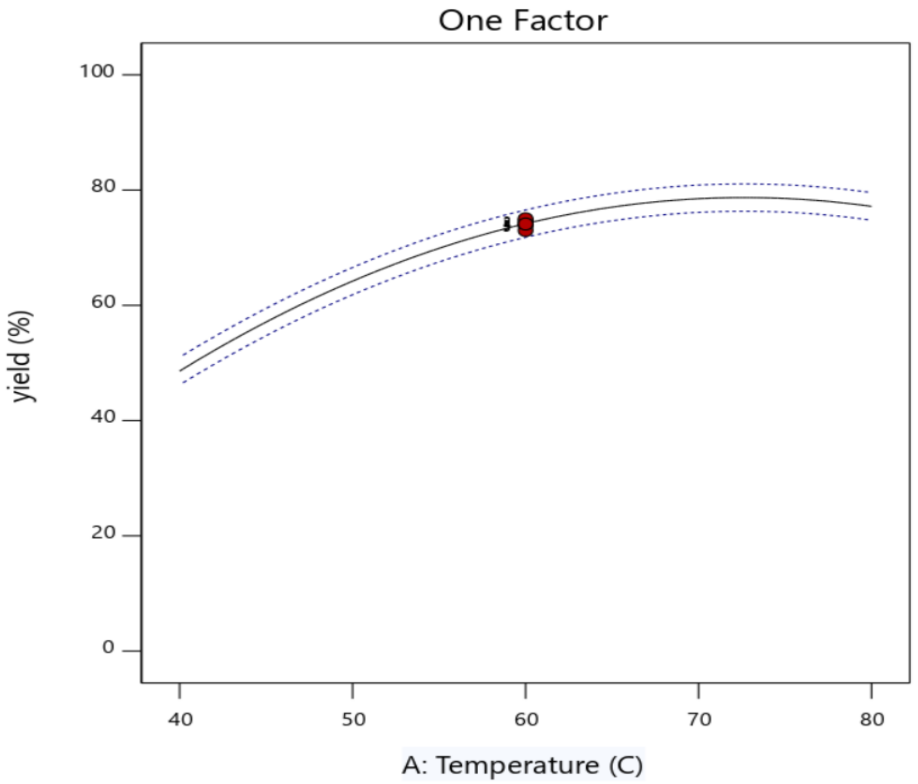


Figure 4. 4 Effect of reaction temperature on extraction of keratin protein of wool

B- Effect of NaOH concentration on keratin protein yield

The concentration of extraction is important for keratin protein extraction (Gindaba et al., 2019; Niraj and Shardendu, 2020). Thus, from figure 4.7 it is observed that as the concentration of NaOH increases from 0.5N to 0.75N protein yield was increases. This is due to the peptide bond of keratin protein in the sheep wool breaking down into simpler molecules. After the optimum concentration, (0.75N) the further increasing NaOH concentration caused decreasing in protein yield. This is due to the appearance of secondary structural changes in the peptide that are followed by the reduced molecular weight of protein polypeptide and after the reaction, NaOH solution may remain in the system and enter into the composition of the product. In addition, as concentration increased disulfide bond decreased (Binti et al., 2017).

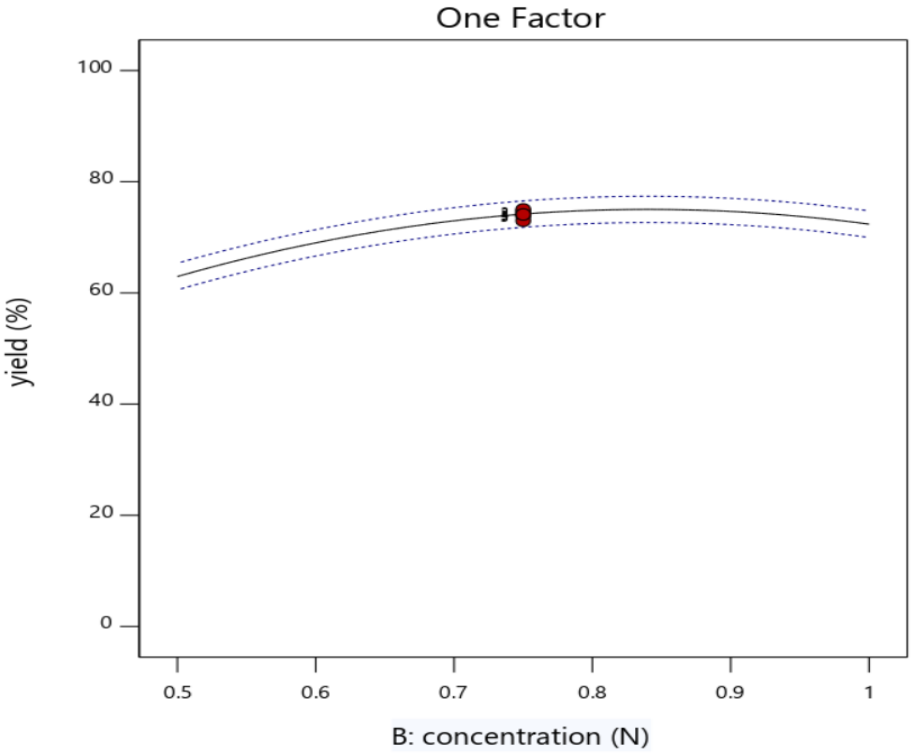


Figure 4. 5 Effect NaOH concentration on extraction of keratin protein of wool

C- Effect of reaction time on keratin protein yield

Extraction time is one of the parameters that affected the keratin protein yield extraction. As revealed from figure 4.8 the keratin protein yield was low at 30min, then it was gradually increased as the time was increased from 30min to 45min. However, the yield was started to decrease beyond optimum reaction time, this reveals that at higher reaction time the keratin protein was decomposed. The keratin would be decomposed after a long period (Wang et al., 2021).

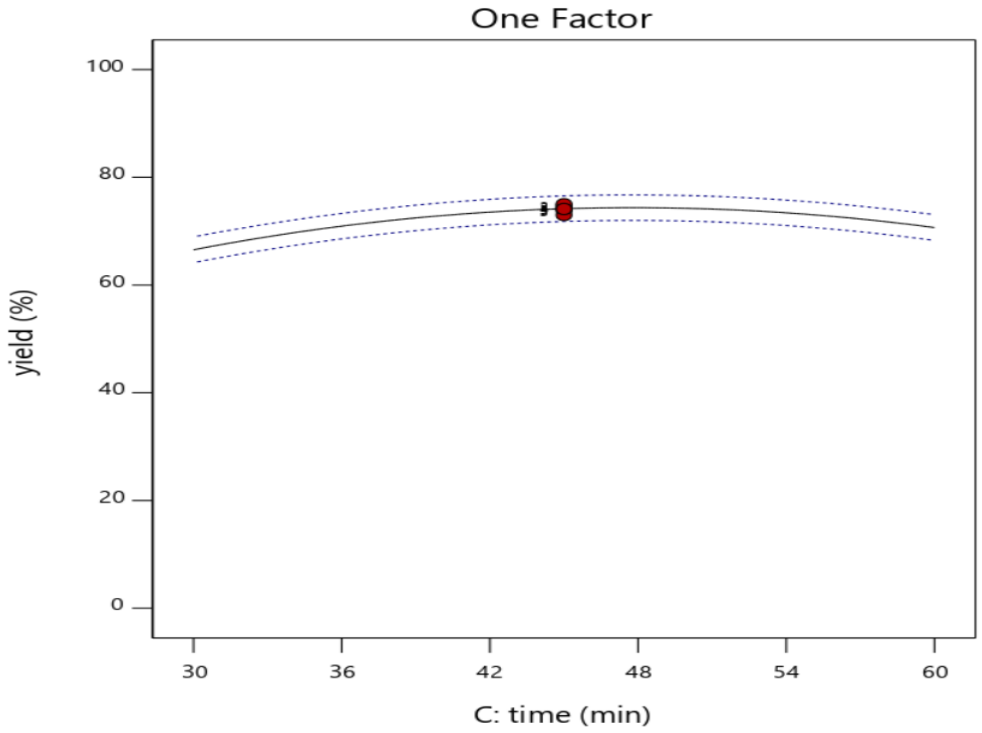


Figure 4. 6 Effect of reaction time on the extraction of keratin protein of wool

4.2.8 Interaction effect of process parameters on keratin extraction of protein yield

An interaction occurs when the response is different depending on the settings of two factors. In this study, from ANOVA it was found that the combined effect between extraction temperature and NaOH concentration, reaction temperature, and reaction time has a significant effect on the extraction of keratin protein yield. Accordingly, from equation 4.1, among these interaction factors, the interaction between reaction temperature(A) and NaOH concentration (B) have a more significant effect on the keratin protein production, because it has a higher coefficient (-3.32) than AC. The negative sign indicates that interaction factors negatively affect keratin protein extraction (as interaction factors increase keratin protein yield decrease).

I) Interaction of effects of reaction temperature and concentration of NaOH on keratin protein yield

Figures 4.9 a), and b) indicates the 3D and contour plot for interaction effects of extraction temperature and Concentration of NaOH on the extraction of keratin protein yield. At normal or lower temperature, the extraction process was lower and consumes higher NaOH. An increasing of reaction temperature decreases NaOH concentration used for extraction process. However, using higher reaction temperature and higher NaOH concentration reduce protein yield. This may be due to after reaction the concentration of the NaOH enters into product and there may be loss of protein structure or amino acid at higher temperature and protein yield was decreased.

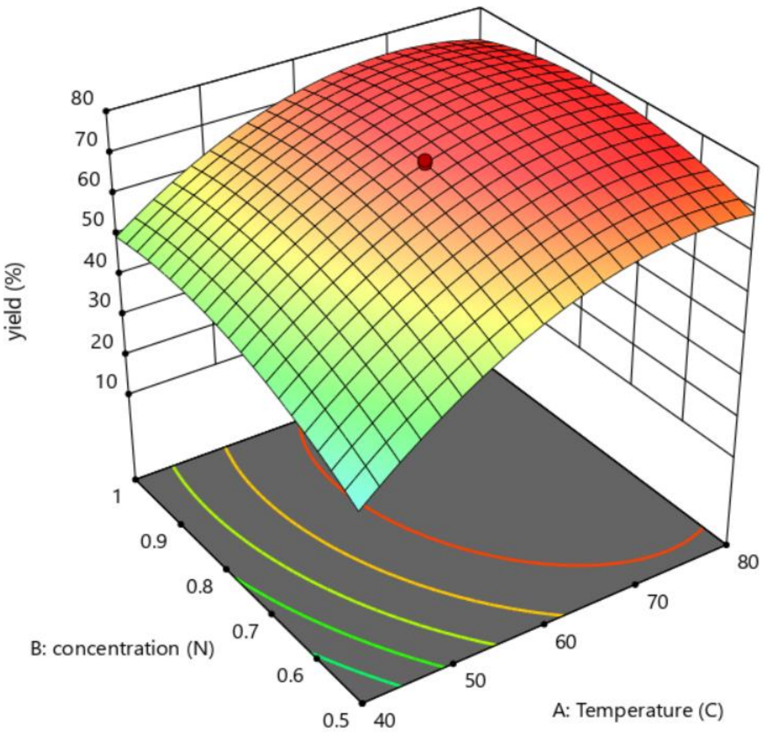
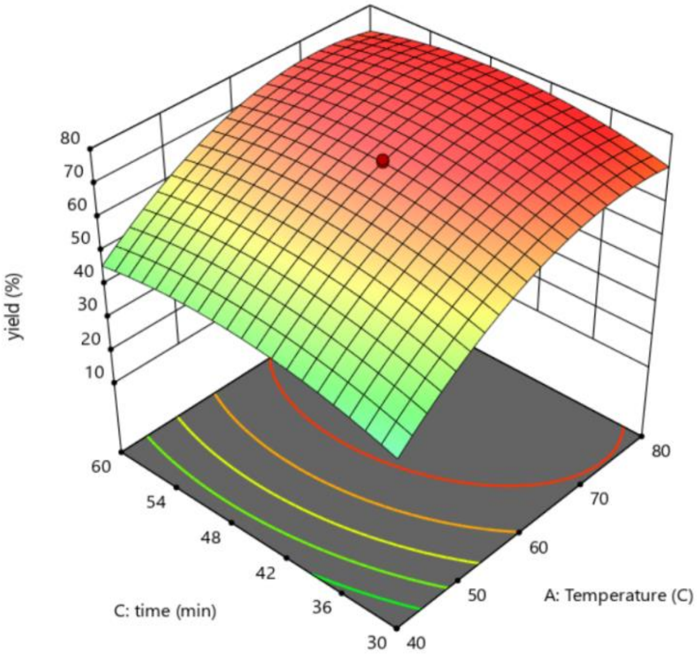


Figure 4. 7 Interaction effect of A and B using 3D plot

II) An interaction effects of reaction temperature and reaction time

An interaction effect of the reaction temperature and extraction time on the percentage of keratin yield is shown in Figures 4.10. lower reaction temperature requires longer reaction time for keratin protein extraction. Higher reaction temperature reduces reaction time used for extraction process. But using higher temperature for longer time reduces protein yield. This may due to protein in the sample being denatured at high temperature for longer period, and its percentage protein yield was decreased.



Figures 4. 8 Interaction effect of A and C using 3D plot

4.2.9 Optimization of process parameters for keratin protein extraction

Optimization of process parameters for keratin protein extraction was important. The optimization of process parameters such as reaction temperature, the concentration of NaOH, and extraction time was under RSM done using CCD. As shown in table 4.5 the main criteria for process parameter optimization were maximized protein yield and keeping the values of the factors in range.

Table 4. 5 Constraints for optimization

Variable	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: reaction temperature	is in range	40	80	1	1	3
B: NaOH concentration	is in range	0.5	1	1	1	3
C: reaction time	is in range	30	60	1	1	3
Keratin protein yield	maximize	18.62	75	1	1	3

The design expert under numerical optimization gave 100 different solutions. Thus by considering the desirability function, the numerical optimization gave optimum points for independent variables that could maximize the response the results shown in table A.1 indicate the possible solution with the desirability of 100% from which the most appropriate value is selected for optimal condition. The consideration of constraints selected for optimal condition by design experts at, 63.97 ºC, 0.70 N, and 46.86min were selected, which gave 75.62 %yield among optimizing alternatives given by design expert software. Correspondingly, (Nustorova, 2014), stated that the protein content of sheep wool was 78.8%. The deviation of the obtained products, from literature may be caused by the inflation of equipment, and measurement error during the experimental work, age, and species of the sheep. The volume of keratin in the skin differs according to the species and age of the animal (Majalah Kulit and Karet, 2017). The optimum possible solution for protein yield was shown in Table 4.6.

Table 4. 6 Optimum possible solution for protein yield

No	Temperature (ºC)	Concentration (N)	Time (min)	Yield (%)	Desirability
1	**63.97**	**0.70**	**46.86**	**75.62**	**1.000**

The desirability ramp for numerical optimization of the response process parameters were shown in figure 4.11.

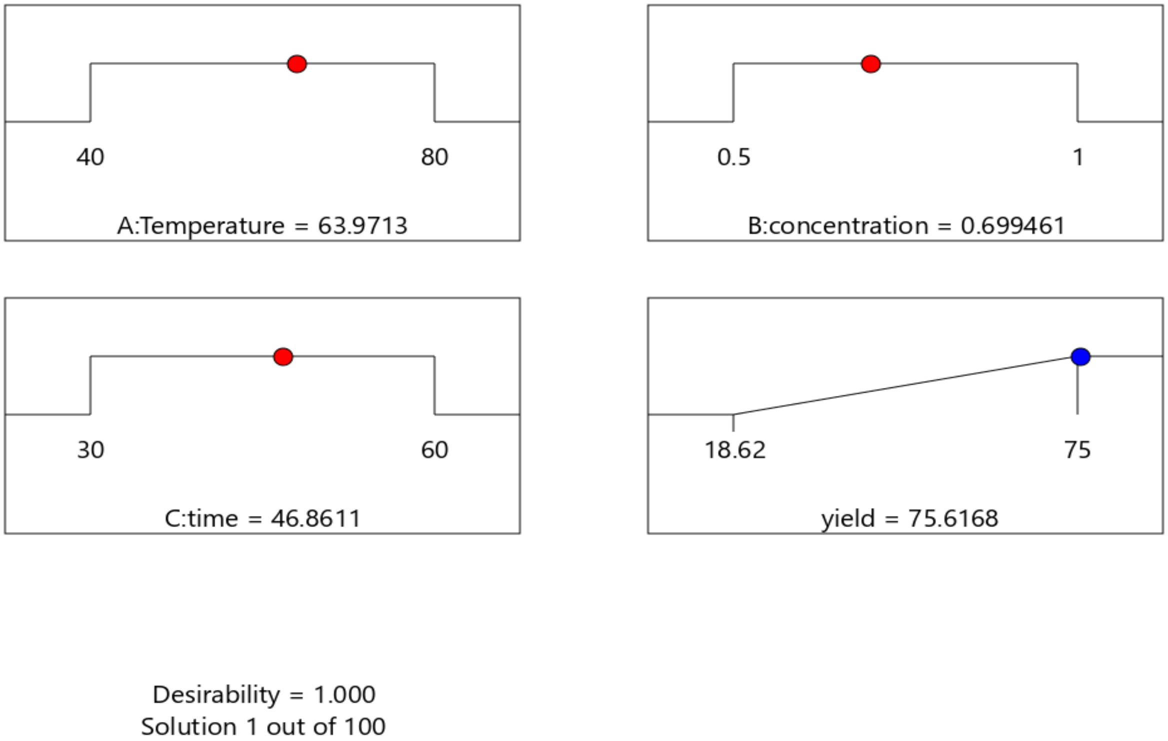


Figure 4. 9 desirability ramp for the numerical optimization of the response process parameters

4.2.10 Model validation

After numerical optimization triplicate experiments were conducted to confirm the validity of the model using the predicted optimized process parameters. At these process parameters, the average percentage of keratin protein yield (75.15 ± 0.17 %) was obtained. It was indicated that the experimental and predicted values were close to each other and the error was 0.45%. Hence, the validity of the model was confirmed.

4.3 Extraction of organic fertilizer from sheep wool and banana peel

4.3.1 Determining the level macro and micronutrients of produced Fertilizer

A) Determination of the primary nutrient level

The level of the primary nutrient is one of the most important criteria that is used to decide the abundance of nutrients provided for plant growth. In this study, the level of primary nutrients was determined by different methods depending on the types of primary nutrient elements. Nitrogen is important for, promotion of rapid growth, increasing leaf sizes, and quality forms an amino acid, ensuring the development of fruit and seed, plays a crucial role in metabolic process, and influences the rate of crop growth and quality. Potassium is used for plants for various biochemical and physiological processes that are responsible for plant growth and development. Besides, it takes part in protein synthesis, carbohydrate metabolism, and enzyme activation, mitigating role in various abiotic stresses such as drought, salinity, metal, toxicity, high or chilling temperature. Therefore, lack of potassium increases plant susceptibility to various diseases and pest infestation which makes a plant vulnerable to damage under various conditions. Phosphorus is used as activating coenzyme for amino acid of protein synthesis, decomposes carbohydrates produced during photosynthesis, it is also mandatory for normal growth, such as photosynthesis, glycolysis, respiratory, and fatty acid synthesis.

For the determination of phosphorus by the spectrophotometric vanadium phosphomolybdate method, a standard curve was prepared. A range of calibration curves was prepared according to the procedure mentioned 3.3.1. section ii, and the standard for estimation of phosphorus was constructed. The best fit linear equation is derived. The calibration curve for the estimation of phosphorus concentration was revealed in Figure 4.12. The equation $y = 0.0961x + 0.1415$ was used for the estimation of phosphorus in the unknown sample. Where y is the absorbance at 880nm and x is the concentration of phosphorus in the sample.

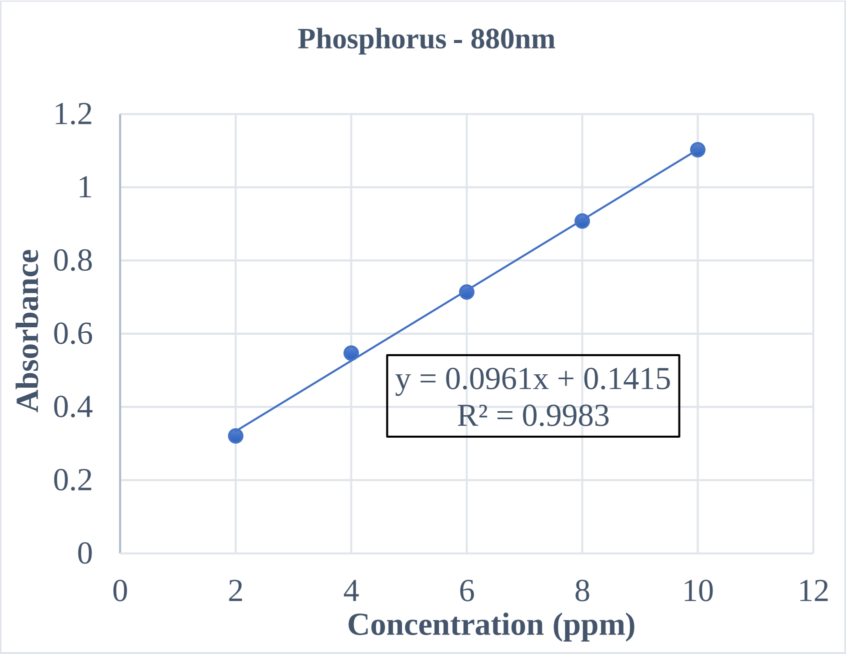
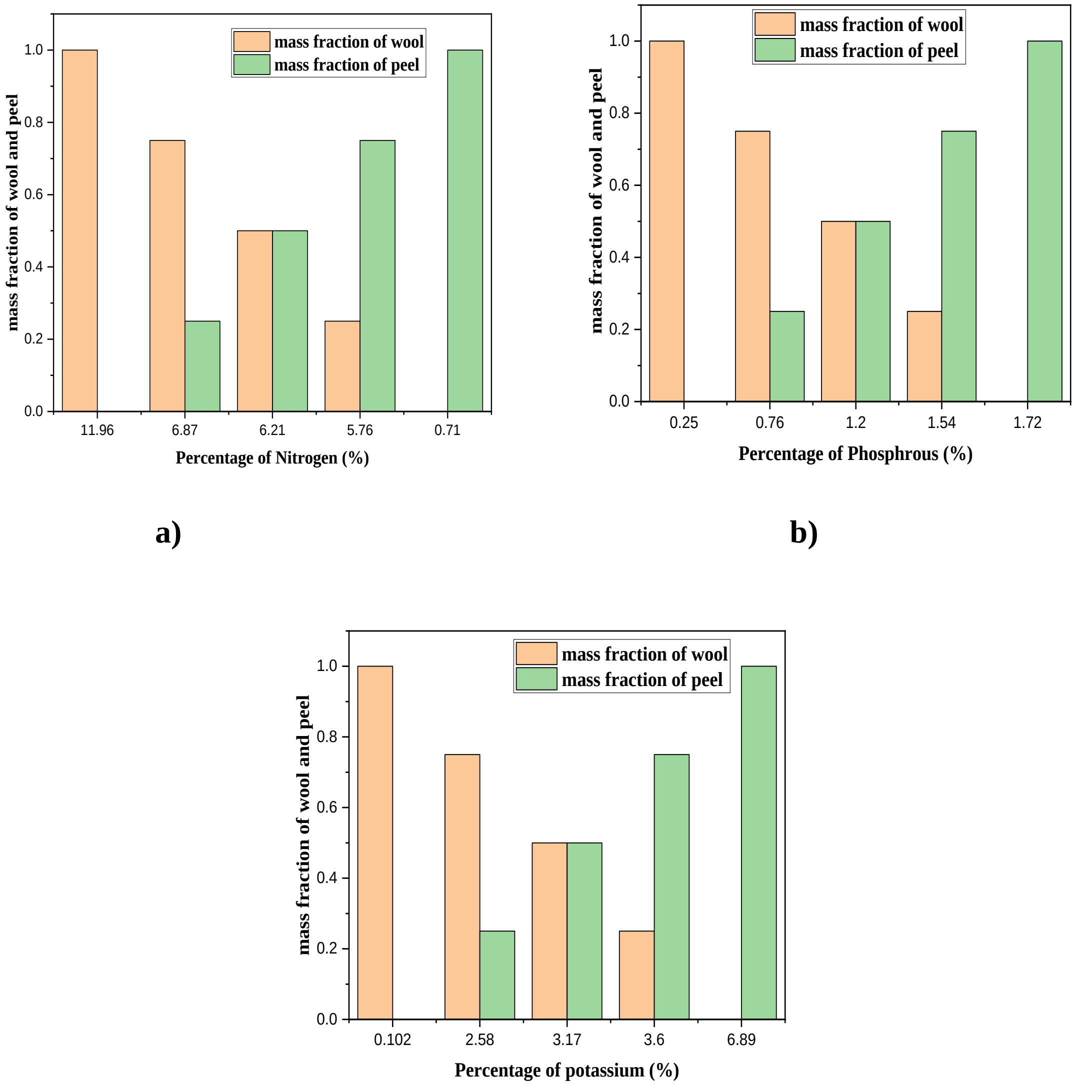


Figure 4. 10 calibration curve for estimation of phosphorus concentration

The linear relationship (R^2= 0.9983) between the absorbance of phosphorus and concentration demonstrates reliability in estimating the phosphorus content of the unknown sample. The equation has a positive slope of 0.1415 (P) implying that a unit increase in sample accounts for the increase in the phosphorus concentration.



a) **b)**

c)

Figure 4. 11 Histogram for a percentage of a) nitrogen, b) phosphorus, and c) potassium

Figure 4.13 shows that the wool alone gives the largest amount of nitrogen (11.96%) and the lowest percentages of potassium (0.10) and phosphorus (0.25%). The percentage of nitrogen obtained in this investigation was comparable to that obtained by (Arlas, 2019) and was lower than that recorded by (Burns et al., 2021). Correspondingly, (Burns et al., 2021), stated that the potassium and phosphorus concentrations in the wool were 0.000045% and 0.0137%, respectively.

Environmental and genetic variables may play a role in the variance of macronutrients in wool (Khan et al., 2012)

The banana peel alone contains a low percentage (0.71%) of nitrogen and a high percentage (6.89%) of potassium and (1.72%) of phosphorus. The relative work was also done by (Mayur Dattatray Khairnar, 2020), 0.78 % of the nitrogen in the banana peel. (Durgo and Mandura, 2020) also claimed that banana peels were contained 7.5% of potassium concentration and 0.29% of phosphorus concentration. The little deviation of this results from literature may be caused lack of equipment performance or error of measurement, and geographical location. The appreciable high content of potassium implies that if peel is taken it will help in regulatory development such as osmoregulation and plant- water relation of plants.

The fertilizer produced from combination of different mass fraction of wool and peel have also contains ranged (5.76 – 6.87) % of nitrogen, (2.58 – 3.6) % of potassium, and (0.76 – 1.54) % of phosphorus composition. The comparison of the results with recommended standard value was revealed in table 4.7.

Table 4. 7 Comparison of the primary nutrients in obtained product with standard

Primary nutrient	Standard (Ayilara et al., 2020)	This study		
		wool	peel	Combination of wool and peel
N (%)	≥ 1	11.96 ± 0.10	0.71 ± 0.14	5.76 ± 0.12 - 6.87 ± 0.57
P (%)	≥ 1.5	0.25 ± 0.31	1.72 ± 0.21	0.76 ± 0.23 - 1.54 ± 0.01
K (%)	≥ 1.5	0.10 ± 0.28	6.89 0.10	2.58 ± 0.16 - 3.6 ± 1.3

± indicates the value of the standard deviation of the mean (n = 3).

As revealed from table 4.7 the percentage of potassium and phosphorus in fertilizer produced from wool alone was very less than the value recommended as standard, whereas the percentage of nitrogen in fertilizer produced from peel alone was less than the standard recommended value. Besides, the percentage of primary nutrients in fertilizer produced from a combination of wool and peel were well achieved the standard values of organic fertilizer recommended by food and agricultural organization. Moreover, the percentage of K_2O and P_2O_5 were calculated using equations C.2 and C.3 in appendixes. The percentage of K_2O and P_2O_5 in fertilizer produced from wool was 0.121 % and 0.573% respectively. Besides the K_2O and P_2O_5 of fertilizer produced from peel was 8.30% and 3.94 % respectively. The fertilizer produced from a combination of wool and peel has contained 3.11 % to 4.34% of K_2O and 1.742% to 3.53% of P_2O_5.

According to the fertilizer production industry, the standard values of N, P_2O_5, and K_2O in organic liquid fertilizers, are > 2%, > 1%, and > 2%, respectively (Bhavsar, 2018). Yet, all primary nutrients in the fertilizer produced from a combination of wool and peel was confirms the standard value of industrially produced liquid organic fertilizers.

B) Determination of the secondary nutrient level

Determining the level of each secondary nutrient are a crucial role in deciding the abundance of nutrients for plant growth. Among the secondary nutrients, the level of Mg and Ca was determined using AAS, whereas sulfur was determined using a U-V spectrophotometer.

A range of standard curves was prepared and the standard for estimation Sulphur was constructed. The best fit linear equation is derived. The calibration curve for the estimation of sulfur concentration was shown in Figure 4.14. The equation $y = 0.1442x + 0.1436$ was used for the estimation of Sulphur in the unknown sample. Where y is the absorbance at 420nm and x is the concentration of Sulphur in the sample

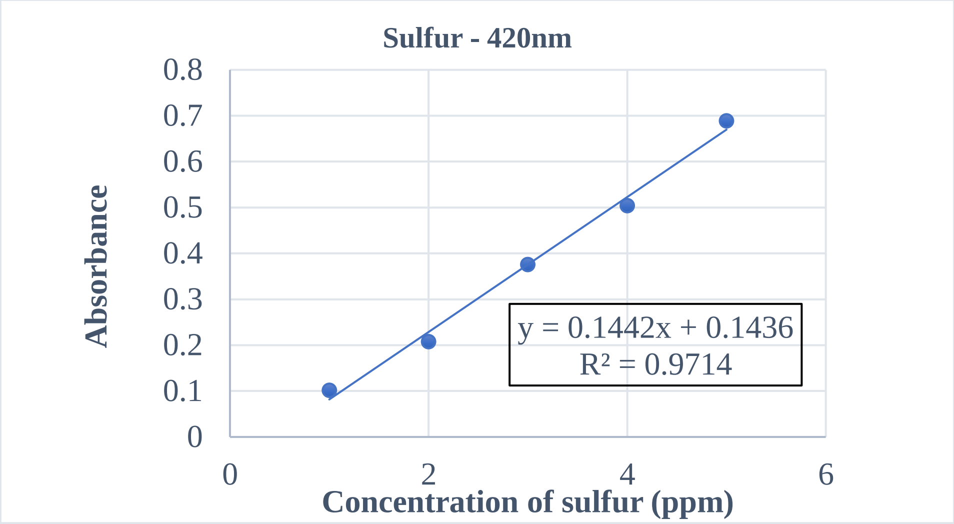


Figure 4. 12 Calibration curve for estimation of Sulphur concentration

The linear relationship (R^2= 0.9714) between the absorbance of Sulphur and concentration demonstrates the reliability in estimating Sulphur content of the unknown sample. The equation has a positive slope of 0.1442 (S) implying that a unit increase in sample accounts for the increase in the Sulphur concentration

Knowing the level of each secondary nutrient in fertilizer was essential for determining the composition of secondary nutrients in organic fertilizers. The total concentration of secondary nutrients in this study was shown in table 4.8.

Table 4. 8 Comparison of secondary nutrient with literature

Secondary nutrients	Reference			
	(**Popko et al., 2015**)	This study		
		wool	peel	Combination of wool and peel
Ca (%)	0.168	0.0394	0.263	0.074 – 0.184
Mg (%)	0.034	0.018	0.146	0.096 – 0.120
S (%)	2.35	1.72	0.0083	0.046 – 0.11

Apart from table 4.8, the Fertilizer produce from wool alone contained, (0.0394 %) of calcium, (0.018 %) of magnesium and (1.720 %) of Sulphur. The present result confirms that the wools are the richest in sulfur among the secondary macronutrient. The obtained result was lower than the value reported by (Burns et al., 2021). The high content of sulfur signifies that if wool is taken it will help for the synthesis of Sulfur-containing amino acid cystine, cysteine, and methionine which are the building block of protein, for plants.

The peel composition results high concentration (0.263%) of Calcium, (0.146 %) of magnesium and low concentration (0.0083 %) of Sulphur. The high level of calcium and magnesium in the peel may be caused due to banana peel being rich in minerals (Hussein et al., 2019). This result was compared well with earlier studies of banana peel mineral composition (Durgo and Mandura, 2020). Also, the fertilizer produced from combination of wool and peel contained, a range of (0.074 -0.184) % of calcium, (0.0960 – 0.120) % of Magnesium and (0.046 – 0.110) % of sulfur.

C) Determination of the micronutrients

Micronutrients are the nutrients that are necessary for plant growth, whereas, their requirement has not been as long as macronutrient elements. There are eight, micronutrients that are used for plant growth, however, under this study, only four micronutrients such as Manganese, copper, zinc, and iron were discussed. Micronutrients are generally available in very small quantities in most soils and plants, and they are essential for plant growth, and their efficiency leads to severe limitations in plant growth.

Table 4. 9 Comparison of obtained micronutrients in product with standard

micronutrients	Standard (Ayilara et al., 2020; Kala et al., 2011; S.Bhavsar, 2018)	**This study**		
		wool	peel	Combination of wool and peel
Cu (%)	0.01 – 0.05	0.002	0.0002	0.0003 – 0.001
Mn (%)	0.01 - 0.05	0.0008	1.6820	0.092 – 0.508
Zn (%)	0.01 - 0.05	0.009	0.0002	0.0057 – 0.007
Fe (%)	0.01 – 0.05	0.0201	0.0582	0.0161 - 0.018

As shown in table 4.9 the composition of Cu, Mn, and Zn n fertilizer produced from wool was below the values recommended as standard, whereas the composition of Fe was found in the recommended values. The fertilizer produced from banana peel was contained high Mn. These high potential contents of Mn may penetrate the soil-plant system and affects the natural root microorganism mechanism which regulates the transport accumulation from spin soil ending in the edible part of plants. Besides, the fertilizer produced from a combination of peels and wool contains a range of micronutrients that are close to the standard recommended values of micronutrients in organic fertilizer.

4.3.2 Effect of mass fraction of wool and peel composition on pH of produced Fertilizer

The pH values of organic fertilizers for nutrient solutions are important parameters in determining the usage of agricultural purposes.

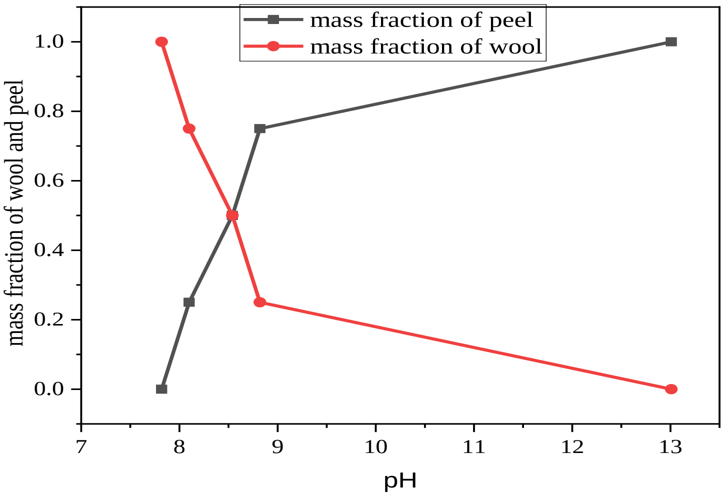


Figure 4. 13 Effect of mass fraction of wool and peel on pH of produced Fertilizers

As revealed from figure 4.15, the pH of fertilizer was decreased when wool mass fraction was increased. This decrease in pH at high wool mass fraction indicates that the digestion of wool keratin produces a certain number of acidic components, such as carboxylic acids, and that the concentration of amino acids and other organic acids in the produced organic fertilizer may be more dominant than ammonium and other formed alkaline compounds. Furthermore, the pH was increased as the mass of peel was increased. The increase in pH due to increased peel mass fraction was observed; banana peels produced alkaline minerals, which resulted in an increase in the pH of liquid-organic fertilizer. The pH of the fertilizer produced from a combination of peel and wool mass fraction was ranged from 8.10 – 8.76, which indicates that the alkali concentration in the fertilizer was gradually increased but, it was also dominated by amino acids or another organic acid. According to the standard for the specification of liquid organic fertilizers, the pH of the Liquid organic fertilizer was in the range of 6.0 – 8.50 (SLS, 2021). So, the little deviation from the standard value occurs due to some factors such as lack of equipment efficiency used for purification or lack of accuracy of the equipment used for measuring and human error. Thus, the obtained result guaranteed, that product is organic liquid fertilizers.

4. 3. 3 Determination of the Density, TOC, EC, C/N, and Functional group

a) Determination of density

The density of the produced fertilizers was determined using procedure 3.3.3 section (a), equation 3.9. Thus, density was ranged from 1.201gm/ml to 1.211gm/ml. Based on the density of the produced fertilizers the 88.36 ml to 89.1ml. This indicates, 88.36 to 89.1 milters of liquid fertilizers were prepared by dissolving 10g samples into 0.7 N of NaOH for 47 min at 64ºC.

b) Determination of density, TOC, EC, and C/N ratio

The TOC, EC, and C/N ratios are the most important property that is used to determine the usage of fertilizer for agricultural production. Table 4.10 shows the TOC and EC of produced fertilizers.

Table 4. 10 Comparison of the TOC and EC, of obtained products with standard

	Standard (SLS, 2021)	**This study**		
		wool	peel	Combination of wool and peel
TOC (%)	≥ 5	16.26 ± 0.65	19.24 ± 0.14	(16.41 ± 0.71) – (17.49 ± 0.23)
EC (dsm^{-1})	≤ 20	10.52 ± 20	8.65 ± 30	(8.74 ± 17) – (9.61 ± 0.34)

± indicates the value of the standard deviation of the mean (n = 3).

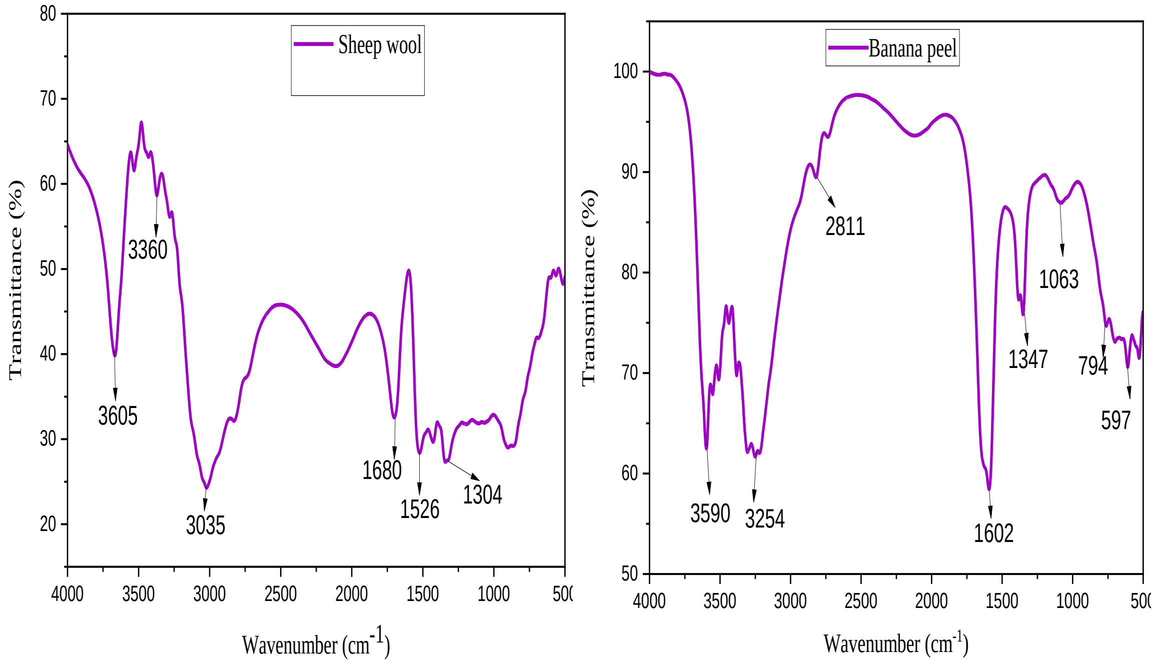
Regarding the TOC results, it was found that it was ranged from 16.26 to 19.24 % for different mass fraction of wool and peel types under study. The lowest value of total organic carbon (16.26 %) was found in fertilizer produced from wool, and whereas the highest value of the TOC (19.24%) was obtained fertilizer produced from.

The EC was ranged from 8.65 to 10.52 for different fertilizer types. The lowest value of EC (8. 65dsm^{-1}) was found for mass fraction of peel alone and the highest value of EC (10.74 dsm^{-1}) was found for peel wool alone fertilizer. The highest value of EC was indicated that the shorter peptides, amino acids, and other compounds) in the wool were solubilized which contributes to increasing the electrical conductivity of liquid-organic fertilizer. The electrical conductivity of banana peels may be occurred due to the high mineral contents of banana peels.

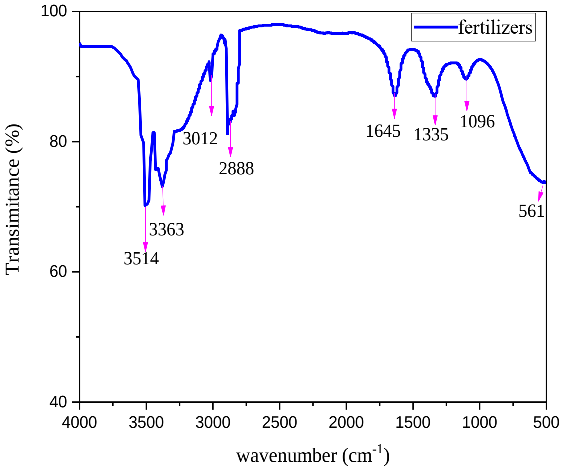
The C/N ratio is an important parameter which deals with information regarding the decay of organic matter in the soil since microorganism that decomposes the organic matter uses carbon as a source of energy and nitrogen for building a cell structure. The C/N ratio ranged from 1.86 to 42.10 for different mass fraction of wool and peel composition types in the study. It was found that the lowest value (1.86) of the C/N ratio found in mass fraction of wool alone and the highest (42.10) C/N ratio value was obtained at mass fraction of peel alone. The fertilizer produced from a combination of wool and peel provides a 1.89 to 3.08 value of C/N. Related value of C/N was obtained by (S.Bhavsar, 2018). The highest C/N values in banana peel alone indicate that it enriches the soil with resynthesized organic molecules, improving soil fertility and structural quality, whereas a low C/N ratio in the mass fraction of wool indicates that it stimulates the process of mineralization organic matter using a large amount of nitrogen.

c) Functional group analysis

In this study FTIR spectrum analysis was used to understand the composition of raw materials and products qualitatively



a) b)



c)

Figure 4. 14 FTIR of a) sheep wool, b) banana peel and c) produced liquid-organic fertilizer from combination wool and peel

A variety of absorption bands are produced by keratin proteins, including amide A, amide B, amide I, amide II, and amide III. The stretching vibration of N-H bonds is linked to amide A and amide B. (Kalaoglu, 2010). The FTIR spectra of amide A, I, II, and III ranged from 3300 to 3500 cm-1, 1600 to 1800 cm-1, 1470 to 1570 cm-1, and 1250 to 1350 cm-1, respectively (Ji et al., 2020). However, according to literature the FTIR spectrum of keratin amide A and amide B was seen at 3287 cm^{-1} and 3072cm^{-1} respectively (Wojciechowska et al., 2004). In this study, the FTIR spectrum of keratin amide A and amide B was observed from Figure 4.14 a) at 3360 cm^{-1} and 3035 cm^{-1} respectively. Amide I is the peptide carbonyl formed due to the stretching vibration of C = O and N-H.(Akhtar et al., 1997; Zoccola et al., 2008) were, claimed that α-helix and random coil structure are perceived at 1640 -1650 cm^{-1}, β-sheets have a strong absorption band at 1610- 1640 cm^{-1} and weaker band at 1680 - 1690 cm^{-1}. As seen from figure 4.1 the amide I band was perceived at 1680 cm^{-1} indicate the presence of weak β-sheets.

Amide II is N – H in-plane and C-H stretching vibration in aid of C-C stretching (Kalaoglu, 2010). Apart from figure 4.1 the amide II is located at 1526 cm^{-1}. Amide III is due to the in-plane connection of N-H plane bending and C-N stretching with contribution from the C-C and C-O in-plane bend. The vibration observed around 1304 cm^{-1} indicates amide III absorptions of wool (figure 4.1). The absorption band located at 3605 cm^{-1} indicates the presence of the O-H of carboxylic acid in sheep wool. (S. Bhavsar, 2018) claimed that the amino analysis of sheep wool, indicates, the wool contains cysteine, glutamine, asparagine, serine, tryptophan, histidine, arginine, alanine, lanthanide, glycine, proline, methionine, valine, and tyrosine type amino acids.

As perceived from figure 4.14 b), the bands at 3590cm^{-1} and 3254 cm^{-1} indicate the O – H of hydroxyl groups due to a complex vibrational stretch of naturally occurring carbohydrate structure and N- H of amine stretching of amino acids respectively. The peaks that appeared at intense band 2811cm^{-1} indicate the presence of the C –H stretch of alkanes (Akter et al., 2021).The absorption peak at 1602cm^{-1}, 1342cm^{-1}, and1064cm^{-1} where corresponds to conjugate C = C, amine due to C-N stretching, and C-O stretch respectively. Correspondingly, (Waweru et al., 2020) also found the presence of these functional groups in the banana peel. The peak at 794cm^{-1} and 597cm^{-1} was assigned to N – H deformation of amines, and C – H stretching of alkenes and aromatics respectively.

Figure 4.14 c) illustrates band shift involvement of hydroxyl group (O-H) at absorption peaks of 3514cm^{-1}. The intense peak perceived at 3363 cm^{-1} and 3012cm^{-1} indicates the presence of amine A and amine B due to the stretching vibration of N-H bonds in the produced fertilizer. The peaks located at intense band 2888cm^{-1} indicate the presence of the C –H stretch of alkanes. The shifted band located at 1645cm^{-1}, 1335cm^{-1}, 1096cm$^{-1,}$ and 561cm^{-1} designate Amide I formed due to the stretching vibration of C = O and N-H, Amide III due to C- N stretching, and C – O stretching, and C-H stretching of alkenes respectively. The FTIR spectra analysis graph shows that the composition of sheep wool and banana peels contain amines and carboxylic acid groups, which indicate that the produced fertilizers were contained, small chains of peptides and amino acids. In addition, the FTIR analysis of liquid organic fertilizer from combination of wool and peel indicates disappearance of amid II, conjugate C =C and N – H deformation amines. This may be probably, the molecular vibration of those groups was dominated by carrier materials, and the light may pass through the sample at high transmittance.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research work was intended to produce liquid organic fertilizer from a combination of sheep wool and banana peel. In this study, the moisture content, ash content, and Functional group of wool and peels were analyzed. The moisture and ash content of wool was high, and it was indicating that wool and peel contain water-soluble enzymes and minerals which are essential for plant growth. The FTIR spectra analysis represents wool and peels that have amines and carboxylic acid that signifies the presence of amino acids and polypeptide chains.

The keratin protein extraction of wool was investigated using alkali hydrolysis and was found that different parameters including reaction temperature, reaction time, and concentration of NaOH were affected the protein yield. RSM with CCD was used for the optimization of keratin protein yield. Besides the optimum extraction of parameters developed by the quadratic model for maximum extraction of Protein yield were 63.97℃, 0.70 N, and 46.86 min reaction temperature, Concentration of NaOH, and reaction time respectively. At this optimum process parameter, the maximum protein yield extraction was found to be 75.62%. The result of the model likewise indicates that all the extraction process parameters affected the protein yield and most dominantly reaction temperature highly affected.

The result of the study indicates that liquid organic fertilizer from wool and banana peels were contained all the primary, secondary, and micronutrients. Among primary nutrients, the wool composition alone contains 11.96%, 0.25%, and 0.102% of nitrogen, phosphorus, and potassium respectively. The banana peels alone also contain 0.71%, 1.72%, and 6.89% of nitrogen, phosphorus, and potassium respectively. Besides the combination of wool and peel composition has a range value of (5.76 -6.87) %, (0.76 -1.54) %, and (2.58 -3.6) % of Nitrogen, phosphorus, and potassium respectively, and it was confirming standard values of organic fertilizers that recommended by food and agricultural organization, and organic fertilizer production industries. The study also reveals that the pH of produced fertilizers was affected by wool and peel composition. The TOC, EC, and C/N, of the products was confirms the standard value. Functional groups of produced fertilizer was also indicating the presence of amino acids in sample.

5.2 Recommendation

The result obtained in this study clearly showed that waste sheep wool and banana peels contained a good level of macro and micronutrients which are essential for soil conditioning and plant growth. The conversion of waste materials into organic fertilizer is not used only for plant production and soil conditioning, but it is also used for waste reduction and economic points of view. Hence the following points are recommended strongly.

- It is recommended that ahead of implementation on the field, further study and feasibility needs to be carried out.
- The inexpensive organic fertilizer is one of the substances studied and used for environmental remediation. Therefore, further study should be carried out on the amino acid composition of produced organic fertilizer using HPLC.
- In this research, it was attempted to show that banana peels can improve the level of potassium, phosphorus, and other macronutrient element contents of organic fertilizer. Therefore, further investigation could be carried out on evaluating an optimum process parameter of improving the macronutrient level of organic fertilizer from combined raw materials.
- Further studies are needed on various topics related to valorization and best incorporation techniques of other waste materials in liquid-organic fertilizer production
- The effect of each process parameter on physio-chemical properties of organic fertilizer needs further study.

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APPENDIXES

Appendix A: Experimental results for keratin protein extraction

Table A. 1 Optimum possible condition selected for keratin protein extraction

No	Temperature (℃)	Concentration (N)	Time (min)	Yield (%)	Desirability	
1	**63.971**	**0.699**	**46.861**	**75.617**	**1.000**	**Selected**
2	62.667	0.783	47.000	76.496	1.000	
3	66.826	0.740	38.434	75.825	1.000	
4	74.874	0.718	55.181	76.311	1.000	
5	77.284	0.885	38.407	75.645	1.000	
6	74.497	0.801	53.189	77.784	1.000	
7	69.310	0.790	35.123	75.322	1.000	
8	64.960	0.784	41.708	76.779	1.000	
9	77.284	0.885	51.593	76.604	1.000	
10	77.463	0.755	37.647	76.294	1.000	
11	79.515	0.739	38.138	75.782	1.000	
12	76.836	0.955	45.746	75.434	1.000	
13	70.156	0.838	54.390	77.541	1.000	
14	70.755	0.805	41.421	78.271	1.000	
15	73.325	0.644	42.749	76.186	1.000	
16	75.816	0.917	41.831	76.400	1.000	
17	73.897	0.677	46.857	77.442	1.000	
18	74.795	0.789	36.470	76.319	1.000	
19	64.622	0.793	52.912	76.773	1.000	
20	74.707	0.835	38.245	76.933	1.000	
21	65.166	0.826	50.163	77.653	1.000	
22	69.872	0.678	41.167	76.357	1.000	
23	73.115	0.748	35.493	75.677	1.000	
24	66.416	0.735	41.822	76.759	1.000	

25	65.307	0.782	56.572	75.596	1.000
26	71.620	0.889	44.035	78.082	1.000
27	66.381	0.961	50.687	75.939	1.000
28	74.944	0.954	43.795	75.898	1.000
29	65.333	0.872	45.042	77.509	1.000
30	71.706	0.811	40.069	77.912	1.000
31	70.725	0.758	40.929	77.902	1.000
32	74.126	0.689	41.825	77.192	1.000
33	71.498	0.886	38.701	76.657	1.000
34	74.434	0.929	39.825	75.797	1.000
35	74.126	0.936	53.897	75.652	1.000
36	69.338	0.786	35.456	75.508	1.000
37	66.513	0.728	39.052	75.750	1.000
38	78.973	0.877	40.502	75.902	1.000
39	75.826	0.797	38.902	77.220	1.000
40	64.862	0.828	51.983	77.220	1.000
41	64.159	0.869	49.538	77.173	1.000
42	77.592	0.710	42.335	77.227	1.000
43	76.221	0.800	37.193	76.428	1.000
44	76.610	0.726	54.354	76.430	1.000
45	79.976	0.827	44.461	76.958	1.000
46	62.753	0.911	43.374	75.686	1.000
47	73.323	0.841	47.972	78.773	1.000
48	77.589	0.691	43.786	77.125	1.000
49	74.368	0.677	37.745	75.591	1.000
50	70.045	0.968	51.866	75.812	1.000
51	71.152	0.966	45.031	76.250	1.000
52	76.057	0.678	41.583	76.752	1.000
53	72.990	0.885	51.442	77.735	1.000
54	65.232	0.748	46.563	77.231	1.000

55	67.924	0.948	49.637	76.681	1.000
56	77.165	0.830	38.114	76.406	1.000
57	78.774	0.698	53.357	75.718	1.000
58	71.094	0.891	53.413	77.275	1.000
59	66.800	0.881	40.889	76.918	1.000
60	65.284	0.944	52.690	75.768	1.000
61	75.021	0.826	48.773	78.555	1.000
62	76.071	0.920	52.032	76.090	1.000
63	74.370	0.711	49.240	77.960	1.000
64	73.832	0.921	38.517	75.635	1.000
65	79.325	0.721	47.187	77.173	1.000
66	63.599	0.747	51.592	76.054	1.000
67	68.632	0.705	47.358	77.533	1.000
68	70.974	0.961	52.981	75.698	1.000
69	73.311	0.701	55.797	75.877	1.000
70	71.384	0.736	36.175	75.794	1.000
71	79.674	0.627	44.987	75.015	1.000
72	74.065	0.853	40.372	77.596	1.000
73	75.787	0.904	43.566	77.074	1.000
74	71.314	0.726	37.092	76.085	1.000
75	66.203	0.685	46.619	76.234	1.000
76	67.079	0.724	53.708	76.425	1.000
77	66.828	0.654	42.963	75.097	1.000
78	69.075	0.840	45.328	78.682	1.000
79	69.898	0.639	47.388	75.913	1.000
80	73.009	0.792	35.556	75.944	1.000
81	74.064	0.824	38.643	77.255	1.000
82	71.809	0.693	56.243	75.493	1.000
83	76.906	0.824	40.845	77.472	1.000
84	72.390	0.717	39.223	76.940	1.000

85	61.548	0.756	50.656	75.274	1.000
86	71.408	0.764	57.831	75.859	1.000
87	72.413	0.756	38.604	77.217	1.000
88	79.049	0.805	52.514	76.615	1.000
89	61.611	0.898	51.351	75.513	1.000
90	71.299	0.776	42.186	78.427	1.000
91	63.658	0.849	46.352	77.148	1.000
92	72.739	0.799	55.724	77.032	1.000
93	72.385	0.673	39.753	76.188	1.000
94	67.558	0.894	55.350	76.345	1.000
95	62.244	0.765	51.662	75.643	1.000
96	60.568	0.844	44.504	75.313	1.000
97	76.819	0.658	40.986	75.996	1.000
98	61.272	0.911	51.615	75.109	1.000
99	75.444	0.719	42.352	77.760	1.000
100	72.183	0.964	45.228	76.260	1.000

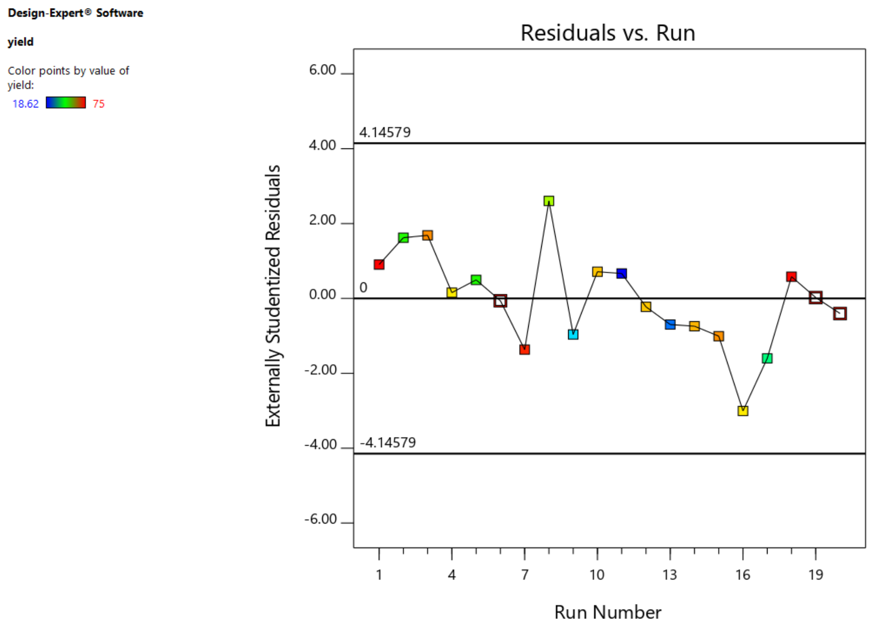

Figure A. 1 Residual vs run plot

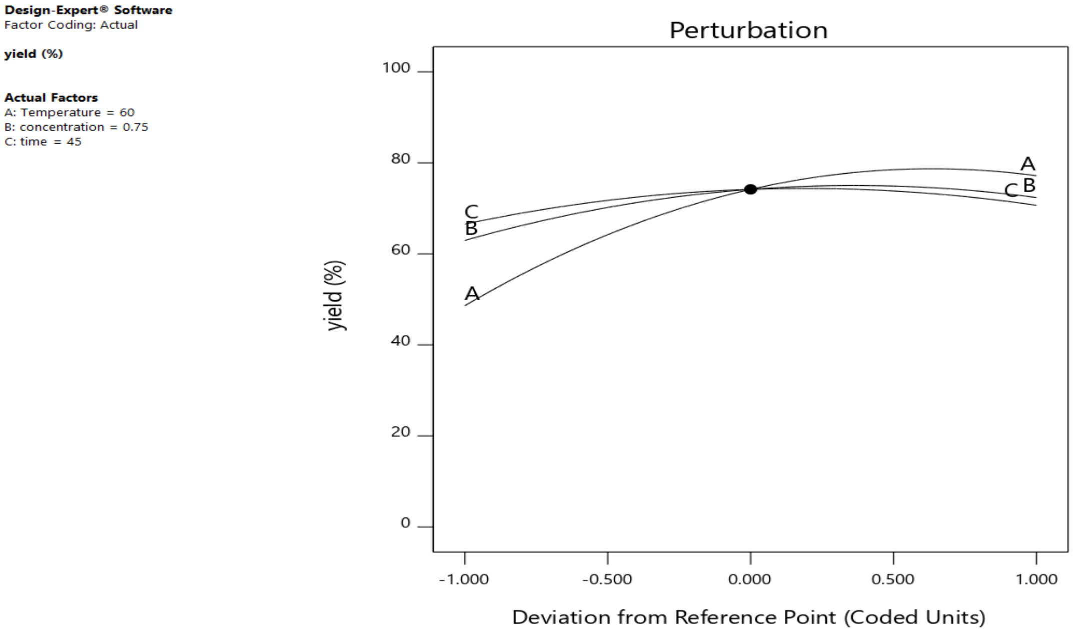

Figure A. 2 Perturbation plot for keratin protein extraction

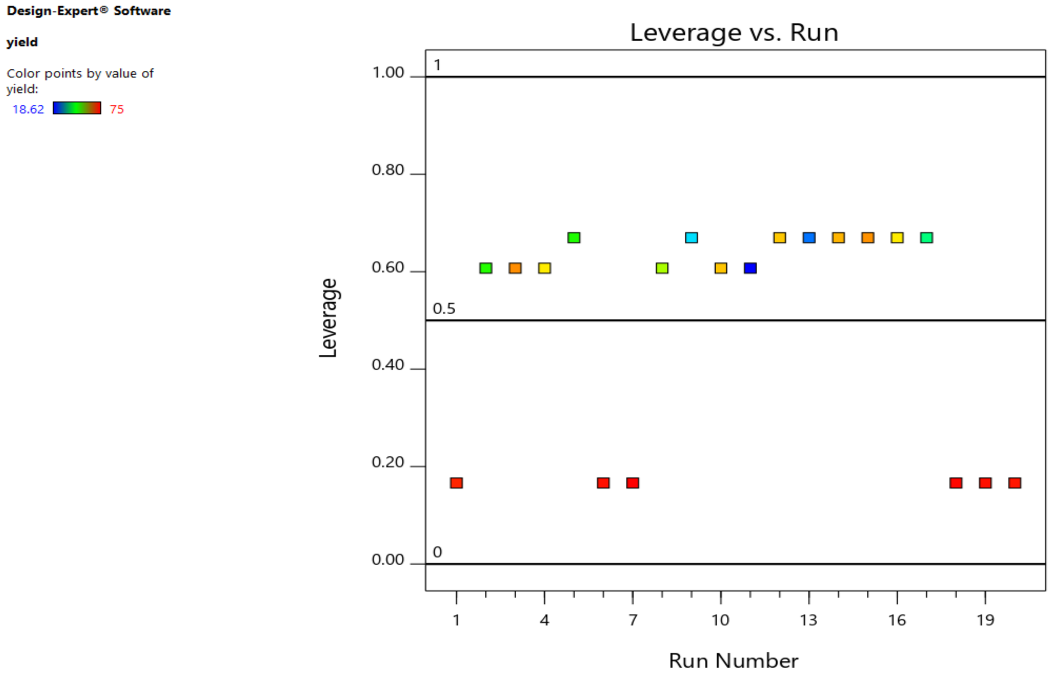

Figure A. 3 Leverage vs run

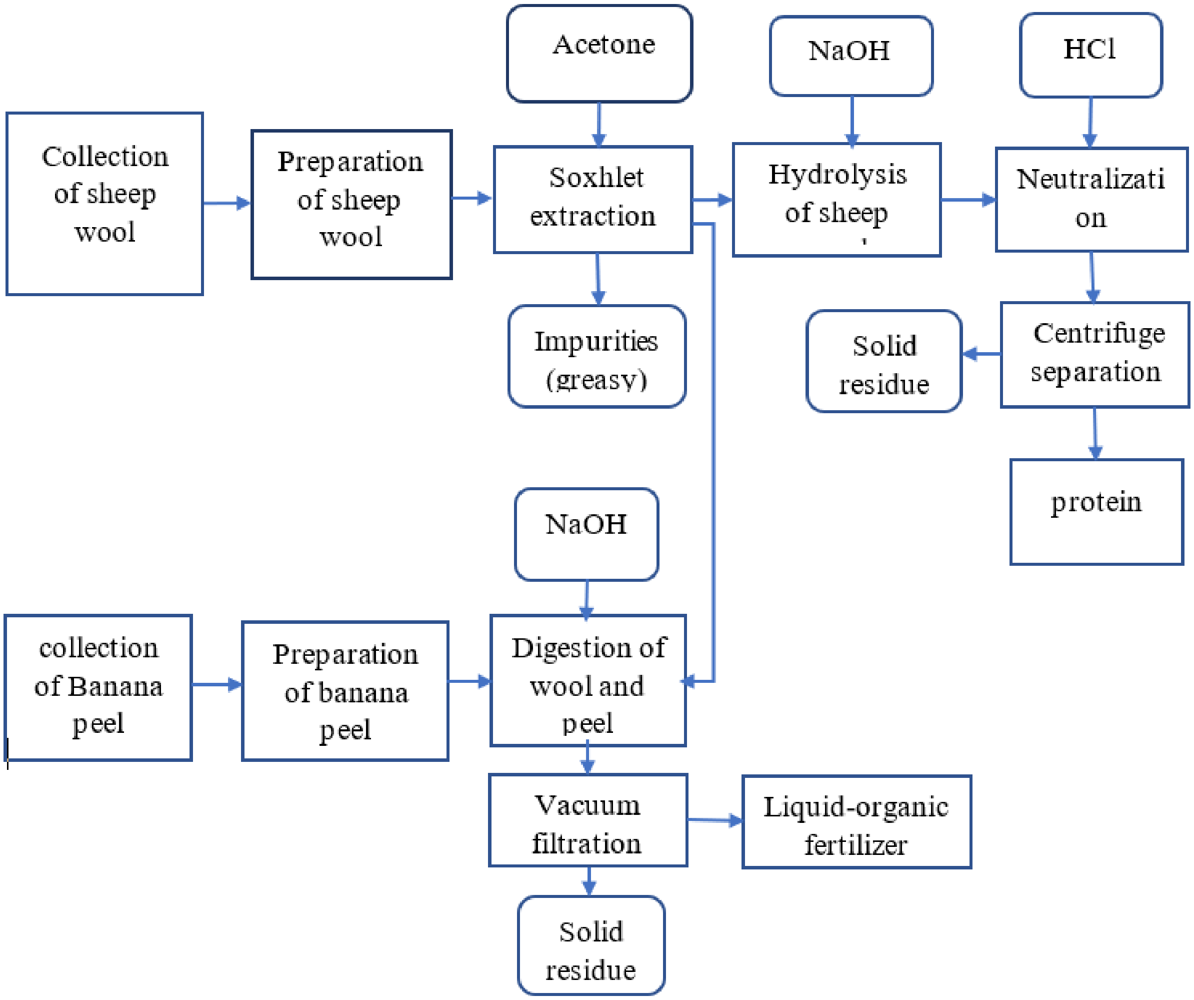

Figure A. 4 Overall experimental frame work

Table B. 1 Combination of wool and peel in experimental data of fertilizer production.

run	**Wool/peel ratio**	
	wool	peel
1	1	0
2	0.75	0.25
3	0.25	0.75
4	0.5	0.5
5	0	1

Table B. 2 Experimental data vs absorbance of phosphorus and sulfur in produced fertilizer

Run	Wool/peel ratio		Absorbance of Phosphorus (880nm)	Absorbance of Sulfur (420nm)
	wool	peel		
1	1	0	0.146	0.684
2	0.75	0.25	0.203	0.521
3	0.25	0.75	0.287	0.23
4	0.5	0.5	0.274	0.252
5	0	1	0.413	0.108

Appendix B.2 Calibration curves

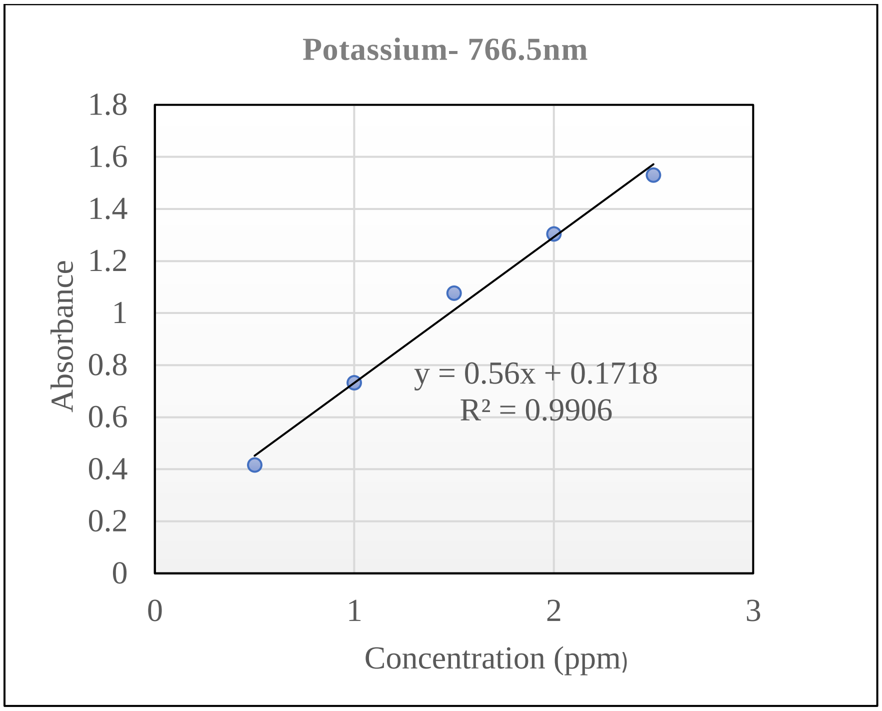

Figure B. 1 calibration curve of Potassium

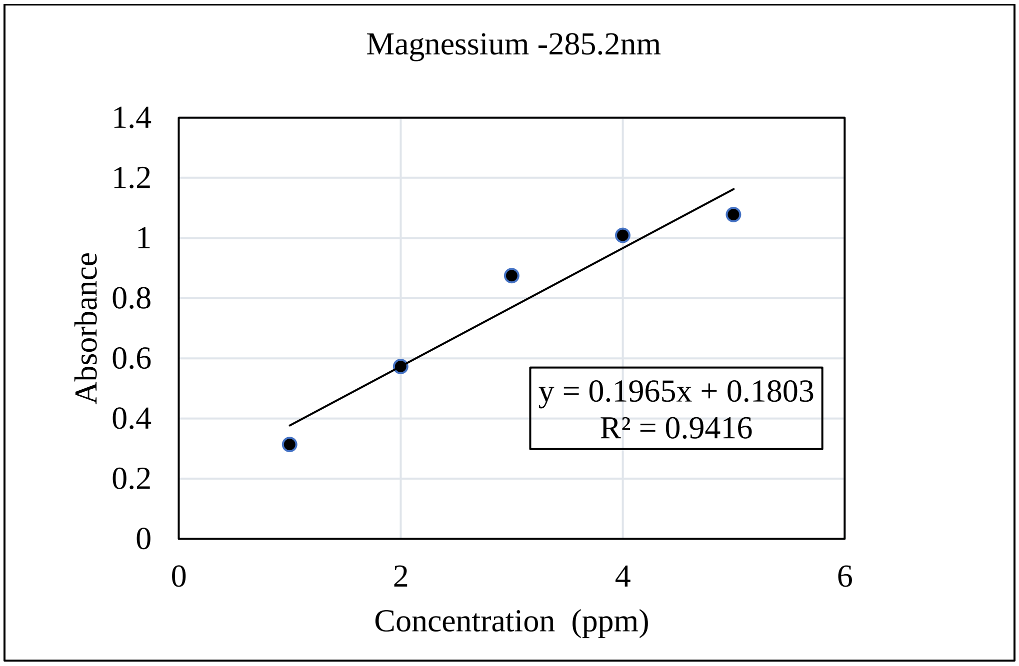

Figure B. 2 Calibration curve of magnesium

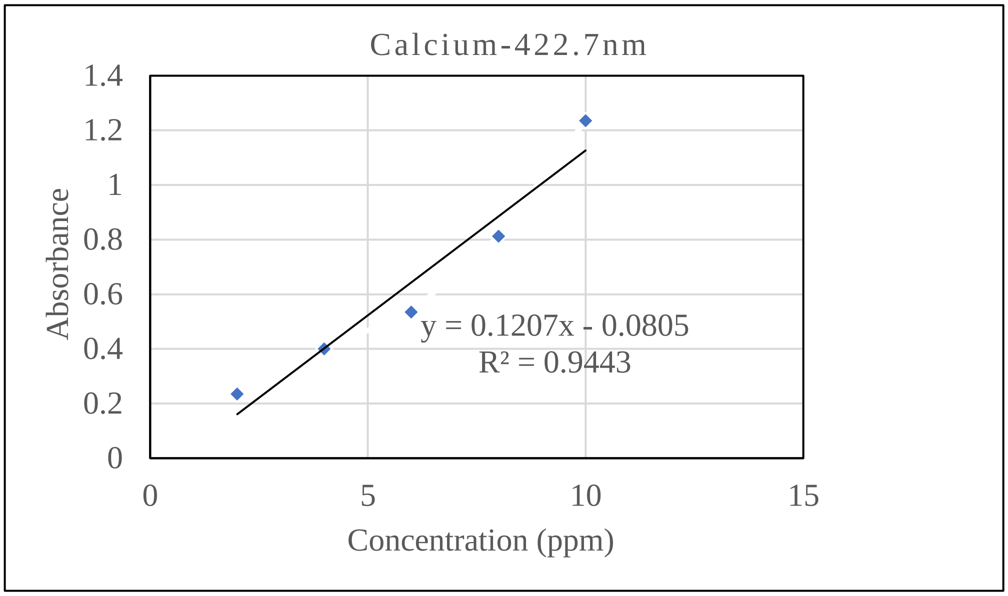

Figure B. 3 Calibration curve of calcium

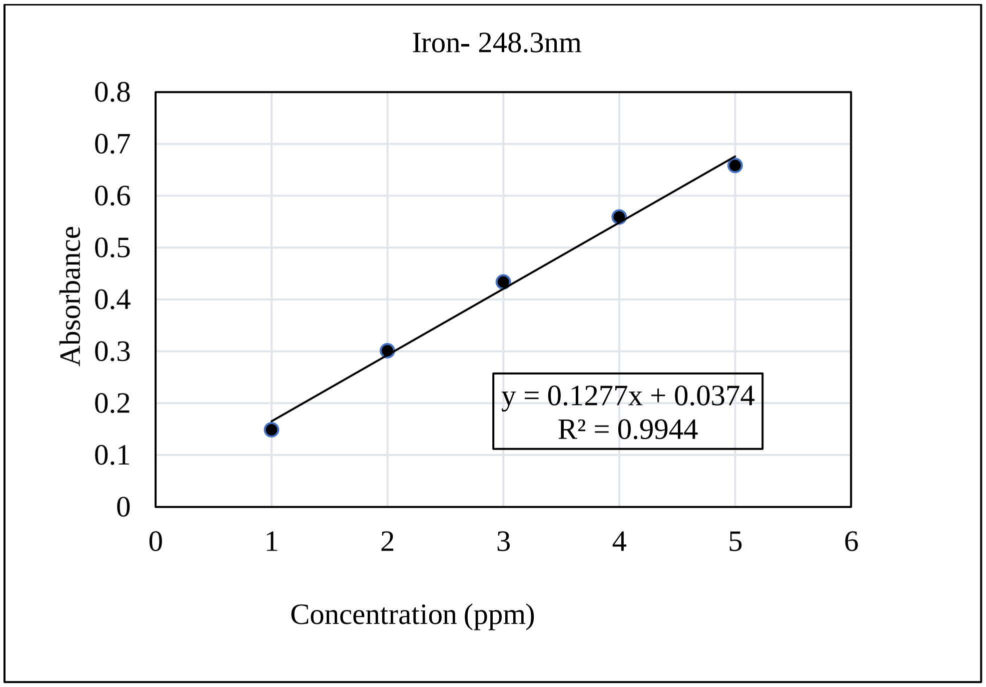

Figure B. 4 Calibration curve of Iron

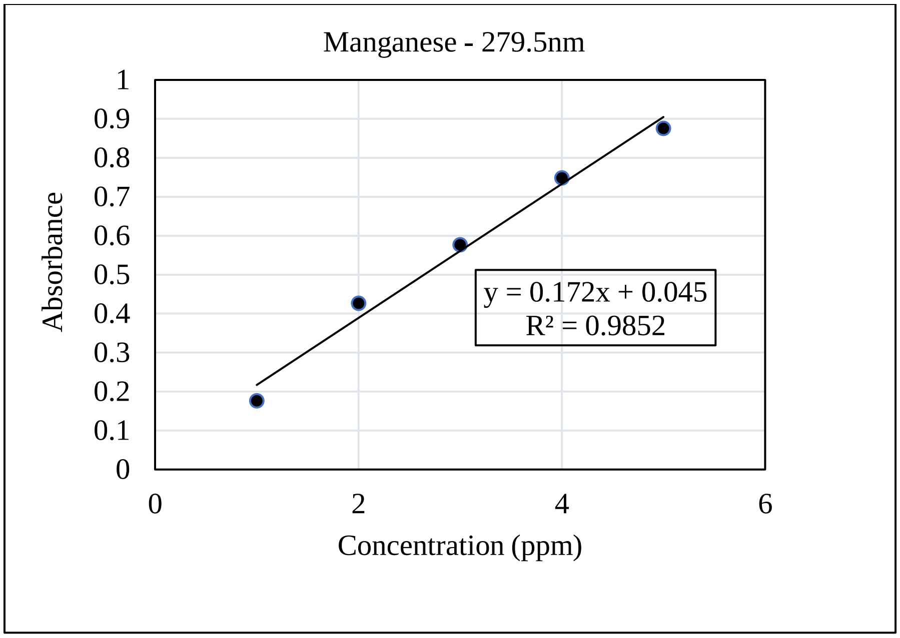

Figure B. 5 Calibration curve of manganese

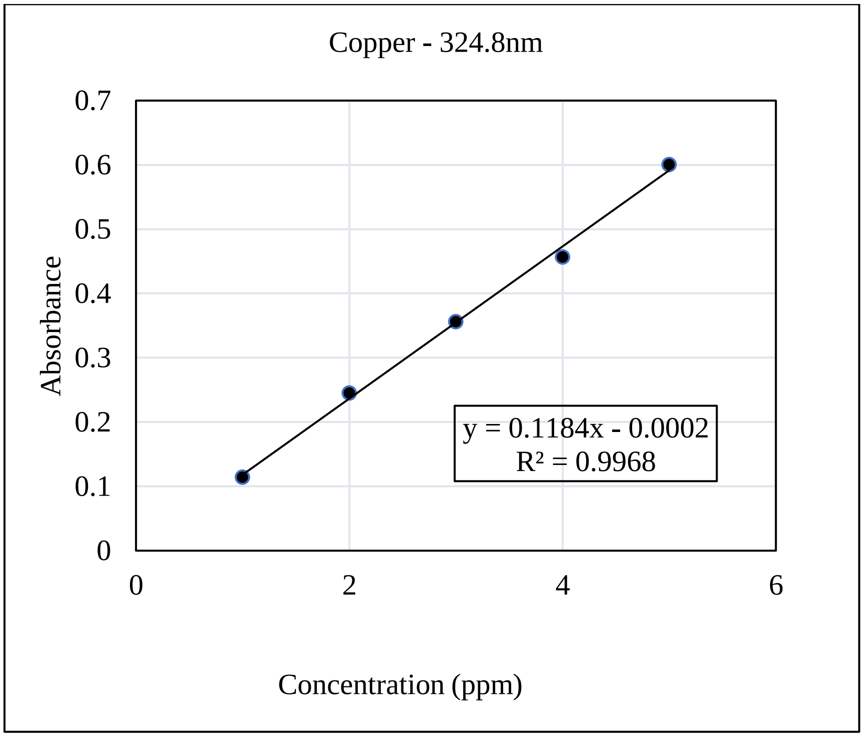

Figure B. 6 Calibration curve of copper

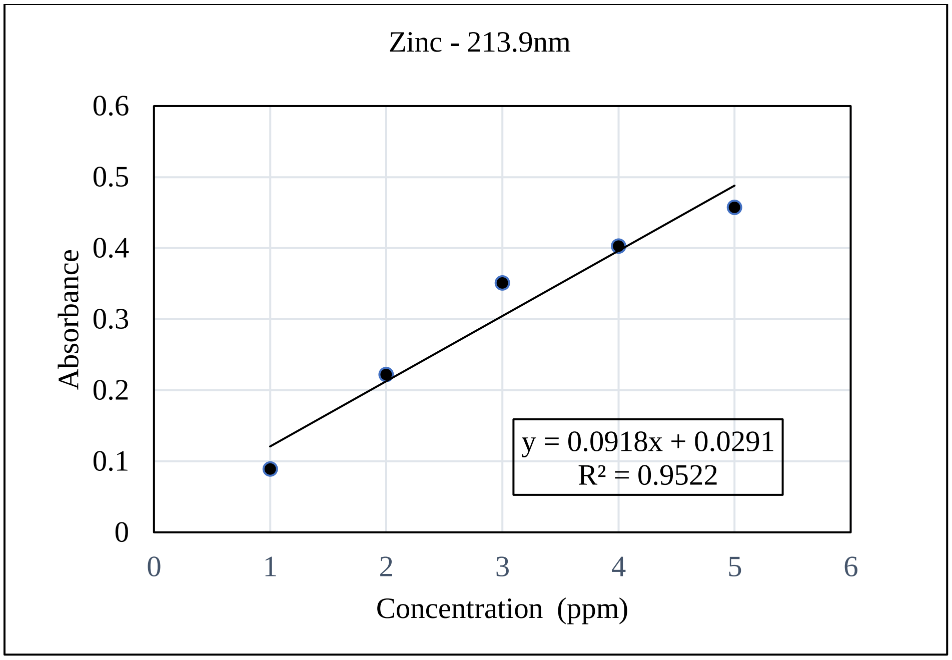

Figure B. 7 Calibration curve of Zinc

Appendix C.1 Percentage of primary nutrient, pH of fertilizers with mass fraction of mass fraction of wool and peel,

Table C. 1 Percentage of primary nutrient Vs mass fraction of wool and peel.

Wool/peel ratio		Nitrogen (%)	Phosphorus (%)	Potassium (%)
wool	peel			
1	0	11.96	0.25	0.102
0.75	0.25	6.87	0.76	2.58
0.5	0.5	6.21	1.20	3.17
0.25	0.75	5.76	1.54	3.6
0	1	0.71	1.72	6.89

Relationship between units

1ppm = 1mg/kg,

1mg/kg = 0.001mg/g, and

1mg/g = 0.1%

Table C. 2 Mass fraction of wool and peel Vs pH of Fertilizers

Wool to peel		pH
wool	peel	
1	0	7.82
0.75	0.25	8.10
0.5	0.5	8.54
0.25	0.75	8.76
0	1	13.01

The volume of produced fertilizer was calculated using equation **(C.1).**

$$V_f = \frac{m_f}{\rho_f} \qquad \text{(C.1)}$$

Were, Vf - Volume of produced fertilizers (ml)

m_f - a mass of produced fertilizers (g)

ρ_f - density of fertilizer (g/ml)

Appendix C.3 Conversion factor for major primary nutrient (FAO, 1991)**.**

$$P_2O_5 = 2.2919 \times P \qquad \text{(C.2)}$$

$$K_2O = 1.2046 \times K \qquad \text{(C.3)}$$

Appendix C.4 Total concentration of an element in the products

The total concentration of elements in the sample was calculated using the equation (C.4)

The total concentration of the element in sample = $\dfrac{(r-b)\times TV\times df}{wt}$ (C.4)

Where,

r- reading value,

b- concentration of blank,

TV – total volume of sample for reading,

df -dilution factor, and

wt. –weight of the sample

Appendix D: Some selected Major apparatus used for the study

a) b)

c) d)

Figure D. 1 a) Kjeldahl’s digester, b) Kjedhal distillation c) AAS and, d) U-V

Appendix E. Some selected experimenter pictures of the study

Appendix E.1 process flow chart for study

Figure E. 1 Process flow chart for this study

Appendix E.2 prepared sample for digestion

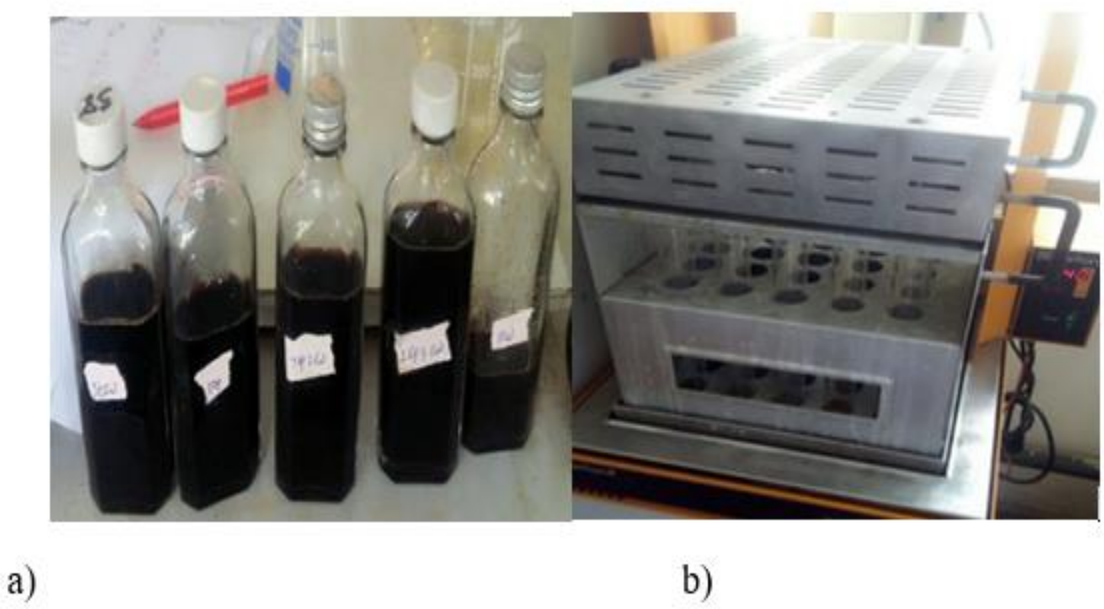

Figure E. 2 a) sample prepared for digestion, b) sample on digestion by Kjeldahl’s method