JIMMA UNIVERSITY

JIMMA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL ENGINEERING

PROCESS ENGINEERING STREAM

TITLE: - SYNTHESIS AND EVALUATION OF A CHICKEN FEATHER KERATIN BASED BIOFILM PLASTICIZED BY SORBITOL BLENDED WITH GLYCEROL.

THESIS PROPOSAL SUBMITTED TO SCHOOL OF GRADUATE

STUDIES OF JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT FOR

THE DEGREE OF MASTER OF SCIENCE IN PROCESS

ENGINEERING STREAM.

BY:- TSION ASSEFA

JANUARY 2021

JIMMA, ETHIOPIA

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Declaration

I hereby declare that this research entitled "synthesis and evaluation of a chicken feather keratin based biofilm plasticized by sorbitol vs. glycerol". is my original work and has not been proposed by any other for award of a degree in this and any other university.

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Abstract

Biomass has the potential to reduce greenhouse gas emissions by replacing fossil fuels. The exponential growth of the human population has led to the accumulation of non-degradable waste materials across our planet. Bioplastic synthesized from only keratin has fragile and less flexible and can be improved by adding starch and plasticizer. In this study, chicken feather keratin, mango seed starch, and glycerol and sorbitol were used. Proximate analysis of chicken feather was crude lipids (0.79%), total ash (1.45%), crude fiber (2%), moisture (11.9%), and crude protein (89.5%). The optimum process variables for keratin yield were 6hr., 50^{o}C temperatures, which gives 79% maximum yield. The composition of the extracted keratin was characterized using FTIR, the crystalline nature of keratin was characterized by using XRD, and keratin's thermal stability was characterized using TGA. The starch extracted from mango seed had 2.17% crude protein, 6.66% crude lipids, 8.5% total ash, 10.5% moisture, 34.37% crude fiber, and 37.8% carbohydrates. The film was synthesized by conducting 20 experiments by using CCD response surface rotatable (k<6) and analyzed by design expert 11, by conducting three factors of dry oven temperature(35-65^{o}C), plasticizer(GLY and SOR) concentration (20-50%)w/w and starch concentration(30-70%) w/w of keratin (7g) basis. The three responses were tensile strength(TS), water absorption(WA), and elongation at the break (EA) of the synthesized bioplastic film. The result obtained in the range were (10-17.86MPa) TS, (8.99-20%) EA and (19.45-27.5%) WA. The obtained results at the optimal point were 17.76MPa TS, 8.98% EA and 19.44% WA at a combination of 49.94^{o}C dry oven temperature, 34.60% plasticizer (GLY and SOR), and 49.94% Starch concentrations. Moisture, solubility, transparency, and thickness of the film were determined. The film was also characterized using TGA, XRD and FTIR. This study indicates that higher strength, low water absorbent, and good thermally stable bioplastic film can be produced from chicken feather keratin via mango seed starch by blending two different plasticizers (GLY and SOR).

Keywords: Feather-keratin, mango seed starch, glycerol, sorbitol, bioplastic, biopolymer

Acknowledgment

First and foremost, I would like to thank GOD, whose many blessing have made me who I am today, for giving me the strength and wisdom to successfully complete this thesis for his protection and strength, an ever-present help in the entire situation and challenge that I face.

I am forever grateful to Dr Femi Olu for having accepted to be my thesis advisor. His patience, guidance, understanding of attitudes and constrictive suggestions, undoubtedly was the utmost supports for me succeed in my thesis work. I also thank my co-advisor Mr-Ketema Beyecha for his continues comment.

I am so grateful to the collage of agricultural at Jimma University, which allowed me to conduct the experiment. I give deep thanks Mr Gemechu for his help during my laboratory work at the post-harvest lab. Also, I deeply thank the chemical department lab technicians Mr defare and Mr Dingeta for their help. And also I thank materials department lab technicians Mr sulutan, for his help and good friendship.I thank Ethiopian Conformity Assessment Enterprise (ECAE) and Adama Science Technology University.

I thank JIT to be my sponsor and giving me a chance to join this master's program. I want to give my heart felt gratitude for Abeba Asefaw, Tsion Ayalaw, Lidiya wubshet, Rediat Terefe and Workiye Getnet for their help and good friendship. Finally, I acknowledge my family with the deepest gratitude who encourage me to strive toward academic-excellence and always wish the best for them.

Table Contents

Declaration..... i

Abstract ii

Acknowledgment iii

List of Table..... viii

List of Figure ix

Acronyms xi

CHAPTER ONE 1

1 Introduction.....1

1.1 Background 1

1.2 Statement of Problem 3

1.3 Hypothesis and research objective.....4

1.3.1 Hypothesis 4

1.3.2 Objective of the study.....4

1.4 Scope of the study.....5

1.5 Significance of the study.....5

CHAPTER TWO 6

2.1 Introduction 6

2.2 Natural protein source 7

2.3 Biomass Keratin 8

2.4 Chicken feather overview 9

2.4.1 Chicken feather production capacity.....9

2.5 Methods of keratin extraction from chicken feather.....11

2.6 Biomass Starch 14

2.7 Mango seed and mango seed extraction criteria.....15

2.8 Mango seed starch extraction and specification criteria 16
2.9 Characterization of keratin protein and starch presence 17
2.10 Plasticizer 18
2.11 Film preparation techniques 19
2.12 Bio-plastic production from the different protein source.....20
2.13 Physiochemical analysis of bio-plastic 20
2.14 FTIR analysis.....21
2.15 TGA analysis of film 23
2.16 XRD analysis of film 23
CHAPTER THREE.....25
3.1 The structured environment of the thesis 25
1.2 Chemicals 25
3.3 Materials.....25
3.4 Laboratory equipment.....25
3.5 Processing methods 26
3.5.1 Methods for Proximate analysis keratin.....26
3.5.2 Method for the extraction of keratin from chicken feathers 27
3.5.2.1 Experimental procedure 27
3.5.3 Characterization of the presence of protein and yield of keratin.....29
3.5.4 Starch from waste mango seed.....29
3.5.5 Characterization of starch extracted from mango seed.....32
3.6 Synthesis of bio-plastic from the extracted keratin via starch 33
3.7 Application studies of plastic film 33
3.8 Characterization techniques 35
3.9 Experimental design and statistical analysis 36

CHAPTER FOUR....37
4.1 The result of proximate analysis of chicken feather....37
4.2 Keratin extraction and yield results....37
4.2.1 Development of a regression model for keratin extraction....41
4.2.2 Optimization of process variable and yield....42
4.3 The result of the characteristics of the extracted keratin....42
4.3.1 Determination of appearance of keratin during the precipitation stage....42
4.3.2 UV-Vis result of extracted keratin....43
4.3.3 FTIR Analysis....44
4.3.4 X-ray diffraction of feather and keratin....44
4.3.5 Thermal geometric analysis (TGA) of feather and keratin....45
4.3.6 Chemical and physical properties of extracted starch....46
4.4 Synthesis of bioplastic and analysis on tensile strength, elongation at the break and water absorption....47
4.4.1 Statistical analysis of factors affecting the response variables....48
4.4.2 Adequacy check for the developed response surface quadratic models....52
4.4.3 Development of regression model equation....58
4.4.4 The interaction effect between process variables and responses....58
4.4.5 Process factors and response variables optimization....67
4.5 Application studies result of the bio-plastic film....70
CHAPTER FIVE....76
5 Conclusion and Recommendation....76
5.1 Conclusion....76
5.2 Recommendations....78
Reference....79

Appendices 83

List of Table

Table 2.1 Ten countries producing the most chicken in the world.....10
Table 2.2 Proximate composition of a chicken feather.....11
Table 2.3 optimum parameter for extracting keratin from chicken feathers by sodium sulfide ..13
Table 2.4 Amylose and amylopectin concentration from various starch sources 14
Table 2.5 World top 10 mango producer 15
Table 2.6 The production capacity of mango in regional states of Ethiopia.....16
Table 2.7 Proximate composition of mango seed 17
Table 2.8 Characteristic of mango seed starch.....17
Table 2.9 the comparison of the tensile strength of keratin blend with different polymer 19
Table 2.10 The tensile strength of commonly used plastic 21
Table 2.11 Infrared spectroscopy correlation 22
Table 2.12 The Major Functional Group Found In Keratin/ Starch Films.....23
Table 3.1 Factors and ranges of the CCD design quadratic model.....36
Table 4.1 The result of the proximate composition of the raw chicken feathers on a mass basis .37
Table 4.2 Analyze the variance for 12 experiments of keratin yield 38
Table 4.3 ANOVA for Quadratic model.....39
Table 4.4 Model adequacy measures for keratin extraction yield response.....39
Table 4.5 Summary of constraint response and goals of optimizations.....42
Table 4.6 Optimization table of extraction.....42
Table 4.7 The result on characteristics of mango seed starch 46
Table 4.8 Design summary.....48
Table 4.9 values of the three response variables associated with the factors.....50
Table 4.10 Analysis of variance [partial sum of squares], for tensile strength 51
Table 4.11 Model adequacy measures for tensile strength 53
Table 4.12 summary of constraint responses and goals of optimizations.....68
Table 4.13 Optimum operating point.....68
Table 4.14 Model validation.....69
Table 4.15 Results on some physicochemical properties for keratin-starch plasticized by glycerol, sorbitol, and both glycerol and sorbitol. 72

List of Figure

Figure 2.1 Classifications of biodegradable polymers 6
Figure 2.2 Different natural protein sources 7
Figure 2.3 Projected demands for poultry meat and eggs in Addis Abeba 11
Figure 2.4 Chemical structure of keratin from chicken feathers 12
Figure 3.1 chicken feather 27
Figure 3.2 Block flow diagram of keratin extraction 28
Figure 3.3 Waste mango seed 30
Figure 3.4 Block flow diagram of starch extraction from mango seed 31
Figure 4.1 Effect of temperature on yield 41
Figure 4.2 Effect of time on yield 41
Figure 4.3 Keratin precipitation 43
Figure 4.4 UV-Vis result of extracted keratin 43
Figure 4.5 FTIR result of keratin and feathers 44
Figure 4.6 X-ray diffraction of feather and keratin 45
Figure 4.7 TGA of feather and keratin 46
Figure 4.8 Mango seed starch powder 47
Figure 4.9 Process flow diagram of film synthesis 47
Figure 4.10 Predicate vs. actual plots for tensile strength 53
Figure 4.11 outliner VS. Run number plot for tensile strength 55
Figure 4.12 Normal probability Plots for A TS, B EA, and C WA 57
Figure 4.13 Residual vs. Run number of tensile strength (TS)plots 58
Figure 4.14 interaction effects of dry oven temperature and plasticizer concentration on tensile strength, elongation and water absorption 62
Figure 4.15 interaction effects of dry oven temperature and starch concentration on tensile strength, elongation and water absorption 64
Figure 4.16 interaction effect of starch and plasticizer concentration on the tensile strength, elongation and water absorption 67
Figure 4.17 FTIR result of keratin-starch glycerol, keratin-starch sorbitol and keratin-starch glycerol vs. sorbitol 73

Figure 4.18 TGA analysis of the produced film 74
Figure 4.19 XRD analysis the produced film 75

Acronyms

ANOVA	Analysis Of Variance
CCD	Central Composite Design
CV	Coefficient of Variation
D.oven Tem	Dry Oven Temperature
EA	Elongation at the Break
JIT	Jimma Institute of Technology
PRESS	Predicated Residual Error Sum of Squares
RSM	Response surface method
FTIR	Fourier Inferred Ray Spectroscopy
FK	Feather keratin
GLY	Glycerol
HCl	Hydrochloric Acid
MCC	Microcrystalline Cellulose
NaOH	Sodium Hydroxide
Uv-Vis	UltraViolet visible Spectroscopy
XRD	X-Ray Diffraction
TGA	Thermogravimetry Analysis
TPS	Thermoplastic starch
SOR	Sorbitol

CHAPTER ONE

1 Introduction

1.1 Background

The world's need for petroleum-based plastic for food packaging applications increases due to the rapid population growth. In people's day-to-day lives, there is the use of plastic and the release of used petrol-based plastic to the environment. The plastic produce from petroleum is the primary environmental pollutant. This is because petroleum-based plastic cannot be degraded in the soil. Plastic has toxic pollutants that affect the environment and cause land, water, and air pollution. It takes hundreds or even thousands of years for plastic to break down, so the damage to the environment is long-lasting. On the other side, agricultural waste is an urgent issue: agricultural residues' biomass by-products originating from production, harvesting, and processing in farm areas. However, biopolymers from agricultural sources or the agricultural waste stream is viewed as a viable alternative to petroleum-based plastic.

The poultry industry is the major source of agricultural waste, the rapid growth of large scale poultry farming cause the release of a large amount of waste chicken feather. On a world scale, it was estimated that approximately 40109 kg of chicken feathers are produced from the slaughter of more than 58109 chicken per day [1]. Since chicken feathers can be processed in fast production time, with a low economic value of poultry per unit, the chicken grows in a short period and the poultry is the major source of human consumption. The development of the poultry industry is rapid. Chickens in Ethiopia contain over 50 million poultry, 96% are non-commercially raised chicken [2].

The current poultry population of Ethiopia is estimated to be around 60 million, out of which the majority (37.9 percent or 22.7 million) are chicks, and only 33.6 percent (20.2 million) are laying hens[3]. About 56 percent (9.6 million) of Ethiopian households have poultry holdings with a varying range of flock size[4]. Regrettably, most of the feathers are cast away as solid waste in landfill sites without any treatment, which results in severe environmental damage. Therefore, recycling and exploiting feather keatin for bioplastic production could also save the environment from harm**.** In general, the chicken feather waste can be eliminated by several methods such as

disposals, burning and burying. These methods, however, are environment-unfriendly. Therefore, the modification of the chicken feather wastes for use as value-added products in packaging applications. Keratin is the major structural fibrous protein providing outer covering, such as hair, wool, feathers, nails, and horns of mammals, reptiles, and birds. More than 91% of keratin can be extracted from chicken feather; Keratin can be synthesized as biomaterials in many forms, including a film.

The synthesis film can be applicable for food packaging; however, Keratin film is highly fragile and has low flexibility. Polysaccharides, or sugar, are a polar molecule that is constituted of many hydroxyl groups in its structure. Starch is a polysaccharide polymer which is bio-based, biodegradable, low-cost, and naturally abundant material. Starch is combined with hydrophobic and ductile biopolymers such as keratin usually presents in a continuous form, whereas starch represents the discontinuous phase [5]. Some researchers solved fragility and flexibility by copolymerizing with starch, but there is still a need to improve the final bio-plastic properties by modifying the plasticizer used. Some reports is done on the production of avocado seed starch crosslinking with keratin. However, the report focuses on the effect of starch and keratin concentration variation on the final film by fixing another effect such as plasticizer concentration and oven drying temperature and most often the plasticizer they use is glycerol too.

The mango seeds are discarded as wastes after the fruits have been eaten. Thus, because of the availability and been a reliable source of starch, this type of agricultural waste could be used as value-added products. The aims of this work are to synthesis and characterize keratin cross-linking with mango seed starch biofilm by using the mixture of glycerol and sorbitol as a plasticizer and see the effect of the concentration variation of starch and glycerol and sorbitol and oven drying temperature on the tensile strength, elongation and water absorption of the final film and characterize the best film by FITR, TGA, and XRD.

1.2 Statement of Problem

Petroleum-based packaging material, and the waste release during agricultural processing is a major environmental pollution, which can harm water, land, and air. Chicken feather release from the poultry industry is a potential source of environmental pollution. There are different disposal techniques such as incineration, landfilling, and composting. The techniques are energy-intensive, required ample landfill space, and contribute to greenhouse gas emissions that are not environmentally sustainable. There are also billions of tons of feather waste from the poultry industry in Ethiopia. It can pose hazards to human and ecological health as they often contain viruses and bacteria. Burying or burning the feathers encounters a severe problem.

Valorizing chicken feathers by converting them into valuable materials is a popular route for dealing with the waste to overcome the above problem. The keratin found in the waste chicken feather can be utilized to prepare the film, which can be used for different applications[1]. Keratin is desirable protein, because of their environmental stability, biodegradability, and biocompatibility characteristics. Some researchers have been working on preparing the keratin-based film, becuase film prepared from keratin alone is highly fragile and has low flexibility need to blend with another polymer such as a polar polymer.

The biodegradable film, synthesized from only keratin, has less mechanical strength and thermal stability[6]. Now biodegradable bioplastic is synthesized from any keratin-rich biomass sources such as horn, wool, etc., the mechanical properties and thermal stability of keratin can be improved by reinforcement with starch. The raw material used must be waste products that do not contradict any food security. Utilizing waste to produce usable products is also advantageous to reduce waste disposal on the environment. Mango seed is one potential agricultural product, and the starch extracted from the seed can be used as filler in the keratin-based film preparation. In this case, chicken feather keratin blended with mango seed starch was synthesized to produce a bioplastic film using solution cast technics.

1.3 Hypothesis and research objective

1.3.1 Hypothesis

A large amount of chicken feather waste is released from the poultry processing industry. This waste can be used as a source of protein, which can be utilized for bioplastic application. The many problems of bioplastic produce from this source is less processability and fragile. The processability of the keratin improves by addition of polar materials such as starch and plasticizer. Plasticizer is used to increase the flexibility of materials by increasing the interspacing between polymer-polymer interactions. When the plasticizer is adds to materials, the tensile strength of the film will decrease. Different plasticizer has a different plasticizing effect. When the plasticizer's molecular weight is low, it is easy to enter between the polymers and increase the material's flexibility. Glycerol has a low molecular weight than other plasticizerss; thus, it has a high plasticizer effect. The one disadvantage of glycerol is it decreases the tensile strength and increases water absorption of the film. Sorbitol is an hydrophilic plasticizer. It has high, molecular weight than glycerol; the molecular weight increase the tensile strength increase and water absorption decreases. Blending these two plasticizers gives the advantage of individual. This study's hypothesis is blending sorbitol and glycerol can increase the tensile strength and reduce water absorption.

1.3.2 Objective of the study

1.3.2.1 General objective

- This study's general objective is to Synthesis and evaluate a chicken feather keratin-based biofilm plasticized by sorbitol blended with glycerol.

1.3.2.2 Specific objective

- To extract keratin and starch from chicken feather and mango seed, respectively
- To characterize the raw chicken feather(moisture content, crude protein, crude fiber, and total ash)and resulting products (UV.Vis anlyasis)
- To formulate a biofilm from resulting keratin and starch
- To evaluate the biofilm's physical and chemical properties.

1.4 Scope of the study

This study's scope is raw material preparation for chicken feather and mango seed, extract keratin from waste chicken feather and starch from mango seed, and then formulate keratin with starch blended film by solvent casting method characterize the prepared film.

1.5 Significance of the study

The study solves the pollution arising from petroleum-based plastic and agricultural waste by converting agrarian waste to a valuable product. The environmental pollution arising from the waste chicken feather, and mango seed can be avoided by preparing keratin-starch blended film that can be used for packaging and solve petroleum-based plastic. Aside from others, it generates additional income for the poultry industry, and open up new opportunities for entrepreneurs to process the raw product into these valuable components. This study serves as a base for future research to be conducted, and decide a better and more effective way of valorizing waste chicken feathers and a mango seed.

CHAPTER TWO

2. Literature review

2.1 Introduction

Biopolymers can be classify into three categories: 1) extracted from natural raw materials, (2) produced by microorganisms, and (3) synthesized from bio-derived monomers. Bio-plastic can be producedd from different naturally occurring polypeptides (e.g. keratin) and polysaccharides, e.g.: cellulose and starch. Among other polysaccharides, cellulose is the abundant natural polymer. It is made up of linear polymer chains of β-1,4-linked glucose residues, and different biomass plant is the wealthy resource for cellulose. The crystalline nature of the cellulose molecules gives high strength and Young's modulus when it is used as filler with other polymer, which results from the hydrogen bonds extended along the molecular chains [7].On the other side starch is the most promising biopolymer because it is economical, most abundant and biodegradable. Starch comprises two main polysaccharides: amylose and amylopectin, with some minor components such as lipids and protein [8]. Many natural structural proteins such as silks, elastin's, collagens, keratins, and resilins have been shown to have distinguished mechanical, chemical, electrical, electromagnetic, and optical properties. Thus, they are highly sought after for protein-based biomaterial development.

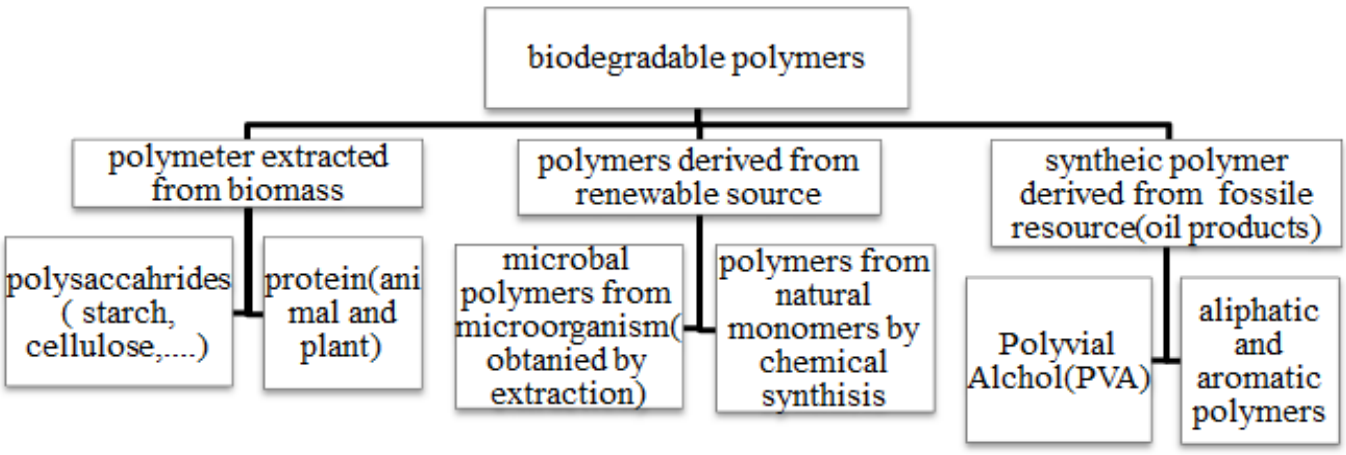


Figure 0.1 Classifications of biodegradable polymers[9]

2.2 Natural protein source

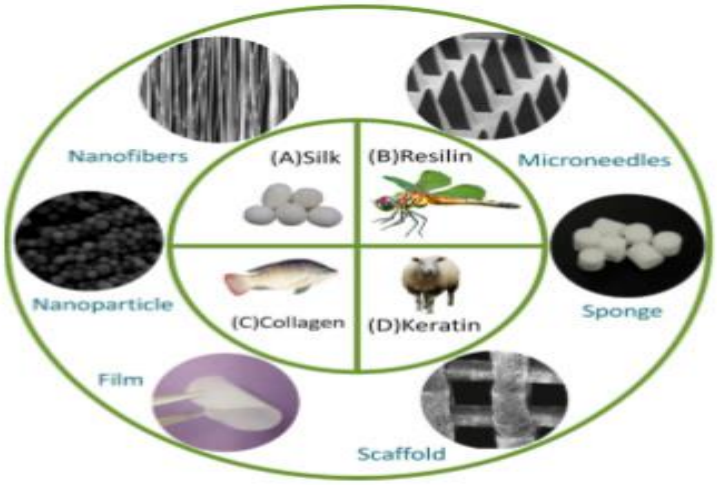


Figure 0.2 Different natural protein sources [14]

1. Elastin

Protein is resistant to enzymatic, chemical, and physical degradation, suggesting long-term stability in biomedical or biomaterial applications. In addition to stability, its self-assembly behavior, elasticity, and biological activity make elastin-based biomaterials exceedingly desirable [10].

2. Soy protein

It is highly biodegradable and environmentally friendly, and it is relatively easy to acquire soy due to its abundance in nature.

3. Collagen

Collagen is the amplest protein in animals, present in the skin, tendons, cartilage, bone, and most internal organs. Collagen is a suitable matrix material for tissue engineering due to its ability to act as a natural substrate for cell attachment and allows for the development of bioengineered tissue. One challenge in treating collagen proteins is heat**.** A relatively high amount of heat is necessary to make collagen water-soluble. Such heat is not suitable for substances in collagen films, microparticles, and other materials [11]

4. Silk

Silk is a fibrous protein produced by animals, from moth sand butterflies to spiders and scorpions. Its hydrophobic side chains, consisting mainly of glycine, alanine, and serine, allow for the formation of β sheets within the protein and give silk its notoriously high tensile strength. Silk is an excellent material for bio-plastic film production with desirable biocompatibility and controllable biodegradability. The development of silk fibers having the properties of spider silks is of keen interest. However, the generation of a spider silk-manufacturing process faces serious problems through spider farming since spiders are quite territorial by nature [11].

2.3 Biomass Keratin

Keratin is the composition of a high content amino acids such as glycine, alanine, serine, and valine. Keratin contains minors amounts of methionine; lysine and tryptophane, most eminent components (7-12%) are cysteine and cysteine, sulfur-containing amino acids. One of the cysteine carboxyl groups makes peptide bonds of a single chain, while other groups, i.e., amino and carboxyl, are included in the peptide bond—polypeptide chain. The chemical activity of keratin is mainly dependent on cysteine that undergoes hydrolysis, oxidation, and reduction. Unlike other proteins, keratin is the most abundant and cheap protein source. From other proteins, keratin-based materials show promise to solve the need for biomaterial because of their biocompatibility, biodegradability, mechanical durability, and natural abundance [12].

Keratin is a fibrous structural protein of hair, nails, horn, hoofs, wool, feathers, and the epithetical cells in the skin's outermost layers. The keratin derived from the chicken feather may be producedd in large quantities because of an inexpensive and environmentally friendly biopolymer. The feather contains 91% keratin, 1% fat and 8% water. Keratin proteins are composed of two protein chains with different compositions and molecular weights (types I and II) [13]. Feathers are composed of 90% keratin, an insoluble, fibrous and structural protein[14]. Keratins are defined by a large amount of amino acid cysteine compared with other proteins. Cysteine (C) is a sulfur-containing amino acid. It can form sulfur–sulfur (S-S) cysteine bonds with other intra- or intermolecular cysteine molecules. Intermolecular cysteine bonds are referred to as “crosslinks.”

2.4 Chicken feather overview

The industry process chicken meat growth rapidly all over the world. The growth rate is due to the higher bowler, including efficient feed to weight gain ratio, the rapid growth rate of chickens, poultry being a rich source of nutrients for human consumption, fast production time, and low economic value poultry per unit[1]. Feathers are the principal waste constituent from the chicken industry. Approximately five million tons of feathers are produced per year in the world. Also, large dumping area is required. They produce a higher portion of heavy metals, chemicals, and pathogens that have detrimental effects on groundwater and environment. Feather keratin cannot dissolve in water, dilute acids, dilute bases, and most non-polar solvents. [15]**.** Feather is inevitably generated during poultry production, therefore a sustained and indigenous source. Utilizing feathers to develop bio-products will add value to feathers and provide inexpensive and renewable raw material.

Compared to other protein, keratin consists of a large amount of amino acid cysteine [16]**.** Chicken feathers are rich in keratin, a strict natural protein-polymer composed of natural monomers. In contrast to other biological sources like plant proteins and modified starch, keratin-based plastics could offer greater strength and tear resistance properties thanks to the tough keratin protein [1].

2.4.1 Chicken feather production capacity

2.4.1.1 Chicken feather production in the world

In the world the production of chicken feather is quickly increasing, the growth rate is due to for the higher bowler include efficient feed to weight gain ratio, the rapid growth rate of chickens, poultry being a rich source of nutrients for human consumption, fast production time, and low economic value of poultry per unit [1]. Among the total bodyweight of poultry, 5% is the feather's weight, which means 2-3 tons of feathers produced from the slaughterhouse with a capacity of 50,000 birds[17]. Every year, about four billons of waste, feathers are produce as by-product in the USA[14]. About $4*10^6$ tons per year of waste chicken feather is generate from the food poultry industry[18].

Table 0.1 Ten countries producing the most chicken in the world [1]

Rank	Country	Metric tonnes/year
1	USA	18.29 million
2	Brazil	13.6 million
3	China	12.70 million
4	India	4.2 million
5	Russia	3.75 million
6	Mexico	3.27 million
7	Argentina	2.1 million
8	Turkey	1.9 million
9	Thailand	1.78 million
10	Indonesia	1.68Illion

2.4.1.2 Chicken production in Ethiopia

Ethiopia is one of five fastest-growing economies globally; more than 50 million poultry is found in Ethiopia. Among this, 96% is non-commercially raised chicken. The Ethiopian Livestock Master Plan has put forward ambitious targets to increase chicken meat production by 24.7%[2]. However, the meat production process's waste release is not correctly handled, mainly the chicken feather. The release of the chicken feathers to the environment without pretreating can affect groundwater because it contains chemicals. If the waste can be changed into a valuable product by extracting keratin, it can solve the problem above.

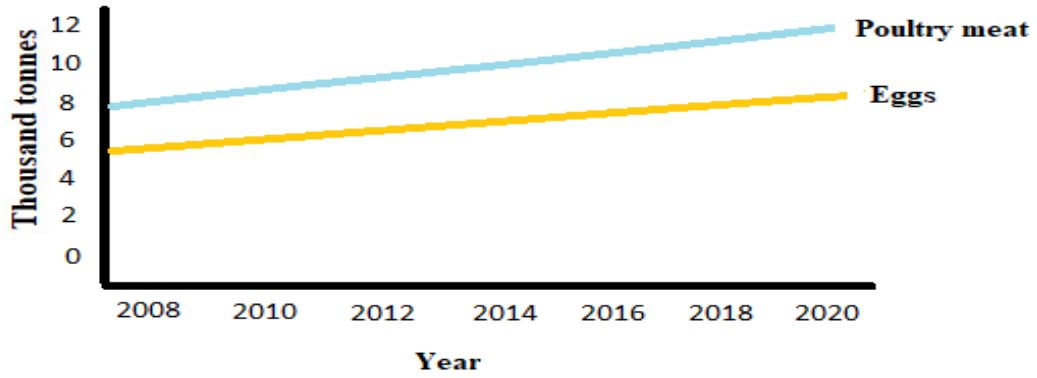


Figure 0.3 Projected demands for poultry meat and eggs in Addis Abeba [3]

Table 0.2 Proximate composition of a chicken feather

Parameter	Composition (%)
Crude lipde	0.83
Crude protein	82.36
Crude fiber	2.15
Ash	1.49
Moisture content	12.33

2.5 Methods of keratin extraction from chicken feather

In different ways, natural protein (keratin) can be extracted from waste chicken feathers. These methods of extractions are the chemical extraction method and enzymatic method. Enzymatic is the most successful and safe for the environment than other methods, but it is costly and not practical at an industrial scale [17]**.** Among these methods, the chemical extraction method is less expensive and effective at an industrial scale extraction. Chemical processes of natural protein (keratin) extraction can be categorized as oxidation, reduction or alkali hydrolysis method. Different reducing agents can break disulfide bonds, such as thioglycolic acid, potassium cyanide, and sodium sulfide. The advantage of reducing agent is fast reaction time and does not bring about any chemical alteration or damage to the protein yield.

Products prepared from the solutions behave as proper proteins and not as products of hydrolysis. Their solutions are precipitated by common protein precipitants such as sulfosalicylic acid, HCl and ammonium sulfate. Oxidizing agents such as bromine, permanganate and hydrogen oxide act very slowly in breaking the disulfide bonds, thus, slowing down the protein extraction process [11]. However, the reducing agents' fast reaction can dissolve keratin at the pH range (pH 10 to 13), but the action is not due to alkali alone. The protein extract from this reaction behaves like an actual protein, not a hydrolysis protein. Reduction of keratin by 2-mercaptoethanol, dithiothreitol (DTT) or dithioerythritol, thioglycolic acid, glutathione, sulfites, and bisulfite generates free cysteine residues, and the resulting cysteine-containing derivatives are called “kerateines” [19]. They are less polar and more stable in acidic and alkaline solutions than oxidized derivatives, and they contain amino acid residues capable of re-crosslinking. Out of different reducing agents used, sodium sulfide gives the highest efficiency in dissolving chicken feathers since the feathers dissolve in a brief period.

Many researchers have been done on the extraction of keratin from chicken feathers by using a reducing agent. Different factors affect the extraction yield during keratin extraction, such as pH, temperature, time, and reducing agent concentration. [15] try to see the effect of temperature, concentration and time on the final yield of keratin (30-65^{o}C), (100 300 500mM) and 1-6h, respectively. They got an 80.2% yield of keratin at 50^{o}C, six hours and 500mM (concentration of sodium sulfate) optimized condition. Using optimized conditions of the above study [14] present the extraction of keratin from chicken feathers by choice optimized condition of PH, temperature and time 3.5, 50^{o}C and six hours respectively the extract 79.6% yield keratin.

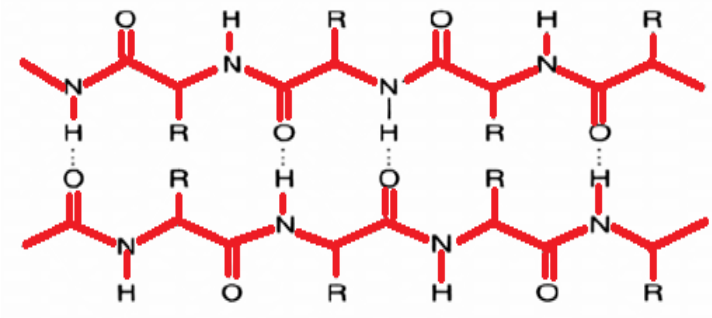


Figure 0.4 Chemical structure of keratin from chicken feathers

Table 0.3 optimum parameter for extracting keratin from chicken feathers by sodium sulfide

Reference	Temperature(^{o}c)	pH	Concentration(M)	Time(h)	Yield(%)
[15]	50	10-13	0.5	6	80.2
[14]	50	10-13	0.5	6	79.6
[8]	40	10-13	0.5	4	

2.5.1 Characterization of extracted keratin from chicken feather

Biuret test to identify keratin's presence:- the presence of keratin can be determined by a biuret test method. It is a chemical test method to identify peptide bonds in the extracted keratin protein. When the copper (II) ion is added to peptides, it forms violet-colored coordination complexes in an alkaline solution. The biuret reaction is often wanted to assess protein concentration because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, consistent with the Beer-Lambert Law. An aqueous sample is treated with an equal volume of 1% strong base (sodium or potassium hydroxide) followed by a couple of aqueous copper (II) sulfate. If the answer turns purple, protein is present, and 5-160mg/mL are often determined. Peptides with a minimum of 3 amino acids with chain lengths are necessary for a significant, measurable color shift with these reagents.

Another method that will show particular amino acids' presence is often studied by FTIR and XRD analysis [8]. Usually keratin, Amino showed C=O stretching vibration with a range of 1800-1600 cm^{-1}, C-H starching vibration at 1530 cm^{-1} shows C-N stretching.

2.6 Biomass Starch

Starch is the most available and cheap biopolymer, acquired from plant tissue such as seed, leave, steam, and root and in some algae and bacteria. Depending on the botanical source, Starches obtained from different sources can be varied, particularly in their qualitative and quantitative makeup and in some of their physiochemical functional properties. [20] investigate the properties of starch extracted from mango seed with different methods. They conclude that the starch extracted varies with their size (with the average diameter range of 13.8um-19.91um) and shape (from oval to irregular), and because of the size of mango seed large, the yield obtained from mango seed is much higher than other sources. There are two types of molecule present in the starch. These are amylose and amylopectin. The starch with high amylose content is preferable in use, as an obstruction in packaging material.

Table 0.4 Amylose and amylopectin concentration from various starch sources

Source	Amylose concentration (%)	Amylopectin concentration (%)
Rice	35	65
Corn	28	72
Arrowroot	20.5	79.5
Wheat	20	80
Cassava	18.6	81.4
Potato	17.8	82.2
Banana	17	83
Tapioca	16.7	83.3
Mango seed	14.82	44.0

2.7 Mango seed and mango seed extraction criteria

In the context of Ethiopia, mango is produced in the southern and western parts of the country. The total production of mango in Ethiopia is 72,187 tons in 2013/14. This accounts for 7219 tons of mango seed kernel annually. The mango production at Arba Minch and Zuria Woreda is 126,800 qt with total area coverage of 634 hectares. Mango farmsteads in Asossa produce an average of 13,500 mangoes per farmstead. The Ethiopian government plans to expand mango production by distributing high yielding varieties for small scale farmers, significantly, in the Southern region and Oromia region, by grafting mangos of known and high yielding varieties. In Ethiopia, there are many large and small scale mango juices processing industry. During mango processing, peel and kernel constitute about 17-22% of the fruit. The production of oil from the mango seed kernel could be an efficient method of utilizing the waste seed kernels (Mustafa Kemal).

Table 0.5 World top 10 mango producer [26]

No	Country	Production (tons/year)
1	India	16,337,400
2	China	4,351,593
3	Thailand	2,550,600
4	Pakistan	1784,300
5	Mexico	1,632,650
6	Indonesia	1,313,540
7	Brazil	1,188,910
8	Bangladesh	1,047,850
9	Philippine	823,576
10	Nigeria	790,200

Table 0.6 The production capacity of mango in regional states of Ethiopia [27]

Region	Area in hectare	Production in quintal	Productivity (quintal/hectare)
Tigray	118.20	-	-
Afar	-	-	-
Amhara	246.85	10,408.67	42.17
Oromia	3,789.47	284,065.79	74.96
Somali	33.52	3,776.26	112.66
Bemishangual-gumuz	652.56	51,411.10	78.78
S.N.N.P	3,375.89	343,910.27	101.87
Gambela	180.41	-	-
Harari	367.24	331.69	0.90
Total	8,764.14	693,903.78	411.34

2.8 Mango seed starch extraction and specification criteria

The preparation method of mango seed starch used a modification of mango seeds starch [20]. The mango kernels were collected and dry these kernels under the sun for two days. The dried seed's kernels were separated manually using a kitchen knife to recover the seeds and dried in the oven at 60^{o}C for 24 hours. The obtained toasted seed was grounded using a grinder, and then the flour was sieved by using a 0.5 mm mesh sieve. 50g of sieved flour weigh then added to 1L of distill water with continuous stirring for 6 hours at room temperature. Next, a cloth bag 0.2 mm mesh was used for filtering mixed and precipitated overnight at 4^{o}C. Finally, the obtained starch was filtered and dried in the oven at 40^{o}C for 24 hours. The starch was grounded with a laboratory grinder, packed in a sealed plastic bag and kept at room temperature until further use.

Table 0.7 Proximate composition of mango seed [28]

Parameter	Composition (%)
Crude protein	6.81
Crude oil	3.63
Crude fiber	8.01
Ash	2.44
Carbohydrate	70.12

2.9 Characterization of keratin protein and starch presence

The presence of starch was identified: The appearance of bluish violet color upon iodine was observed by adding iodine [21]. The chemical test method is a simple way to identify keratin and starch's presence more acceptable in the lab-scale experiment.

Table 0.8 Characteristic of mango seed starch

Types of test	Specification
1. Qualitative test	Bluish violet color
2. Physical test	
Form	Powder
Color	White
Odor	Odorless
Test	Tasteless
3. PH	4-7
4. Moisture content	< 20%
5. Loss on drying	< 1%
6. Ash content	< 15%
7. Amylose content	< 17-20%

2.10 Plasticizer

The plasticizer is a nonvolatile, high boiling. When added to the fabric, it could change the physical or chemical properties of materials. The plasticizer gives flexibility and processibility to the forming film by lowering the glass transition temperature. To get the specified effect of plasticizer on the produced film, plasticizer's choice must be made carefully. The plasticizer selected is supported on the polarity, compatibility, structural configuration (shape) and relative molecular mass. Plasticizer should be low relative molecular mass, low toxic and low volatility, polar plasticizer blend with polar polymer for compatibility purpose [22].

The essential film properties like water absorption, tensile strength and elongation at break are highly influenced by the sort and, therefore, the concentration of plasticizer used. The intermolecular bond (hydrogen bond) found within the hydrophilic plasticizer like glycerol and sorbitol is compliable to polysaccharide and protein polymer. Glycerol, which is an extract from biodiesel from vegetable processing used as a plasticizer in biopolymer processing. The glycerol helped form a homogenous mixture with clear signs of plasticization within the keratin matrix, with no separation and single phase morphology produced, which agrees with the previous research [15].

The glycerol is a hydrophilic plasticizer, and it is hygroscopic properties that influence the produced film's water absorption properties. Since glycerol has low molecular properties gives good mechanical properties than other polyols. However, the hydrophilic nature of glycerol increase the water permeability of the film and when condition at high relative humidity, some migration of plasticizer but within the case of sorbitol the water permeability of the film is become lower.

Sorbitol is a sugar alcohol with chemical formula $C_6H_{14}O_6$ and the relative molecular mass, density and flash point 182.17g/mol, 1.49g/cm^3 and >100^{o}C respectively. This work attempt to see the effect of plasticizer by mixing both glycerol and sorbitol in 1:1 ratio with 0, 20 and 40% keratin basis and to enhance tensile strength elongation at break and water absorption of the produced film by taking the advantage from both plasticizers.The mixture of sorbitol and glycerol give improve properties effect on the mechanical properties, water permeability of the

ultimate film. This is often thanks to the relative molecular mass of the plasticizer[23]. The tensile properties of material increase with the relative molecular mass.

2.11 Film preparation techniques

There are different film preparation techniques, e.g., wet process, dry process and two-step process are the main ones. The solubilization of proteins supports the wet process in a solvent (water, ethanol, and infrequently acetone), called solution casting. Casting, which sets hot solutions on a surface upon cooling and oven drying a solution, is a simple method for producing films with uniform thickness. The solution casting method utilized in many academic research is often because the film is produced with a short period. A uniform thickness dry process involves thermoplastic processing during which protein matrix is mixed with the Nano-particles within the molten state during heating and shearing. Therefore, the formation of a uniform melt is required for this process, and processing temperatures are usually above the protein denaturation point.

Chemical additive and reducing agents such as sodium sulfite, sodium dodecyl sulfate, and urea have a tendancy to disrupt the covalent and non-covalent interactions with plasticizers as processing aid[24]. The two-step process involves combining both processes, i.e., solution casting and dry processing via melt extrusion or melting. During this method, the nanoparticle is mixed with water then added to the protein emulsion with constant mixing. During this mixture, the plasticizer is added and blended. After which, this blend is subject to melting. During this case, the primary technique was selected owing to its effectiveness, and it required a low-temperature environment. Additionally, during this work, the quantity of starch used for reinforcement is small; therefore, the method is effective to conduct at the lab scale.

Table 0.9 The comparison of the tensile strength of keratin blend with different polymer

Sample	Additive	Tensile strength(MPa)	Reference
Feather	MCC	4.2	[25]
Feather	Starch	15.91	[6]
Feather	No	9.59	[14]

2.12 Bio-plastic production from the different protein source

Starch is a low-cost, biodegradable and biocompatible renewable natural source. Starch is a hydrophilic discontinues phase. The starch contains an OH group, which can form a bond with the NH group of keratin, which is found in continuous form. The starch modified by keratin can have better physicochemical and mechanical properties. Keratin processability and flexibility properties can improve by blending with starch. Since keratin can form a 3D interlinking bond by the disulfides, it can form a bond with starch. Since keratin has hydrophobic properties, keratin in the starch-chitosan gives modify water solubility[5].

The blended ratio of keratin and starch affects the physicochemical and mechanical properties. When the starch content increase, the film has roughed and homogenized structure, and the moisture content, the solubility, density and elongation at break increase as the starch content increase too, whereas, the film smoothness, and non-homogenized structure and tensile strength increase as increase the keratin content increase whereas, moisture and water solubility of film decrease as the keratin content increase because of the keratin have hydrophobic properties. The functional group indicates an angle shift, which shows compatibility between keratin and starch[6]. Reference [7,28] tried to see the effect of keratin addition on mechanical properties of TPS, and they conclude the addition of keratin to starch significantly affects the final film properties such as the water solubility of the film, which decrease as the keratin content increase this is because of the hydrophobic properties of keratin.

2.13 Physiochemical analysis of bio-plastic

Mechanical properties:-The main problem of keratin-based bio-plastic is the mechanical properties of the final film. To solve this problem, it is blended with other polysaccharides. The mechanical properties of film such as tensile strength and elongation at break are important properties of film for packaging material. The material's mechanical properties depend on the film-forming condition such as temperature, heating, cooling rate, applied force, deformation, chemical composition plasticizer, filler type, and concentration.

In this work, we tried to look at the effect of plasticizer and starch concentration on the film's mechanical properties. Different literature tries to look at the effect of glycerol and sorbitol on the produced bioplastic. The purpose of plasticizers is to increase the flexibility of bio-plastic by

decrease the intermolecular interaction between polymers. The molecular weight of plasticizers has a high impact on the flexibility and mechanical properties of the film.

The flexibility and mechanical properties of bioplastic have inversely related. As the flexibility of film increase, the mechanical properties of film decrease vices versa. Glycerol has a low molecular weight, and it is a hydrophilic plasticizer. Because of this property, it is the most selective plasticizer by many researchers. However, the mechanical properties of bioplastic plasticized by this plasticizer are less than the bioplastic plasticized by sorbitol. The study done by [27] shows that the bioplastic produced by sorbitol has high mechanical properties than glycerol. The reason given for this result was that the sorbitol has the same structure as starch.

Table 0.10 The tensile strength of commonly used plastic

Name	Tensile strength(MPa)	Reference
Low density polyethylene	8.2-31.4	[6]
Polyvinylidene chloride	19.3-34.5	“ “
Polyvinyl alcohol	44-64	“ “
High density polyethylene	22-31	[28]
Polypropylene	31-38	“
Polystyrene	45-83	“

2.14 FTIR analysis

The secondary structure of the final film identifies by an FTIR analyzer. The infrared spectrum has two basic regions above and below 1500, called functional group regions and fingerprint regions. The functional or diagnosed region contains different peaks used to identify which functional group is present in the substance. There are also quick peak found in the fingerprint region indicative of functional group but its need to be careful when the analysis done.

Table 0.11 Infrared spectroscopy correlation [29]

Functional group	Wavenumber(cm^{-1})
C-H	2850-3300
C=O	1680-1750
C-O	1000-1300
O-H(alcohol)	3230-3550
O-H(Acid)	2500-3300(very broad)
N-H primary amine	3400-3500 and 1560-1640
N-H secondary amine	>3000
N-H ammonium ion	2400-3200

The different substance has a different peak. In the different literature, they try to identify the major functional group of keratin is 3000-2800, 1700-1600, 1580-1540, 1300 and, 1220, which represent CH_3 stretching vibration, C=O stretching (amide I), amide II, and amide III which derived from C-N stretching and N-H bending[15,[6]. In the current study, the two basic plasticizers used are glycerol and sorbitol. The FTIR analysis of glycerol contains 3350-3310, 2930 and, 2880, 1650, 1400-1460, 1450 to 1100 and 920 $cm^{-1,}$ which indicate that O-H stretching, C-H stretching, H_2O bending, C-O-H bending, C-O stretching from primary alcohol to secondary alcohol and O-H bending.

Another hydrophilic plasticizer is sorbitol, which has a high molecular weight compared to glycerol. The functional group of sorbitol was O-H stretching since the sorbitol has fewer hydroxyl groups, the O-H peak was boarder due to many hydroxyl groups than glycerol C-H stretching at 2938 cm^{-1} and C=O stretching at 1645 cm^{-1} [1]. The other raw material used in this study is starch. Looking at the functional group, the band 1007-897 cm^{-1} C-O-H bending stretching vibration, 1150-1005 cm^{-1} C-C stretching, 1235-1150 cm^{-1} C-O-C stretching of Easter, 1734-1631 cm^{-1} bounded water, 3000-2826 cm^{-1} C-H stretching, and 3600-3000 cm^{-1} OH stretching due to polymeric involvement of hydroxyl group and O-H stretching vibration present in starch was observed.

Table 0.12 The Major Functional Group Found In Keratin/ Starch Films [1]

Absorption peak,cm^{-1}	Functional group
800	Amide IV
900-1000	Disulphide bonds in keratin
1200	Amide III
1250	C-O stretching
1550	Amide II(NH bending)
1680	Carbonyl group
1700	C-H stretching
2900	C-H stretching
3100-3600	C-C
3000-3500	3000-3500 OH and/ NH stretching

2.15 TGA analysis of film

TGA is a technique in which the mass of a sample is recorded as the function of temperature or time. The phenomena used for qualitative as well as quantitative analysis, only solid material can be analyzed by this method. Loss in the mass due to H_2O water present with the reactants and this water may be adsorption, the water of crystallization or water of consistency. The bioplastics produced by protein and starch have three major weight loss temperatures. Reference [31] tried to compare the structure and thermal properties of films of pure protein and protein starch. In this study, the protein starch blend film has three weight loss stages. 8% of weight loss happens at 100^{o}c. This is because of the loss of water and at 100 to 250^{o}c about 10% weight loss at this stage there is a loss of plasticizer; however, about 47% weight loss occurs at temperature 300-350^{o}c substantial weight loss happen. Plasticizer affects the thermal properties of produce film.

2.16 XRD analysis of film

It is the key characterization to know whether the materials are crystalline or amorphous. Crystal means when the atoms arranged periodic pattern of three dimensions. However, some solids that do not possess any regular interior arrangement of atoms called amorphous.XRD is an extremely powerful tool for material characterization. XRD data (intensity vs. 2Θ) gives a lot of

information. For instance, some peaks are high in intensity because there is more periodicity than other directions. The height of peaks will be high if there is a preferred crystal orientation; however, if crystals are arranged in a chaotic or random order, the peak of the peak will be below. The higher the electron density variation, the higher the intensity for that plane reflected in the XRD pattern.Polymers are available in many forms, including highly crystalline, semi-crystalline, microcrystalline, or amorphous, and it's possible that during a single polymer sample, all three could also be observed. The presence and measure of those forms depend on how the polymer was formulated and processed and this, in turn, is understood to affect mechanical properties like compression, lastingness, buckling, and creep. Consequently, the degree of crystallinity is a crucial property to accurately determine. The intensity of films depends on the polymer, a plasticizer used, the investigation done on the effect of plasticizer concentration on functional properties of chicken skin gelatin films show that the addition of glycerol had decreased the intensity of peak this is because adding glycerol make the film stable and also the glycerol has hydrophilic properties this make the film more amorphous.

CHAPTER THREE

3. Materials and Method

3.1 The structured environment of the thesis

This study's main experiment framework included sample collection and transportation, sample preparation and storage, extraction of the keratin and starch, production and characterization of the bioplastic film. The experiment works done in Agri campus microbiology laboratory rooms and chemical engineering laboratories in JIT. The physicochemical property of the film and proximate composition of keratin was done at the Agri campus.

1.2 Chemicals

- All the chemical used this study was analytical grade

I. **Sodium sulfide**:- used to extracte keratin from chicken feather
II. **Sodium Hydroxide**: used to dissolve keratin ,
III. **Sorbitol, and Glycerol**:- used as plasticizer
IV. **Hydrochloric acid**:- used to adjust the pH and precipitate keratin
V. **Copper sulfate**:- used to identify the precence of keratin
VI. **Deionized water** :- used to washe and dissolve

3.3 Materials

This research's raw material was locally availably waste chicken feather and mango seed, collected randomly from Jimma town. The chicken feather was used as the raw material to extract keratin.

3.4 Laboratory equipment

The equipment used to conduct this study was: Sieve, Digital mass balance: Heater, Centrifugal separator, conical flask, Filter paper: Separator funnel, Stirrer, pH meter, Thermometer, Digital titration and burets, Miller, Petri dish, cotton cloth, hydrometer, Viscometer, silica crucible, desiccators, drying oven, water bath, heating mantle, stopwatch, scissor, glove, goggles, spoon, Kjeldahl digestion unit, Kjeldahl automatic distillation unit, tensile machine, FTIR, TGA. XRD, UV-Vis Spectrocopy.

3.5 Processing methods

3.5.1 Methods for Proximate analysis keratin

Proximate analyses: The proximate analysis was carried out using the standard analysis of official analytical chemists. The parameters determined were: moisture content, crude protein, total ash. Triplicate was done for each parameter.

1. **Ash content**: - The ash content is defined as the inorganic residue that remained after the organic matter was burnt away. 1.0 g of sample was weighed and placed overnight in a muffle furnace set at 575 ^{o}c. after the ash form, the sample was removed from the furnace. The crucible was cooled in a desiccator and then reweighed. The total ash content was calculated using the following equation:

$$\text{Ash Content (\%)} = \frac{(w_2 - w_0)}{w_1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

Where w_0 = crucible weight; w_1 = sample weight and w_2 = crucible and sample weight

2. **Moisture content:-**Three different mass of each 35g, 40g, and 45g of pretreated chicken feathers was weighed and dried in an oven for 6hr at 60^{o}C, and the weight of the sample was measure at every 2hr during the drying time process. This process of measuring continued until a similar weight was obtained. After the whole, the moisture content of the feather will calculate as below:

$$\text{Moisture Content}(\%) = \frac{\mathrm{W_I} \times \mathrm{W_F}}{\mathrm{W_I}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

3. **The Crude protein:-**Kjeldahl analysis will determine the crude protein content using a Kjeltec analyzer, which measures the total nitrogen content in the sample. This is then converted to crude protein by using the factor 6.25 based on the assumption that the average protein contains about 16% nitrogen.

$$\text{Nitrogen In Sample}(\%) = \frac{\mathrm{W_2} \times \text{Normality of Acid} \times 14/\mathrm{W_1}}{\mathrm{W_1}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

Where w_1= Sample weight measured by milligram and w_2= volume of HCl. A conversion factor of 6.25 will be used to convert total nitrogen to protein for all the waste substrates as follow

$$\text{Crude Protein}(\%) = \text{Nitrogen}(\%) \times 6.25 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

3.5.2 Method for the extraction of keratin from chicken feathers

3.5.2.1 Experimental procedure

1. **Pretreatment of a chicken feather:-** The waste chicken feather was collected from Aseba welde Aragawu hotel in Jimma town. To remove blood and dirt, the collected feather was washed with water and detergent. To remove fat, the washed feather was socked in petroleum ether for 12 hr. and then washed with water and detergent. The cleaned feather was dry in the oven at 60^{o}C for 6hr, it was grounded into powder by using miller and put in a plastic bag at 4^{o}C until it used further process.

Figure 0.1 chicken feather

2. **Keratin dissolution process: -** 25g of feather powder weight and add to 1L of 0.5M Na_2S solution, the mixture was heated at (50-70^{o}C) for (1-6 hours), PH maintained (10-14). The solution centrifuge at 10,000 rpm for 15min and then the liquid was collected. The collected liquid filter by using filter paper to sure the liquid is particle-free.
3. **Keratin precipitation and purification**: - The collected filtrate liquid was precipitated by adjusted with PH to 3.5-4 using 1% HCl, the keratin was precipitated by the isoelectric point. The solution then centrifuges at 10,000 rpm for 5min. The solid particle was collected carefull. Then the solid particle was collected carefully. The collected solid

particles dissolve in 100ml of 2M sodium hydroxide further centrifuge at 10,000 rpm for 5min, the liquid is collected and stored for further analysis.. Twelve experiments were done to observe the effect of main factors (by varying extraction time and temperature by fixing sodium sulfide concentration and PH).

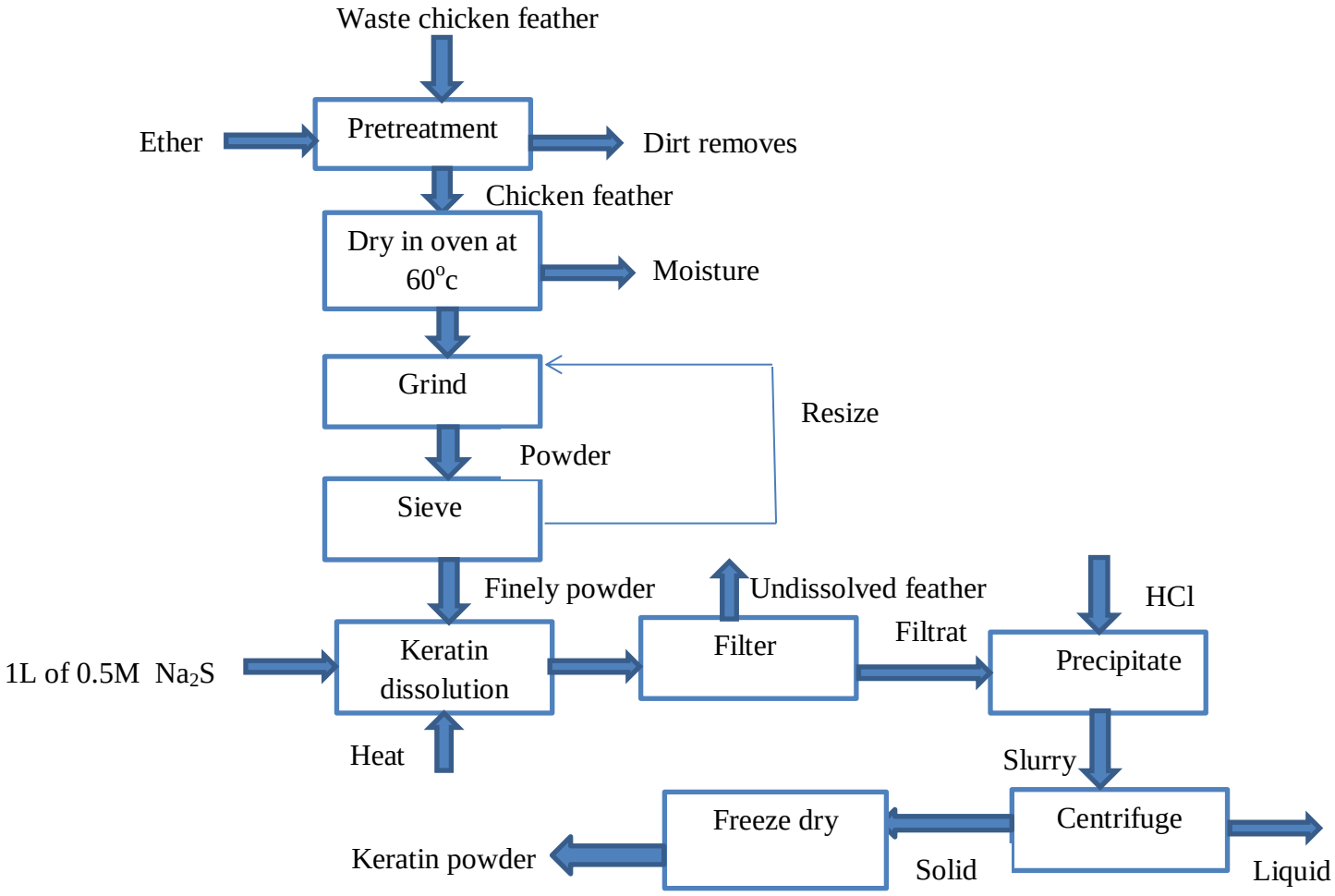


Figure 0.2 Block flow diagram of keratin extraction

4. **Determination of percent yield of the keratin**:-The yield of keratin present in the chicken feather was calculated by measuring the mass of feather powder before extracting keratin and measuring the mass of extracted keratin the two masses were compared. Twelve experiments were done, and the CCD response surface method was used to analyze the response's yield.

$$\text{Yield Of Keratin} = \frac{\text{Product Obtained}}{\text{Raw Feed}} \times 100 \dots\dots\dots\dots\dots\dots\dots (3.5)$$

3.5.3 Characterization of the presence of protein and yield of keratin

1. **Characterization of the presence of protein: -**The presence of keratin will be determined by using Biuret test method characterization procedures as follows 1% copper sulfate solution and 1% potassium hydroxide solution will prepare, and 5ml of the pure keratin solution will collect and mix with potassium hydroxide solution with 1:1 ratio. After the whole, three drops of copper sulfate solution will add to the mixture solution, and changes in the solution will be observed and recorded.
2. **FITR spectra**: - FITR can identify the chemical combination of extracted keratin by looking at the absorption bands that appear for the keratin and feather fibers.
3. **X-ray diffraction (XRD)**:- the different crystal structures of feather and keratin extracted were compared by mini flex II(model) X-ray diffractometer.The patterns were obtained with a Cu K_α radiation source. The 2Θ Bragg angles were scanned for a range of 5-80^oC using 0.02^o step size and 1.0 s per step scan speed.
4. **Thermogravimetric analysis(TGA)**:- thermal analyzer was used to determine the melting temperature and to obtain the thermograms.TGA was performed to determine degradation temperature using TGA Q 500 thermogravimetric analyzers under nitrogen atmosphere, in a temperature range between 10 and 900^oC at ramping time of 10^oC/min. The samples were put in an aluminum crucible, and thus, the data were analyzed. The change in weight differential-difference with temperature was observed.

3.5.4 Starch from waste mango seed

1. **Pretreatment of mango kernels:-**Waste mango kernels collected from natural fruit shop Jimma town was washed by using tap water to remove dirt, and it dries under the sun for two days. The seed was separated from the shell by using scissors, and the seed was dry in the oven at 60^{o}c for 24hr. The dry seed is then ground by using a miller and sieve in a 0.5 mm sieve. The obtained flour is packed in a plastic bag and store in 4^{o}c until it is used for further analysis.

Figure 0.3 Waste mango seed

2. **Extraction of starch from mango seed flour**:- The extraction of starch was done using the distillation method and the procedure adopted from [20] with some modification. First 50g of mango seed flour was measure and add to 1L of distilling water stir for 6 hours at room temperature. Next, the slurry filterer by using nylon mesh and the reaming residual washed with distilled water. Then, the filtrates were mixed and precipitated overnight. Lastly, the acquired starches were filtered and dried in the oven at 40^{o}c for 24hours. The starch was ground with a mortar and pestle, packed in a plastic bag and keep it under room temperature until further use.

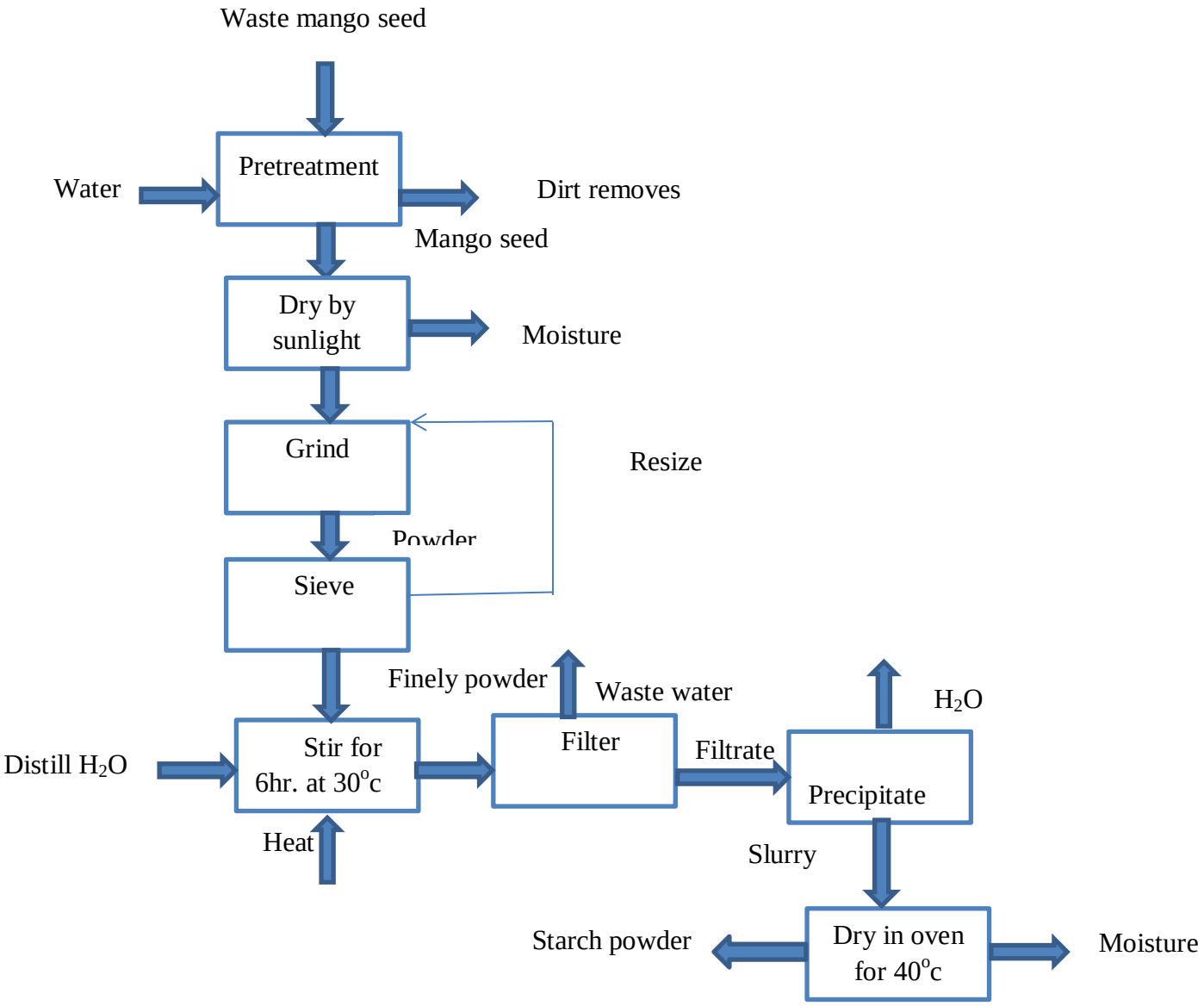


Figure 0.4 Block flow diagram of starch extraction from mango seed

3.5.5 Characterization of starch extracted from mango seed

The mango seed starch was evaluated qualitatively for its properties, including moisture content, ash content, density, and drying [32].

1. **The presence of starch was identified**: the appearance of bluish violet color upon the addition of iodine was observed by adding iodine.
2. **The moisture content of starch powder:** Three gram of starch was weighed into a crucible and placed in an oven with a temperature of 105^{o}C and dried for 24 hours to constant weight. Moisture content in the dried starch was determined by keeping the weighed quantity of sample in a thermostat-controlled oven at 105^{o}C for 24 hours. The dry weight of each sample was taken on weighing balance[33]. The percentage of the moisture content and dry matter was then calculated by the formula as presented below. The percentage of the moisture content and dry matter was then calculated by the formula as presented below.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

The moisture content of starch should be approximately less than 20 % [34].

3. **Determination of loss of drying**:- one gram of starch was weighed and put into a tarred pre-heated(105^{o}C for 30min.) weighing bottle with its lid, and then placed into an oven(105^{o}C) and dried until a constant weight is archived.

$$\text{Loss On Drying} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100 \ldots\ldots\ldots\ldots\ldots (3.7)$$

4. **Determination of ash content:** The sample was heated at the temperature of 550^{o}c as such that organic compound and its derivatives were destroyed and evaporated, yielding mineral elements and organic compound residue. The ash content should be less than 1%. The ash content was calculated using the following formula:

$$\text{Ash content}(\%) = \frac{w_2 - w_0}{w_1} * 100 \ldots\ldots\ldots\ldots\ldots\ldots (3.8)$$

3.6 Synthesis of bio-plastic from the extracted keratin via starch

The sample will prepare by the procedure adapted from the method described by [6] with some modification. The starch (5g) extracted from mango seed was added to 100ml of distilled water. The dispersion was stirred manually on the shaker water bath set at 70^{o}C for 30 minutes while stirred at the same rate until it becomes gelatinized. This step provides homogeneous dispersion by disintegrating the starch granules, and temperatures were selected based on starch's gelatinized temperature. A 7g sample of keratin powder was added to 100 ml of 0.1M NaOH and then heated at 70 °C for 10 minutes with constant stirring with a magnetic bar. The plasticizer (GLY and SOR), a mixed 1:1 ratio, was added at different concentrations (20%-50%) w/v keratin basis. A film-forming dispersion was prepared by mixing the starch solution with different concentrations (30% -70%) w/w based on keratin weight basis was added to the keratin solution. The mixture was heated at 70°C for 15 min with continuous magnetic stirring to prepare keratin/starch blend dispersions. Each mixture was stirred for homogeneity and made the gelatin very strong and allowed cooling to 50^{o}c before being cast on a petri dish. Film-forming solutions (40mL) will transfer into casting in glass Petri-dishes having 10 cm diameters. Then dishes will place in an oven set at (35 ^{o}C -65^{o}C) until the film will dry. The analysis was duplicated three times.

3.7 Application studies of plastic film

Before starting any test, the sample was conditioned for 48hr in a specified temperature and humidity for every physical testing. For this purpose, we use the standard test method (ISO 2418:2005 and ISO2419:2005, sampling and conditioning).

1. **Water solubility test**:- The film's solubility in water was determined according to the method reported by [21]. Disks of the film (2cm × 2cm) were cut, weighed (M_1), and immersed in a beaker containing 50 ml of distilled water. After 24h of immersion at 25^{o}c with a slow agitation, the sample pieces were taken out and dried to constant weight (M_2) in an air circulated oven set at 105^{o}C for 24hr.

$$\text{Water solubility}=\frac{M1-M2}{M1}\times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.9)$$

Where M_1= is the initial mass and

M_2= is the final mass of the sample

2. **Water absorption test**: Water uptake was investigated by cutting film with approximately 2x2 cm and then weighed the mass. The film was put into a container filled with distilled water for 24 hours. After water immersion, the film was removed from the water and weighed to measure the wet weight. Water uptake was calculated as follows.

$$\text{Water Absorption}(\%) = \frac{\text{Wet}_{\text{Weight}} - DRY_{Weight}}{\text{Wet}_{\text{Weight}}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.10)$$

3. **Density test**: The bulk density (D) was determined by dividing the mass (m) per unit volume(v) of the plastic sample.

$$D = \frac{M}{V} \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.11)$$

4. **Transparency of produced bio-plastic**: The film's transparencies are determined using a spectrophotometer (UV 7804C). The transmittance of films was determined at 600 nm as described by [35]. The film samples are cut into rectangles and make it a solution form, then put into the spectrophotometer cell's internal side.

$$\text{Transparency (\%T)} = \frac{-\text{Logt}(600\text{nm})}{\text{X}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.13)$$

Where T600= is the transmittance at 600nm and X is the film thickness (mm)

5. **Moisture**: Before determining film properties, samples were conditioned at 25^{o}C and 53% relative humidity (RH) for 48 h. film moisture content was determined through the weight loss through which the film went after a 24hrs oven drying at 90^{o}c. Preliminary experiments showed us that was enough to dry up samples. The temperature was chosen to avoid the loss of plasticizer [36].

$$\text{Moisture content (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Final Weight}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.14)$$

6. **The thickness of films**: The film thicknesses were measured using digital micrometers, and measurement was made in at least three random locations for each film, and an average value was calculated.
7. **Tensile Properties Test**: Tensile strength and elongation at break are the most important mechanical properties of the packaging bioplastic. Tensile strength is defined as the strength of material concerning force per unit area of cross-section while applying force in a linear direction. The test specimens were conditioned to conform to standard requirements of thin plastic sheeting following ASTM D882. From the developed plastic sheet, filmstrips of uniform width and thickness (10 mmx50 mm) were cut. Tensile testing of the packaging films was performed according to ASTM D882 using a universal testing machine equipped with a 1KN load cell at a crosshead rate of 50mm/min. All tested specimens were required in rectangular shape differing from the conventional dumbbell shape of tensile testing. The test specimens were placed within the Instron universal tester grips, tightened evenly and firmly to the degree necessary to minimize slipping during the test. The machine is operated until the specimen fails under load. At rupture, the force and deformation were determined. Finally, the tensile strength, percentage (%) elongation and young modulus of each sample were calculated using Equations 3.15 and 3.16, respectively.

$$\text{Tensile Strength } = \frac{9.81 \times Z_I}{\text{Breadth } \times \text{Width}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ Eq(3.15)}$$

$$\text{where } z_i = \text{know the weight of film}$$

$$\%\ \text{Elogation} = \frac{L - L_O}{L_O} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ Eq(3.16)}$$

Where L=new length of the sample, Lo= original length of the sample

3.8 Characterization techniques

1. **FTIR analysis of the plastic film**: The functional groups (chemical bonds) of the produced film was identified by Fourier transforms infrared spectroscopy (FTIR). Then the FTIR spectrum was allowed to pass through the prepared sample, and the spectrum responses will

record. Finally, the peak plot of wavenumber (400-4000cm^{-1}) versus Transmittance (%) was plotted using origin software and identified the functional grounds.

2. **Thermogravimetric analysis (TGA)**: The thermal stability of the was studied using their analysis to determine the glass transition and the melting temperature for the produced plastic film.
3. **X-ray diffraction (XRD) of films**: To further clarify the effect of keratin/starch addition on the properties of the films, XRD analysis was investigated

$$\text{Crystallinity Index} = \frac{\text{Maximum Crystal Lattice Diffraction With } 2\theta \text{ At Around } 9°}{\text{Minimum Diffraction Intensity With } 2\theta \text{ At Around } 14°} \ldots\ldots (3.17)$$

3.9 Experimental design and statistical analysis

For experimental design and analysis of the data, the design expert 11 portable was used by using design expert of CCD response surface with rotatable (k<6) 3 level models the total experiment to be conducted is 20 for the synthesized film. Independent variables were starch content (30-70%)w/w keratin basis, dry oven temperature (35-65 ^{o}C) and the plasticizer content (20-50%) w/w keratin basis. 7g keratin was taken for each trial, and the response was tensile strength, elongation and water absorption. The interaction and individual effect were analyzed by design expert 11. The three independent variables select based on the effect on the final produce film. Randomization of the experimental run and an appropriate analysis technique were ensured through proper utilization of software design expert 11 and versions.

Table 0.1 Factors and ranges of the CCD design quadratic model

No	Factors	Level
1	Starch	(30-70 %) w/w based on a keratin weight basis.
2	Glycerol vs. sorbitol(1:1)	(20%-50%) w/w keratin basis.
3	Dry oven temperature	(35-65 ^{o}C)

CHAPTER FOUR

4.Result and discussion

4.1 The result of proximate analysis of chicken feather

The proximate analysis is used to determine the major components of biomass. The parameters determined were: moisture content, crude protein, ash, volatile matter, fixed carbon. The obtained result compared with other researchers was summarized in Table 4.1.

Table 0.1 The result of the proximate composition of the raw chicken feathers on a mass basis

Parameter	Result(%)[a]	Result(%)[b]
Ash (%)	1.45%	1.49%
Moisture (%)	11.90 %	12.33%
Crude protein (%)	89.50%	82.36%
Cured fiber (%)	2.00	2.15
Crude lipid (%)	0.79	0.83

Source: [a](current study)[b][1]

A. **The total ash content: -** the lower ash content may suggest low mineral content. On the other hand, the high value of the ash was indicative of high mineral content.
B. **The moisture content:** The result shows a small value compared to the value obtained; this variation may be due to handling problems. Moreover, high moisture content causes food items to enhance microbial spoilage and short shelf life, leading to its deterioration. As much as possible, the moisture content should be small, and the above result was somewhat excellent and acceptable.
C. **Crude protein**: the result shows the crude protein is 89.5, which is near 90 %. The total protein of chicken feathers is >90 %.

4.2 Keratin extraction and yield results

Extraction time and temperature were the two factors, and keratin yield was the response. Moreover, CCD RSM was taken to analyze the variance for 12 experiments. The obtained result with each trial is summarized in Table 4.2.

Table 0.2 Analyze the variance for 12 experiments of keratin yield

Std	Run	Factor 1 A: temperature(^{0}c)	Factor 2 B: time(hr.)	Response 1 Yield %
4	1	70	6	68.03
5	2	50	3.5	64.5
8	3	60	6	73.5
11	4	60	3.5	62.08
12	5	60	3.5	62.9
1	6	50	1	52
3	7	50	6	78.8
10	8	60	3.5	62.2
9	9	60	3.5	63.95
6	10	70	3.5	63.25
7	11	60	1	56
2	12	70	1	59.29

The above table shows that dissolving feathers for shorter time results in incomplete digestion and a low production yield. On the other side, leaving the feather in a reducing agent for a long time causes unspecific peptide bonds' cleavage. In this study, the maximum yield was obtained at 50^{o}C and 6 hr incubation period, 78.8% from the 7th run. The keratin yields are affected by the temperature and the extraction time. When the temperature is high, the disulfide bond can be broken easily, but the incubation time must be control, this is because when a long incubation time and high temperature is applied to extract keratin, it can affect the peptide bond, so there is a need to give attention while during the process[37]. As extraction time increases, the yield increases slightly but is significantly affected by extraction temperature. As shown in the Table 4.2, the extraction yield depends on both extraction time and temperature[17]. To get a high yield at the minimum temperature, the extraction time must be extended or to get a high yield at a short time, and the temperature must be high.This study shows that the maximum yield of keratin was obtained at 50^{o}C and 6hr.

Table 0.3 ANOVA for Quadratic model

Response 1: yield

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	561.02	112.20	194.43	< 0.0001	Significant
A-temperature	3.73	3.73	6.46	0.0440	
B-time	468.87	468.87	812.50	< 0.0001	
AB	81.54	81.54	141.30	< 0.0001	
A^2	0.5075	0.5075	0.8794	0.3846	
B^2	4.59	4.59	7.95	0.0304	
Residual	3.46	0.5771			
Lack of Fit	1.25	0.4176	0.5669	0.6737	not significant
Pure Error	2.21	0.7366			
Cor Total	564.48				

The **Model F-value** of 194.43 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 0.57 implies the Lack of Fit is not significant relative to the pure error. There is a 67.37% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 0.4 Model adequacy measures for keratin extraction yield response.

Std. Dev.	0.9073	R^2	0.9888
Mean	63.64	Adjusted R^2	0.9846
C.V. %	1.43	Predicted R^2	0.9796
		Adeq Precision	55.3618

The **Predicted R^2** of 0.9796 is in reasonable agreement with the **Adjusted R^2** of 0.9846, i.e., the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 55.362 indicates an adequate signal. This model can be used to navigate the design space.

The Effect of temperature on yield of keratin Figure 4.1 below shows as the temperature increases up to 50^{o}c, the yield increase. It decreases when a time is kept constant 6hr. Temperature is a significant factor affecting the amount of keratin extracted because too low temperatures or too short periods quickly caused incomplete solubilization. In contrast, too high temperatures or too long periods leads to the scissoring of the peptide bond. At higher alkali concentration, the time the keratin yields might slightly increase.

Physical conditions such as temperature, pH, reducing agent, time and temperature have a high effect on the final yield. This study tries to see the effect of temperature and time by making constant other effects. The incubation of a feather for a long time can damage the bond of keratin. The incubation time depends on other factors such as temperature. If the temperature taken is high, the incubation time must be minimized. In this study, the temperature range was 50-70^{o}c, a high temperature, so the incubation time must be minimized. As we mentioned in the literature, the temperature that can obtain the maximum yield is 50^{o}c. This study tries to see the effect of temperature by taking the maximum yield temperature and increasing the top limit by 50 ^{o}c. The maximum yield obtained in this investigation was 78.8% at 6 hr. Furthermore, since the temperature was high, the incubation time must decrease. The present study yield (78.8) is lower than the previously reported study using Na_2S.

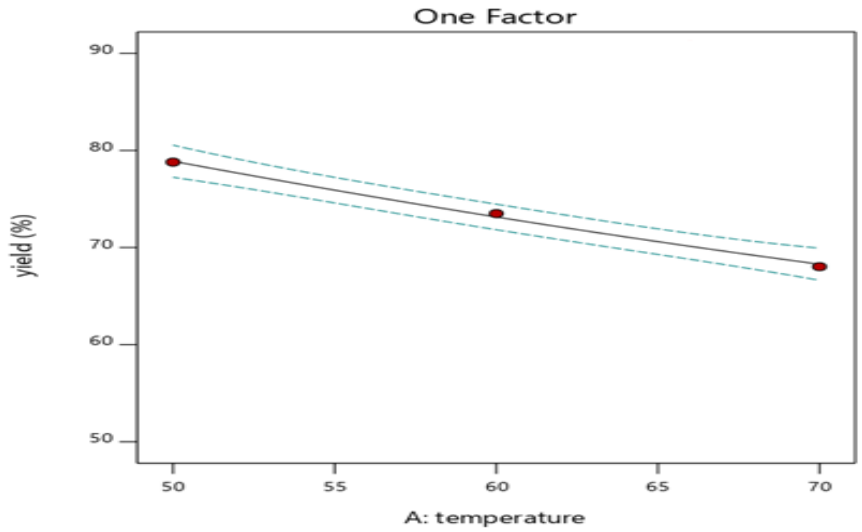


Figure 0.1 Effect of temperature on yield

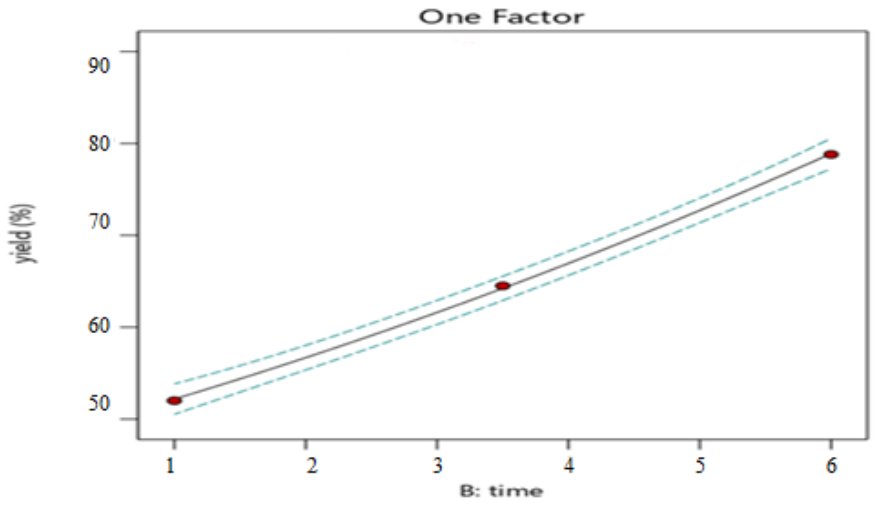


Figure 0.2 Effect of time on yield

4.2.1 Development of a regression model for keratin extraction

The regression coefficients of the developed model were determined from the regression analysis. As indicated in fit summery which is given from the appendixes Table B_8, for each factor, the quadratic models are suggested, as the p-value of this model is smaller than that of other models and no aliased terms. The model equation that correlates the response to the process variables in terms of actual value and codded was formulated. Final equations in terms of actual and coded factors for all responses are given in appendix C.

4.2.2 Optimization of process variable and yield

The main objective of the optimization is either to minimize effort or to maximize benefit. The effort or benefit can usually be expressed as a function of certain design variables. Hence, optimization is the process of finding the conditions that give the maximum or the minimum value of a function. Under this, The process variables, both extraction time and temperature, should be in range to yield maximum keratin.

Table 0.5 Summary of constraint response and goals of optimizations

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:temperature	in range	50	70	1	1	3
B:time	in range	1	6	1	1	3
Yield	maximize	50	79	1	1	5

Using numerical optimization, three solutions were found, the possible combination which contains a maximum yield was selected.

Table 0.6 Optimization table of extraction

Extraction Temperature(^{0}c)	Extraction time(hr.)	Yield (%)	Desirability
50.06	5.99	78.8062	0.996

The predicated result using numerical optimization was validated by doing an experimental (triplicate) at the predicated parameter, and the yield obtained was 78.8062%. This is close to the predicted yield.

4.3 The result of the characteristics of the extracted keratin

4.3.1 Determination of appearance of keratin during the precipitation stage

Appearance is one of the physicals parameters for the characterization of keratin protein. Based on visual inspection, the appearance of keratin during the stage of precipitation seems a milky color. As observed from the Figure 4.3 below, the milky color precipitate was formed when the

pH was adjusted to the isoelectric point of protein (3.5). Thus, this shows the existence of keratin protein in chicken feathers.

Figure 0.3 Keratin precipitation

4.3.2 UV-Vis result of extracted keratin

The absorption spectra to answer keratin's presence exhibited a good fit within the range of 200-280 nm (figure 4.4). The extracted keratin solution's UV-Vis results showed the maximum peak at 280 nm was caused by the aromatic ring portion of amino acid groups. Generally, the fluorescence of keratin was mainly thanks to tryptophan and tyrosine residues. Keratin is absorbed predominantly within the far UV-Vis but had an absorption extension as far as 400 nm. The most chromospheres absorbing within the UV region are aromatic compounds of amino acids.

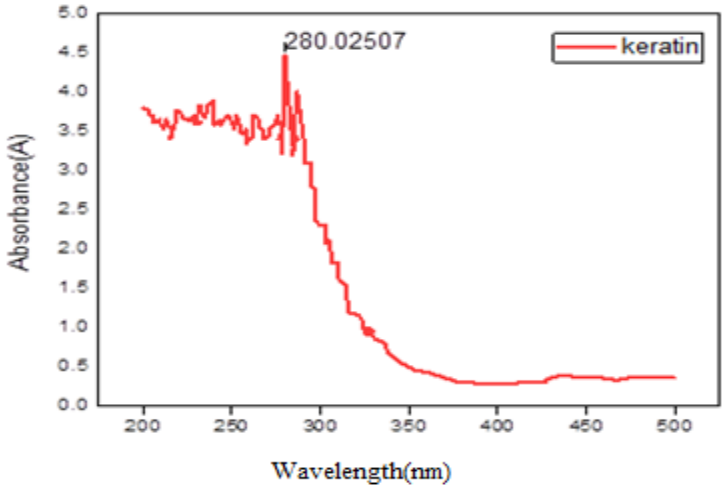


Figure 0.4 UV-Vis result of extracted keratin

4.3.3 FTIR Analysis

The compositional change of both keratin and feather was identified by using FTIR spectroscope 2. The transmission bands appeared in the range of 3000-2800 cm^{-1} related to CH_3 stretching vibration's proportional arrangement. The strong transmission band was attributed to C=O stretching (amide I), between 1700 and 1600 cm^{-1}. The amide II transmission band occurred in 1580-1540 cm^{-1} representing the N-H bending and stretching. The weak band between 1300 and 1220 cm^{-1} indicated the amide III band, derived from C-N stretching and N-H bending and signal from C=O bending and C-C stretching vibration. The peak at 990 and 580 cm^{-1} was associated with C-S and S-S bonds. Amide I-III predicts vital information about protein conformation and alteration in the backbone structure of the protein. The transmission band in-between 750 and 600 cm^{-1} is related to N-H out of plane bending[29].

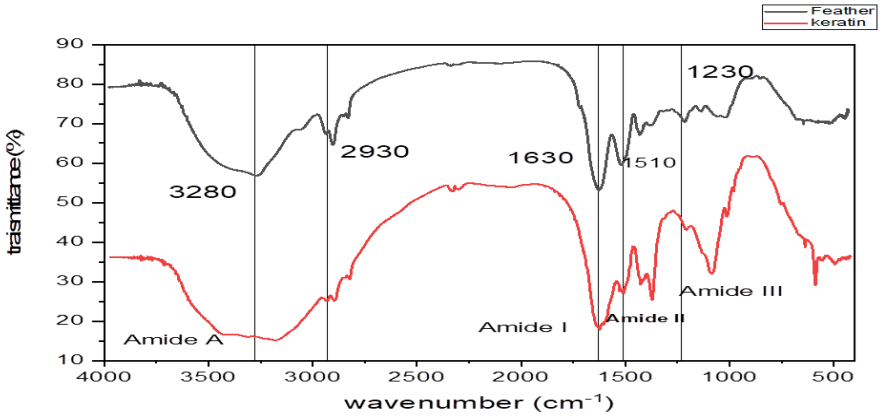


Figure 0.5 FTIR result of keratin and feathers

4.3.4 X-ray diffraction of feather and keratin

The analysis done was by multipurpose X-ray diffractometer to work out of the crystal phase of the sample. Figure 4.6 shows the XRD. The result obtained from XRD analysis tells that both keratin and feather appear in the semi-crystalline form. There are three sorts of crystal diffraction peaks; the meridional reflection of 0.51 nm (2Θ between 15^o and 31^o) for α-helix structure, the equatorial reflection of 0.465 nm(2Θ between 16^o and 31^o) for β-sheet structure[38], and therefore, the equatorial reflection of 0.98 nm(2Θ=9^o) for α-helix and β-sheet structure. The peaks at 8^o-9^o indicated the diffraction patterns of α-helix configuration. The change that occurs within the molecular structure is shown fig 12. the two strong peaks at 2Θ=9^o-10^o and 15^o-31^o

were allocated to α-helix and β-sheet, respectively[14]. Both feather and keratin show diffraction characteristics of α-helix appearing at $2\Theta=9.7^{o}$ and of β-sheet at $2\Theta=21.2^{o}$ and $2\Theta=19.8^{o}$, respectively[6]. The diffraction of the peak at $2\Theta=13^{o}$ was allocated for the amorphous region. The study indicated that partial crystalline of the keratin particles is retained after the regeneration process.

The diffraction peak at 19.6^{o} and 21.2^{o} was indexed for the β-sheet crystalline structure of keratin, and therefore, the peak at 17.8^{o} was indexed for α-helix diffraction pattern. The intensity of peak specifies that the feather and extracted keratin contained a great deal of β-sheet conformation and a bit of α-helix conformation; also, the extracted keratin has more content β-sheet than the chicken feather.

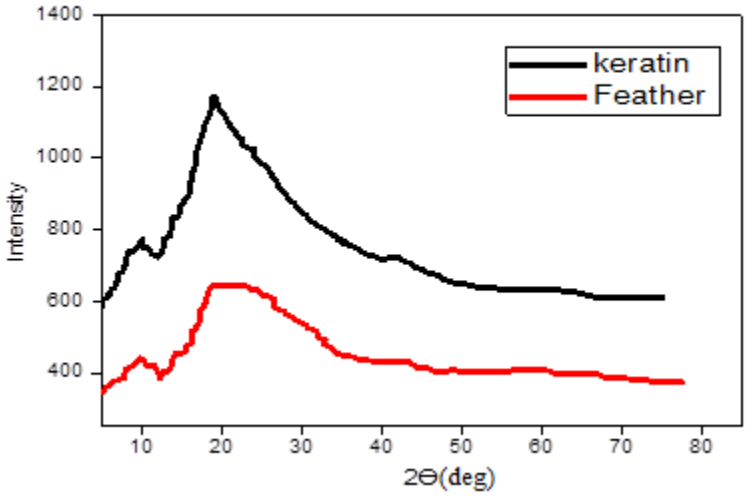


Figure 0.6 X-ray diffraction of feather and keratin

4.3.5 Thermal geometric analysis (TGA) of feather and keratin

The thermal behavior of both keratin and feather was studied using TGA 400 under nitrogen atmosphere at the temperature range 10-500^{o}C at ramping time of 10^{o}C/min to which the sample was heated. The thermal degradation onset temperature and the fiber's thermal degradation weight loss were recorded and analyzed. The TGA analysis of both keratin and feather is shown in Fig 4.7

The feather and keratin had two main weight loss regions. The initial weight loss caused by the loss of free water absorbed in the fibers occurred in the range of 100^{o}C. The weight loss

percentage was about 8%. The second weight loss in the temperature range of 100°c-230°c mainly due to breakage of the disulfide bond and β- sheet conformation. Since the disulfide's cleavage, the volatile compounds including SO_2 and H_2S were released between 230 and 250°c.

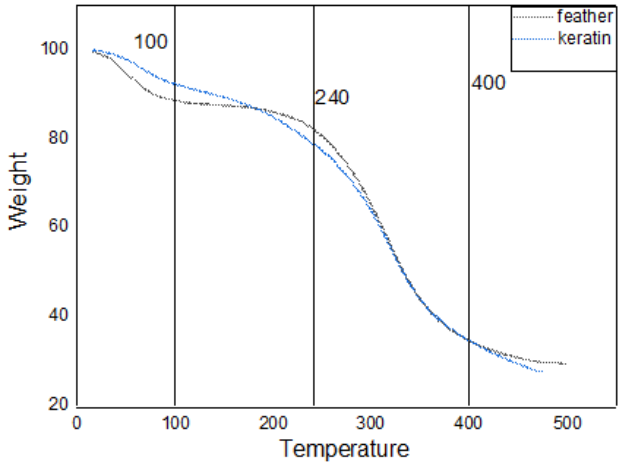


Figure 0.7 TGA of feather and keratin

4.3.6 Chemical and physical properties of extracted starch

The resulting starch in this study is as shown in the table 4.7. The starch was obtained in powder form after drying and grinding.

Table 0.7 The result on characteristics of mango seed starch

Types of test	Results	Specification
Qualitative test	Bluish violet color	Bluish violet color
Form	Powder	Powder
Color	White	White
Oder	Odorless	Odorless
Test	Tasteless	Tasteless
a. Moisture content b. Loss on drying c. Ash content d. Amylose	6.2% 11.20% 0.8% 19.278%	<20% <15% <1% (17-25)%

1. **Moisture content:** the moisture content of starch powder may determine its quality and stability. Starch powder with high moisture content is vulnerable to bacterial growth, compared with that low moisture content. The moisture content of starch should be less than 20%. The moisture content of mango seed starch powder was found to be 6.2%. This result met the above specification, as shown in table 4.7.
2. **Loss on drying:** testing of loss on drying is performed to measure water loss upon heating. The result showed that the loss of mango seed starch's drying was 11.20%, meeting the required specification (Table 4.7). In general, the loss on drying of starch is less than 15%.
3. **Ash content:** it was below 1% that is 0.8%, which satisfies the above specification. The color of the resulting mango seed starch was white by visual inspection. It is possible to change into pure white by using hydrogen peroxide as a bleaching agent.

Figure 0.8 Mango seed starch powder

4.4 Synthesis of bioplastic and analysis on tensile strength, elongation at the break and water absorption.

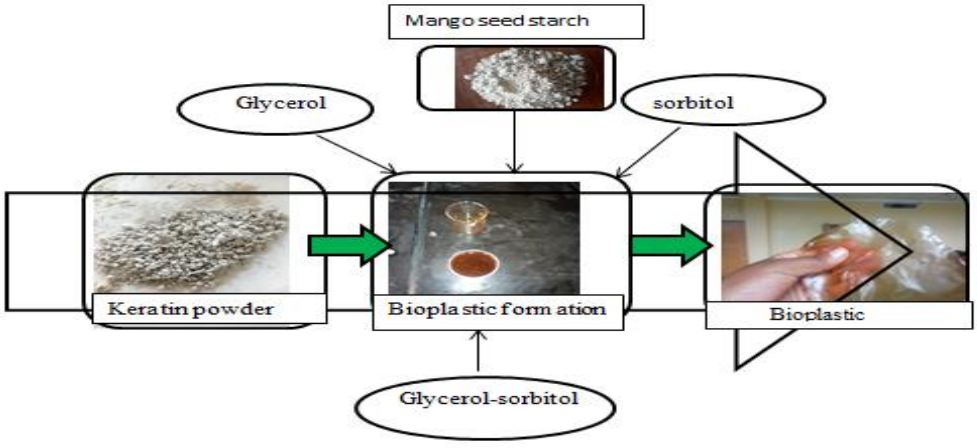


Figure 0.9 Process flow diagram of film synthesis

The experimental values of the tensile strength(TS) elongation at the break(EA) and water absorption (WA) obtained under different conditions are presented in Table 4.8.

These results were inputs to the DESIGN EXPERT software version 11, and the statistical analysis of the conditions is oven-dry temperature GLY vs. SOR and starch concentration. The overall design summary is summarized in Table 4.9.

Table 0.8 Design summary

Study type	Response surface
Initial design	Central composite(rotatable k<)
Experiments	20

4.4.1 Statistical analysis of factors affecting the response variables

The design matrix and the corresponding results of RSM/CCD experiments to determine the effects of the three independent variables dry oven temperature, GLY/SOR, and starch in concentration on tensile strength, elongation at the break and water absorption were shown Table 4.9. The results were analyzed using ANOVA (analysis of variance) appropriate for the experimental design used and shown in Table 4.9 and Appendix Table B_1 and B_2. Empirical models for the output response of films regarding the dry oven temperature, GLY/SOR concentration and starch concentration in actual and coded factors were developed using RSM/CCD methodology. The sequential model sum of squares is given from the appendixes Table B_9 for tensile strength as a sample, and it was found that the quadratic model was the most suitable model for the present study.

The model is significant, had higher polynomial order, high R-squared, adjusted R-squared and predicted R-squared for all response variables. The ANOVA of the quadratic regression model indicates the model to be significant. The model F-value of 107.02, 99.49 and 105.05 implied the model to be signed for tensile strength, elongation at the break and water absorption. Only a 0.01% chance that a “model F-value this large could occur due to noise. Model P-value (Prob>F) is very low [<0.0001]. This reiterates that the model is significant.

The P values are used to check each of the coefficients' significance, which is necessary to understand the mutual interaction pattern between the test variables. The smaller magnitude of the P, the more significant is the corresponding coefficient. The values of P less than 0.0500 indicate the model terms to be significant. For tensile strength, the coefficients estimate and corresponding P value suggest that, among the test variables used in the study, in this case, A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms as to show in Table 4.10.

For Elongation, at the break, the coefficients estimate and the corresponding P values suggests that, among the test variables used in the study, in this case, A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms and for the water absorption the coefficient estimate and the corresponding P values suggests that, among the test variables used in the study. In this case, A, B, C, AB, AC, BC, A^2, B^2, and $C^2$are significant model terms. Values greater than 0.1 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.9869, 0.6699., 0.6426 Implies the lack of fit is not significant relative to the pure error for tensile strength, elongation at the break and water absorption, respectively. There is a 98.69%, 66.99% and 64.26 % chance for tensile strength, elongation at the break and water absorption, respectively, that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good because the model needs to fit.

Table 0.9 values of the three response variables associated with the factors

`Std	Run	Factor 1 A: temperature ^{o}c	Factor 2 B: Gly/Sor conc %	Factor 3 C:starch conc %	Response 1 (TS) MPa	Response 2 (EA) %	Response 3 (WA) %
19	*1*	*50*	*35*	*50*	*16.7*	*10.2*	*19.52*
2	2	65	20	30	14.3	13.52	22.99
1	3	35	20	30	13.5	14.5	24.25
8	4	65	50	70	15	13.1	22.61
3	5	35	50	30	12.55	14.18	24
7	6	35	50	70	11	17.83	26.5
11	7	50	9.77311	50	10.98	19	26.5
12	8	50	60.2269	50	10	20	27.5
20	9	50	35	50	16.88	10.7	20.5
9	10	24.7731	35	50	12.4	15.4	25.1
10	11	75.2269	35	50	16.45	11.23	20.1
15	*12*	*50*	*35*	*50*	*17.86*	*8.99*	*19.45*
5	13	35	20	70	12.05	15.99	25.23
13	*14*	*50*	*35*	*16.3641*	*17.4*	*9.93*	*19.7*
6	15	65	20	70	15.5	12.18	22
14	16	50	35	83.6359	16	11.68	21.5
18	17	50	35	50	17.56	9.89	20.17
4	18	65	50	30	14.9	12.96	22.6
17	19	50	35	50	17.5	9.99	19.74
16	20	50	35	50	17.5	9.9	20

Table 0.10 Analysis of variance [partial sum of squares], for tensile strength

Source	Sum of Squares	Mean Square	F-value	P-value	
Model	118.30	13.14	107.02	< 0.0001	Significant
A-temperature	20.33	20.33	165.51	< 0.0001	
B-Gly/sor conc	0.5872	0.5872	4.78	0.0536	
C-starch conc	1.98	1.98	16.15	0.0024	
AB	0.1128	0.1128	0.9186	0.3605	
AC	3.19	3.19	25.96	0.0005	
BC	0.4753	0.4753	3.87	0.0775	
A^2	14.68	14.68	119.56	< 0.0001	
B^2	82.81	82.81	674.26	< 0.0001	
C^2	0.6061	0.6061	4.93	0.0506	
Residual	1.23	0.1228			
Lack of Fit	0.1148	0.0230	0.1031	0.9869	not significant
Pure Error	1.11	0.2227			
Cor Total	119.53				

The **Model F-value** of 107.02 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, A, C, AC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 0.10 implies the Lack of Fit is not significant relative to the pure error. There is a 98.69% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

4.4.2 Adequacy check for the developed response surface quadratic models

The predicated R^2 0.9664, 0.9495, 0.9578 is in reasonable agreement with the adjusted R^2 of 0.9796, 0.9754 and 0.9801 for tensile strength, elongation at the break and water absorption, respectively. Adequate precision measures the signal to noise ratio. A ratio of greater than 4 is desirable. This model can be used to be navigating the design space. The fit of the model was also expressed by the coefficient of regression R^2, which was found to be 0.9893, 0.9871 and 0.9895 indicate that 98.93%, 98.71% and 98.95% the variability in the response could be explained by the model for tensile strength, elongation at the break ad water absorption respectively. The closer the value of R^2 (correction coefficient) to 1, the better is the correlation between the experimental and predicted values. Here the value of R^2 (be 98.93%, 98.71% and 98.95%) being close to 1 indicated a close agreement between the experimental results and theoretical values predicted by the model equation. This implies that the prediction of experimental data is quite satisfactory. The coefficient of variation (CV) indicates the degree of precision with which the experiments are compared. Generally, the higher the value of CV is, the lower the reliability of the experiment. Here a lower value of CV (2.46, 3.89 and 1.66) for tensile strength, elongation at the break and water absorption, respectively) indicates greater reliability of the experiments performed. The predicted residual sum of squares for the model, which measures how well a particular model fits each point in the design; Adequate precision measures the range in predicated response relative to its associated error. In other words, a signal to noise ratio and its desired value is four or more. In this case, the ratio of 28.4421, 27.7863 and 28.6385 for tensile strength, elongation at the break and water absorption, respectively, indicates an adequate signal which could be used to navigate the design space or decide whether the model can be used or not. Model adequacy measure for tensile strength, elongation at the break and water absorption shown in Table 4.11 and Appendix B_2 and B_3 part.

Table 0.11 Model adequacy measures for tensile strength

Std. Dev.	0.3504	R^2	0.9893
Mean	14.86	Adjusted R^2	0.9796
C.V. %	2.36	Predicted R^2	0.9664
		Adeq Precision	28.4421

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 28.4421 indicates an adequate signal. This model can be used to navigate the design space. The model's adequacy was further checked through graphical analysis of predicated versus actual, outlier T versus run number, normal probability plot of residuals versus standardized residual, and residual versus the run number.

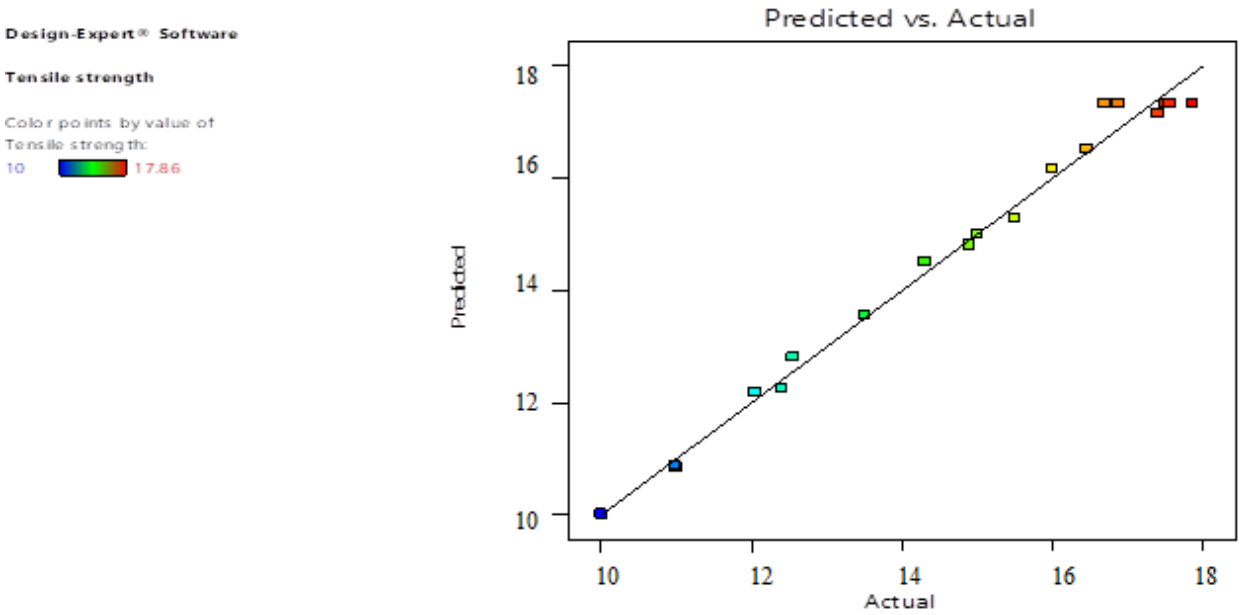
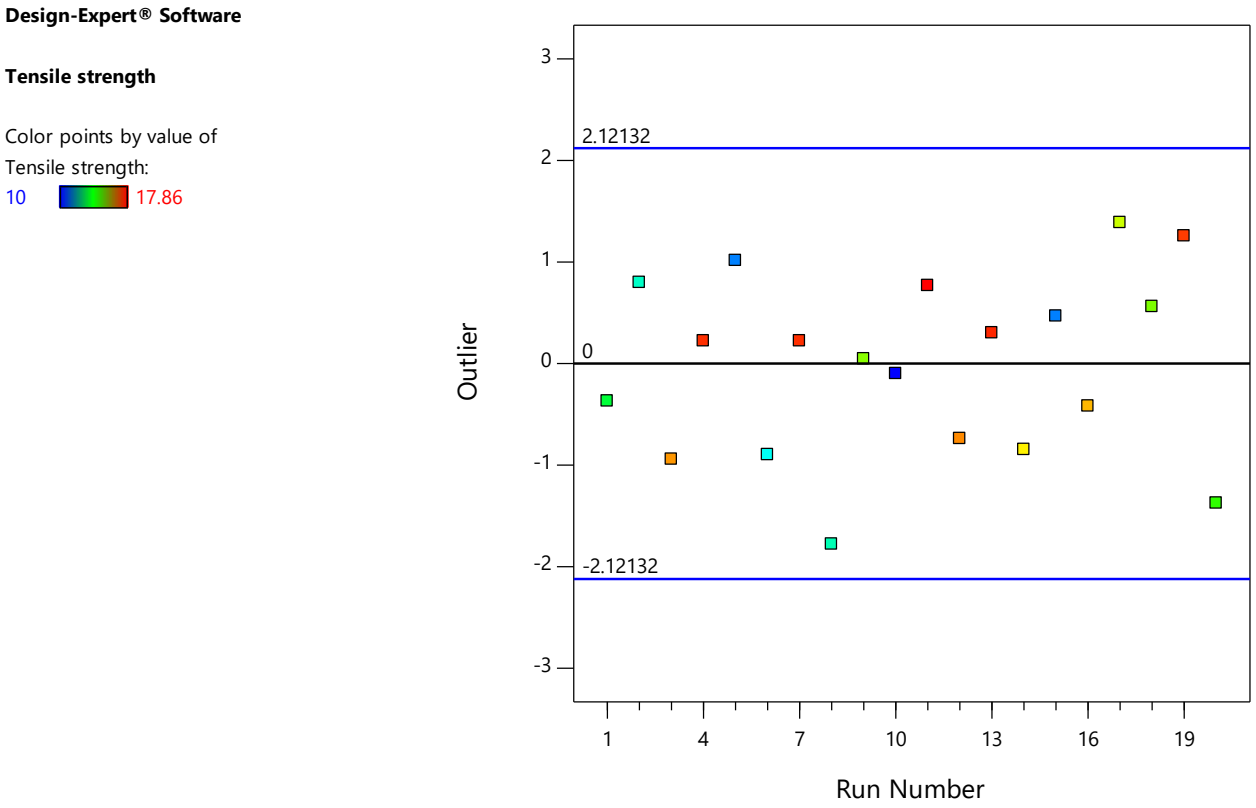


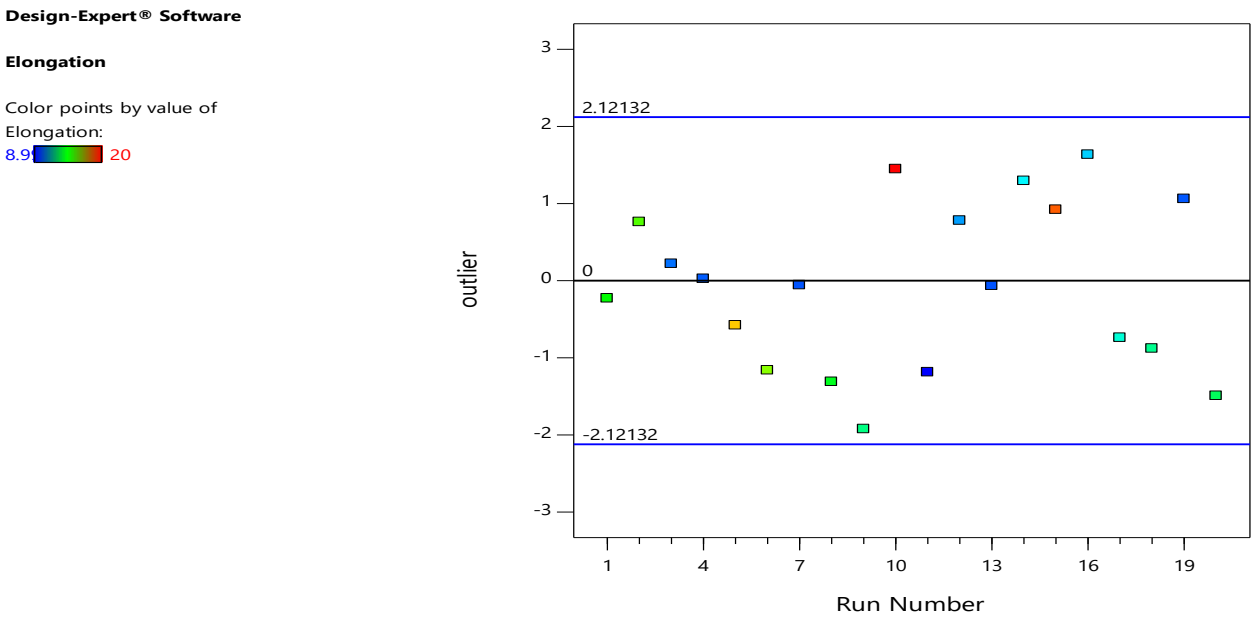
Figure 0.10 Predicate vs. actual plots for tensile strength

The plots represented the line of a perfect fit with points corresponding to zero error between predicate values and actual values from the above Figures 4.11 and appendix K. They demonstrated that the regression model equation provided an accurate description of the experimental data for all responses, in which all the points are close to the line of the perfect fit.

Also, model adequacy can be checked by the plot of outlier T vs. run number, which gives information about whether experiments were done in appropriate condition or not.



A, tensile strength (TS)



B, Elongation (EA)

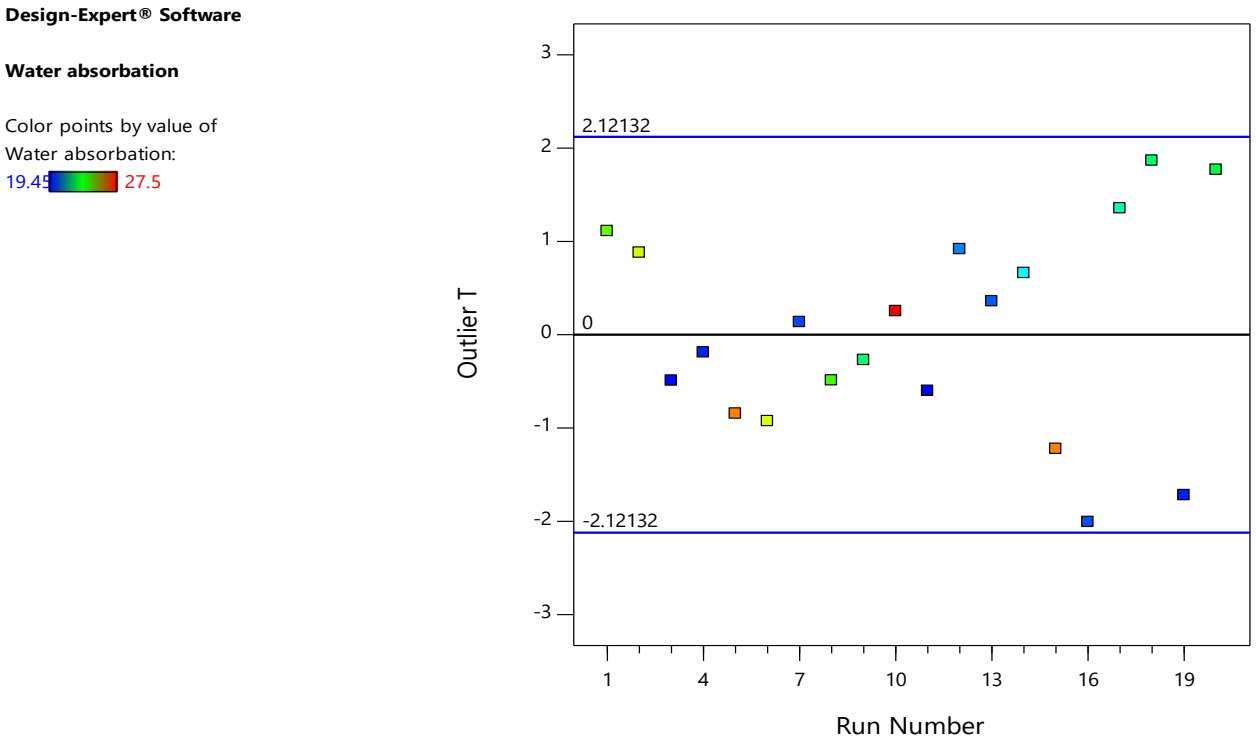
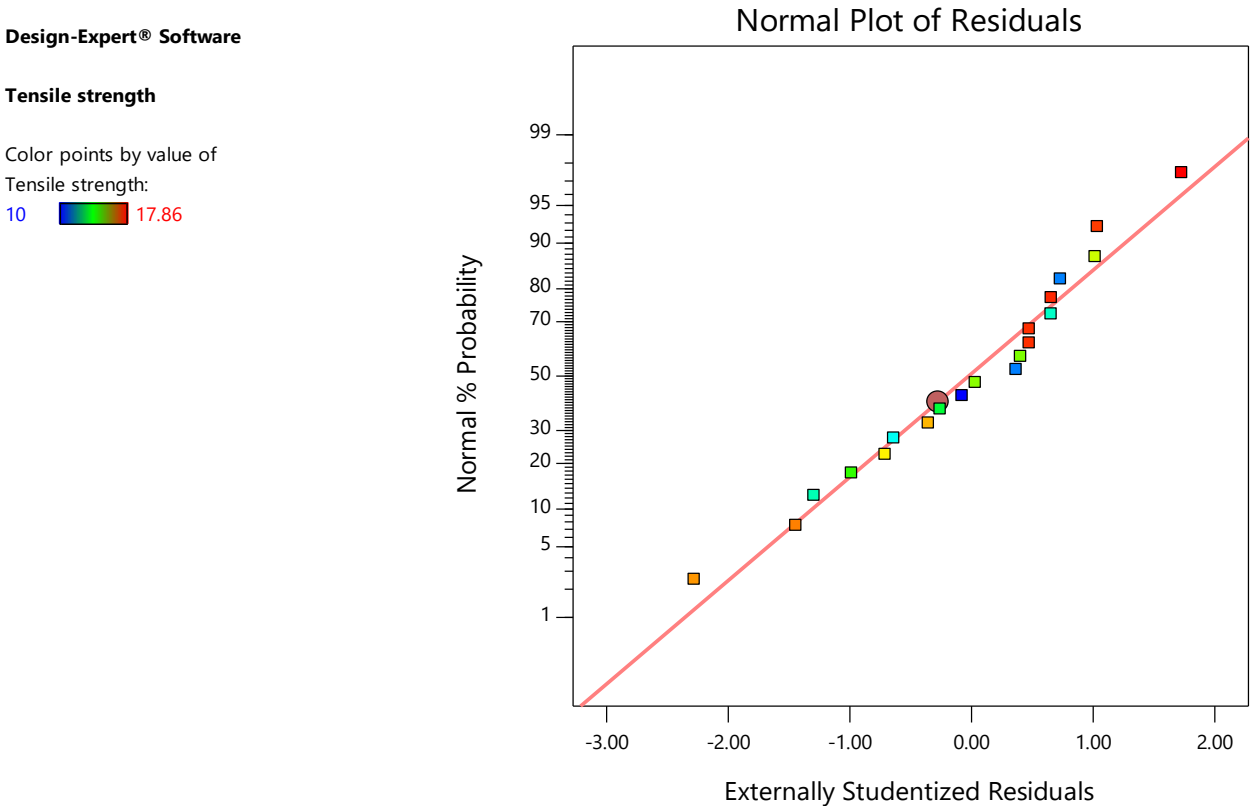
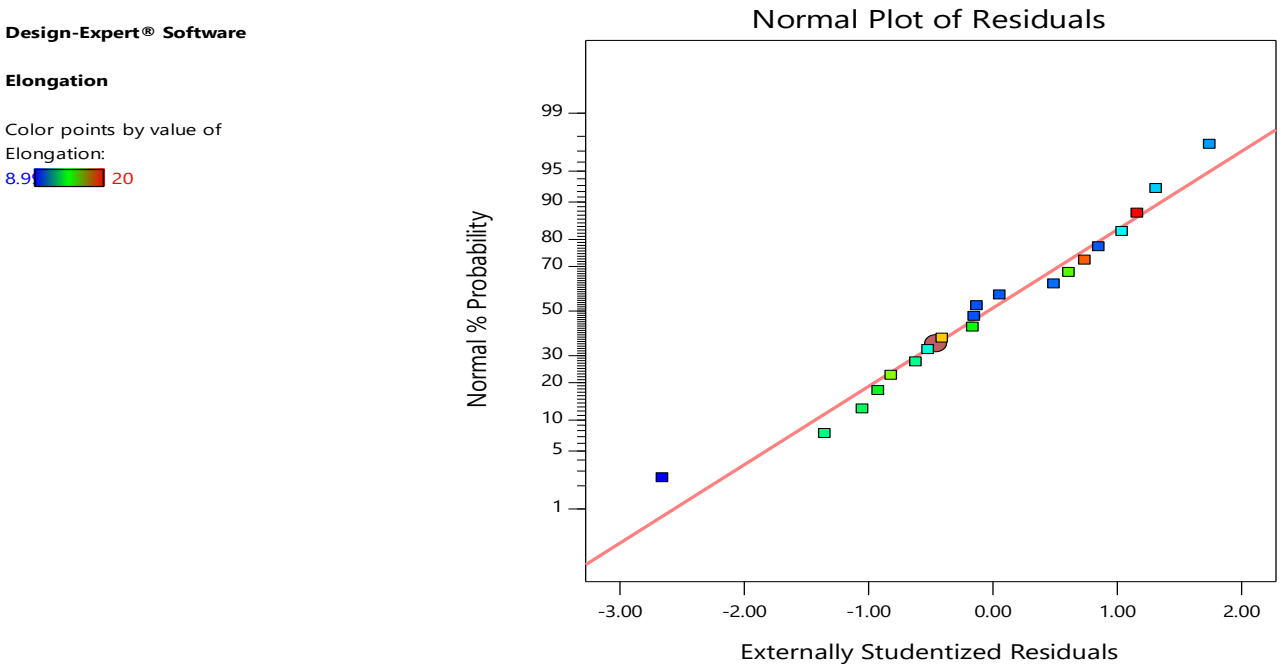


Figure 0.11 outliner VS. Run number plot for tensile strength

The plots of outlier T vs. run number are presented Figure 4.12 above and appendix L. Hence from the outlier vs. run number show, each experiment is within the outlier T border. It implies that no bad data and all experimental runs were done in the appropriate condition. Hence no experiments repeat.



A, Tensile strength (TS)



B, Elongation (EA)

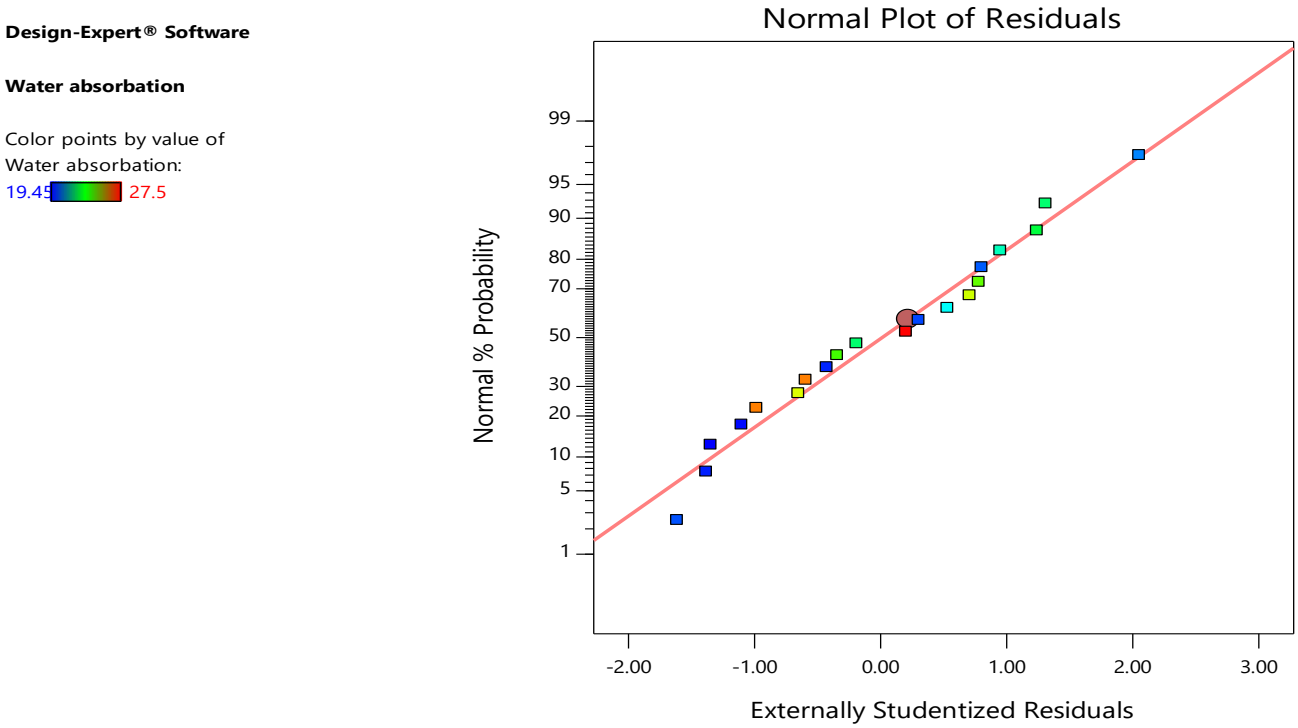


Figure 0.12 Normal probability Plots for A TS, B EA, and C WA

C, water absorption (WA)

Data have been collected in a randomized run order or some other order that does not increase or decrease in the predictor variables used in the model to emphasize experiment design. Hence, it is better to check whether the data collection method affects the adequacy of the model by the graphical method as shown in Figure 4.14 below and appendix M.

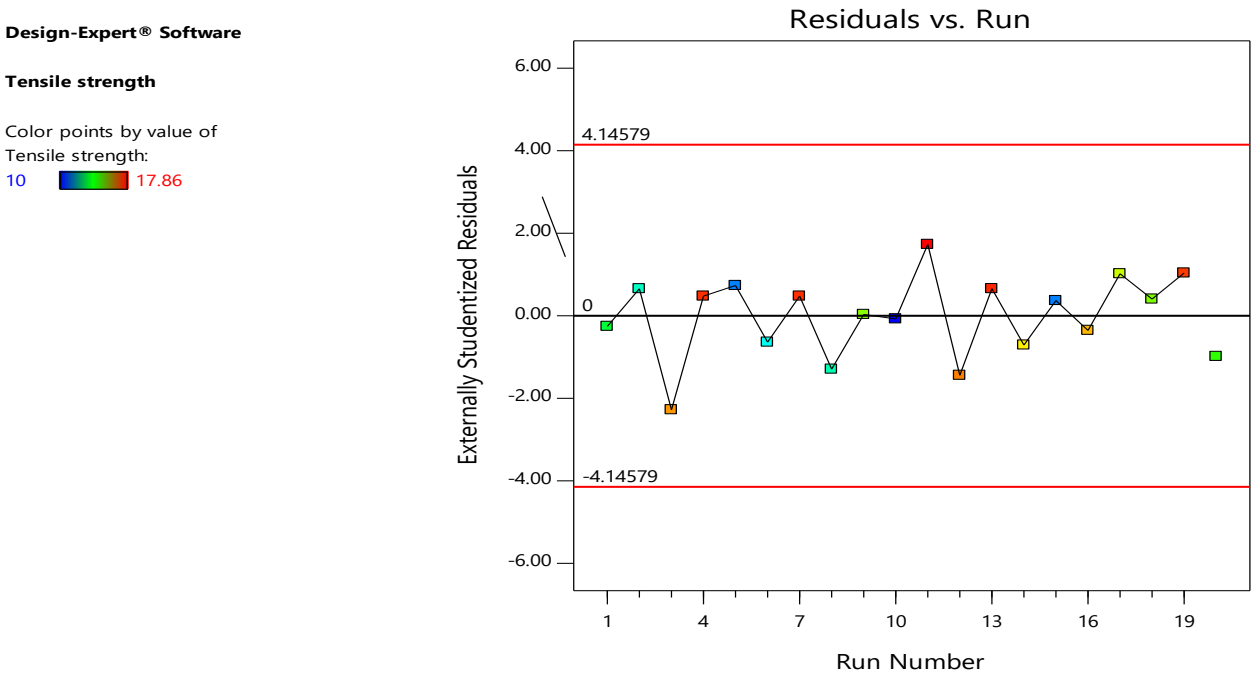


Figure 0.13 Residual vs. Run number of tensile strength (TS)plots

4.4.3 Development of regression model equation

The regression coefficient of the developed model was determined from the regression analysis. AS indicated in fit summery, which is given from the appendixes Table B_8 ,$B_{9,}$ and B_{10} the quadratic model is suggested, as the p-value of this model is smaller than that of other models and no aliased terms. The model equation that correlated the response to the process variables in terms of actual value and codded was formulated. Final equations in terms of actual and coded factors for all responses are given in Appendix C.

4.4.4 The interaction effect between process variables and responses

To study the interactive effect of factors on the tensile strength, elongation, and water absorption, the response of surface methodology was used, and the 3D surface was drawn. Response surface as a plot as a function of two factors at a time, maintaining another factor at fixed levels, helps

understand the interaction effects of these factors. The 3D response surface graphs, as shown in the figure, shows that a significant interaction between every two variables. As can be seen from the coded equation(Appendix C), the interaction factor effects on tensile strength, elongation and water absorption can be understood easily by the coefficients of interaction factors. In this section, the significant interaction effects of factors are discussed. There are three interaction factors analyzed by the model equation. These are AB(temperature and Gly/Sor), AC(temperature and starch concentration) and BC (GLY/SOR and starch concentration). Among these three interaction factors, the one which consists of higher coefficients at the code regression models is the most significant interaction factor for the response. The sign of the interaction factor's coefficient indicates the effect of the interaction factors with a negative sign has a negative effect on the responses (TS, EA, and WA). The 2D graph easily showed the interaction graph in the appendix part of appendix E. the significant interaction factors are discussed in the following sections.

1. **Interaction effects of dry oven temperature and plasticizer (glycerol/sorbitol) concentration on tensile strength, elongation and water absorption**

Figure 4.15 of the 3D represents the interaction effects of dry oven temperature and plasticizer on tensile strength and elongation at a constant starch concentration (50%). Varying dry oven temperature and plasticizer concentration had a significant (P-values less than 0.0500) effect on tensile strength and elongation value. The increase in dry oven temperature at constant initial plasticizer concentration increases the tensile strength and becomes maximum at a center point(35% conc.). Dry oven temperature increase and plasticizer concentration decrease the elongation and water absorption increase, on the other hand, dry oven temperature decrease and plasticizer concentration raises both elongation and water absorption increase, this is because the higher mobility of the polymer due to increase plasticizer, enables the film to absorb moisture over time which is likely due to the hydrophilic nature of plasticizer [39].

As show from Table 4.9 the maximum EA was recorded at center point dry oven temperature (50^{o}c) and maximum plasticizer conc. (60.2269%). On the other hand, the minimum EA was recorded at the center point ($50\ ^{o}$c) and 35% plasticizer concentration. The maximum WA has been recorded at 35^{o}c oven-dry temperature and plasticizer's center point (50%). Generally, as

dry oven temperature and plasticizer concentration increase up to the center point the tensile strength increase, and then it decreases. This can be caused by the influence of higher temperature that can cause the intermolecular bonds between weaker. The covalent polypeptide bond between the amino acid break when the temperature is high, and the hydrogen bonds between amylose chains undergo the bond's termination. Further heating will break glycosides bonds (bonds between monomers) in amylose.

An increase in the heating temperature can cause depolymerization in the amylose chain. The straight-chain amylose falters and becomes shorter, thus decreasing amylose content. Generally, as drying temperature increased, tensile strength increases up to a center point of 50 ^{o}C, tensile strength decreased, and finally it becomes parabolic; this implies the center point favors the film's tensile strength.in similar way . As the plasticizer concentration is beyond the range or rises, it is difficult to peel off the film, and the film becomes highly flexible. So controlling plasticizer concentration is important. The result agrees with the previous works on chicken feather keratin[40] as the plasticizer concentration reaches beyond the saturation/increases, tensile strength declines. The possible reason for the high tensile strength at low plasticizer concentration is the domination of strong hydrogen bonds produced by keratin-keratin and keratin-starch intermolecular interaction over keratin-plasticizer attraction [36].Generally, the medium plasticizer (35%) favors getting maximum tensile strength (17.86Mpa) of the film.

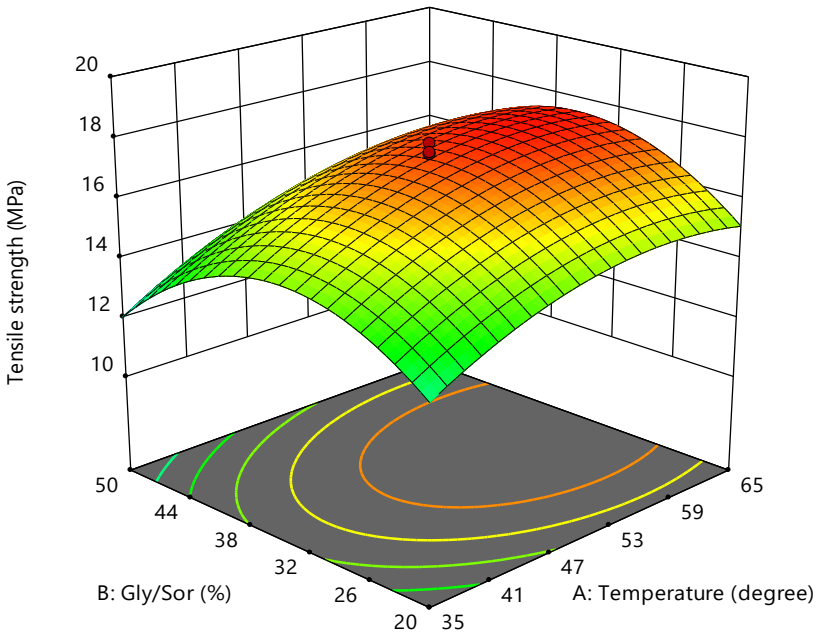
The result of elongation at break decreased with the increase in the temperature shown in figure 4.15. This is because the heat that is given causes an increase in the molecules' kinetic energy in which the molecules vibrate and create a free volume to allow larger molecule chain rotation. So, as the temperature increase %EA decreases, this implies lower elongation is a favor for the synthesized film.As the temperature rises, both the distilled water and plasticizer (glycerol and sorbitol) evaporate. To some extent, the film dries very well, and the film's water absorption is reduced. The decrease in extension at break value was probably due to more plasticizer content, which can cause lower interaction between polymer chains.And as dry oven temperature and plasticizer increase, both EA and WA decrease. The interaction effect was shown below in figure 4.15.

Design-Expert® Software
Factor Coding: Actual

Tensile strength (MPa)
Design points above predicted value
Design points below predicted value
10 17.86

X1 = A: Temperature
X2 = B: Gly/Sor

Actual Factor
C: Starch = 50

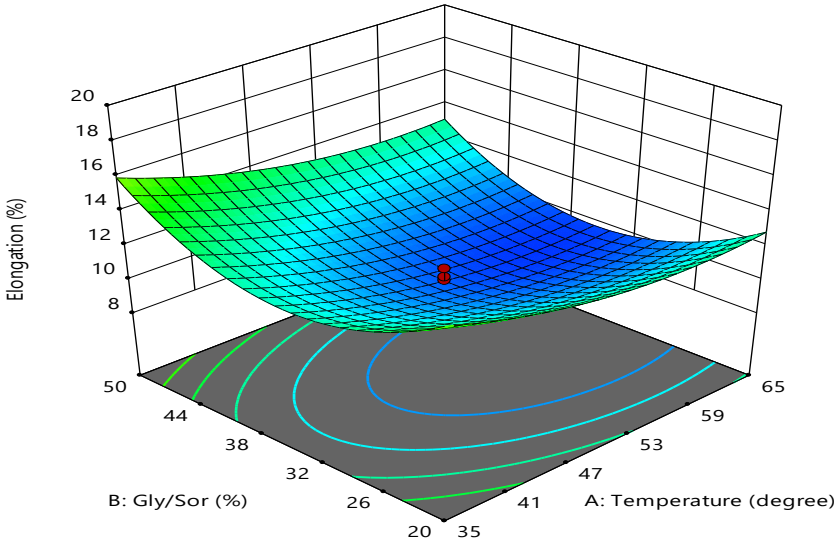


Design-Expert® Software
Factor Coding: Actual

Elongation (%)
Design points above predicted value
Design points below predicted value
8.9 20

X1 = A: Temperature
X2 = B: Gly/Sor

Actual Factor
C: Starch = 50



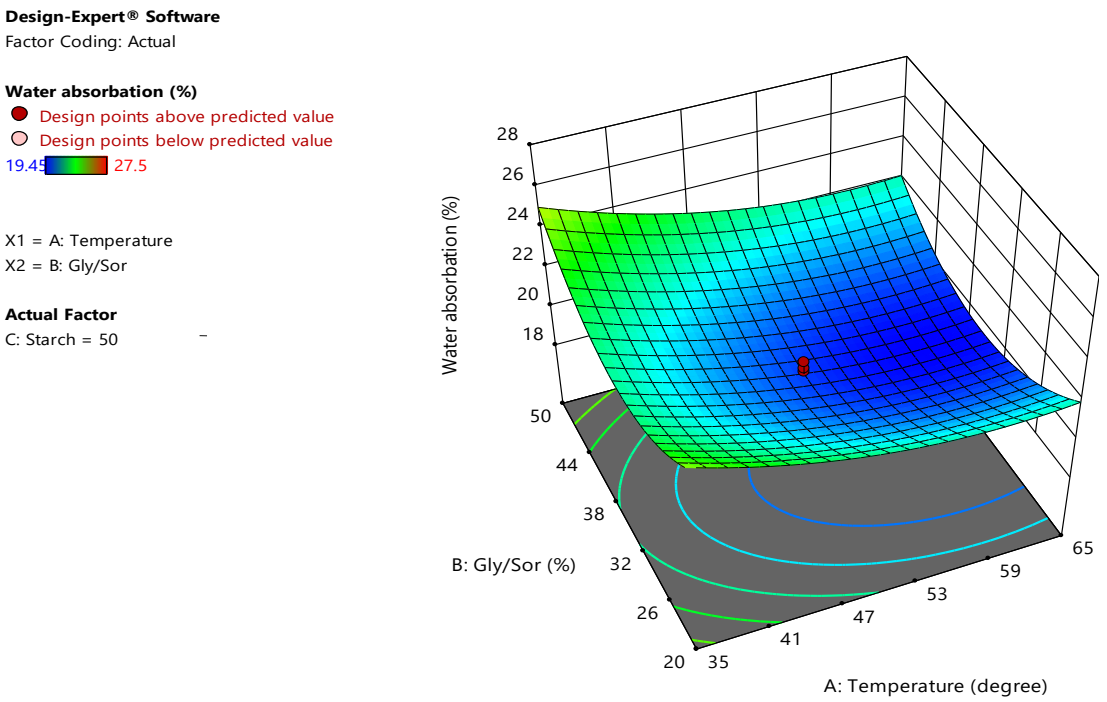


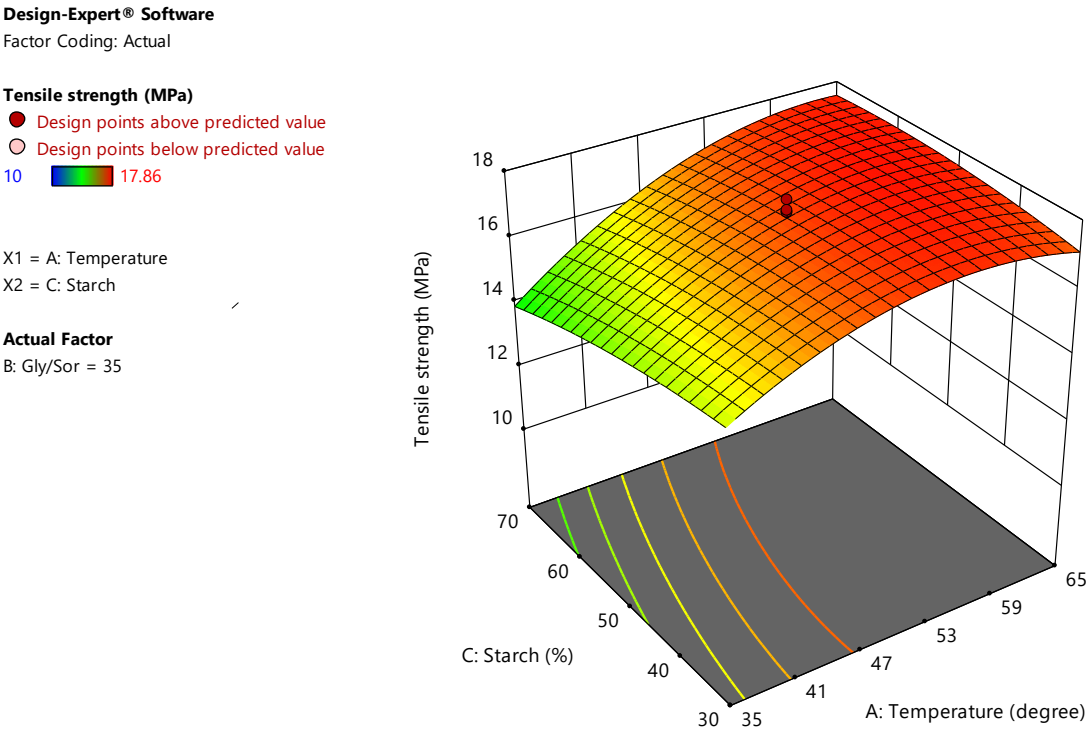
Figure 0.14 interaction effects of dry oven temperature and plasticizer concentration on tensile strength, elongation and water absorption

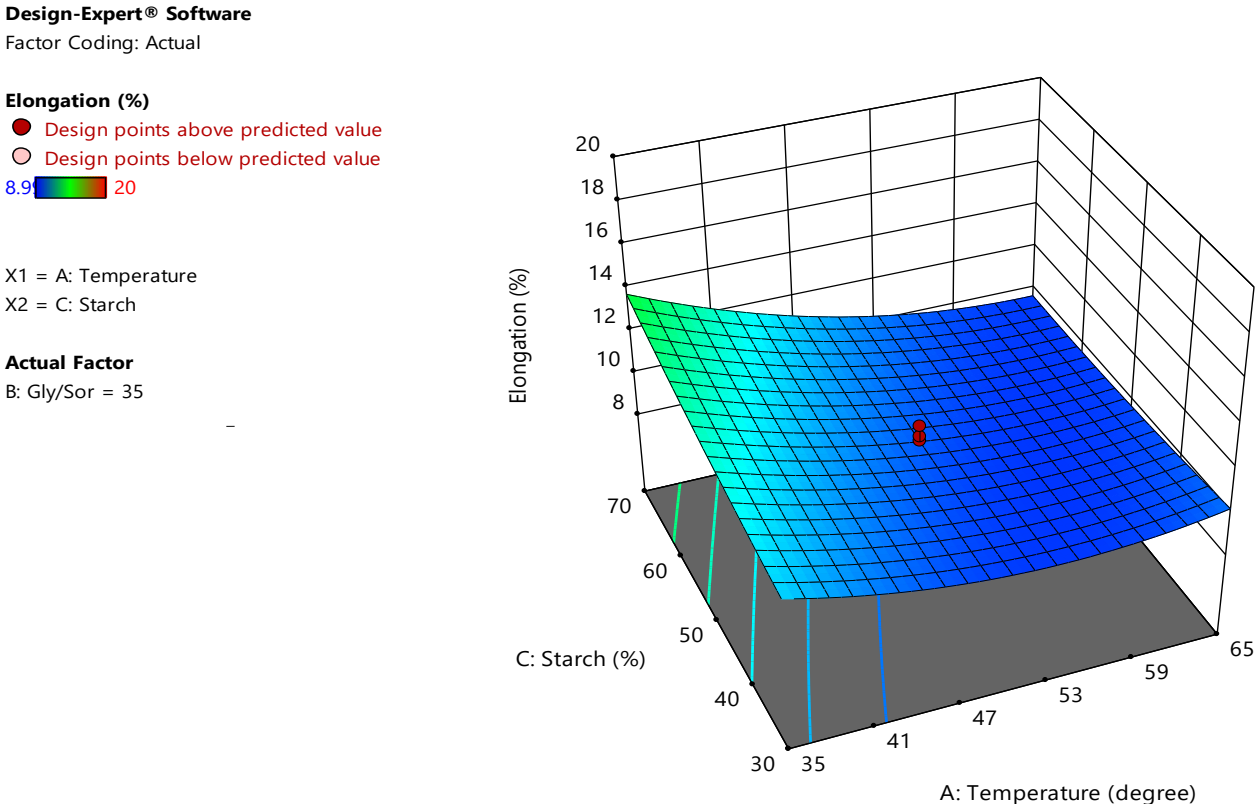
2. Interaction effects of dry oven temperature and starch concentration on the tensile strength, elongation and water absorption

Figure 4.16 of the 3D shows the effects of dry oven temperature with starch concentration and their mutual interaction on the tensile strength and water absorption at constant plasticizer concentration (35%). The increases in dry oven temperature and the decrease in starch concentration increase the film's tensile strength. The highest response value, 17.86, was observed at 50^{o}c dry oven temperature and 50% starch plasticizer concentration, further increased in the temperature, the tensile strength decrase due to the degradation of protein and starch. The tensile strength increases up to the center point of dry oven temperature, but the tensile strength decreases when the starch concentration increases. This happens because the hydrophilic nature of starch can destroy the hydrophobic nature of keratin. The interaction graph tells this situation. The analysis of variance also indicates that AC/interaction of dry oven temperature with starch concentration affects the response tensile strength significantly (p-value less than 0.05).

When the oven-dry temperature above the center point and the starch concentration increases the tensile strength decreases; on the other hand, dry oven temperature increases and the starch

content decreases, both EA and WA decrease because of the tensile strength increase. This is because of the polymer's higher mobility due to increased starch enables the film to absorb moisture over time, which is likely due to the hydrophilic nature of starch [6]. From table 4.9 the maximum for EA and WA were recorded at the minimum dry oven temperature (24.7731^{o}c) and center starch concentration (50%). On the other hand, the minimum for EA and WA have been recorded at 50^{o}c oven-dry temperature and 50% starch concentration. Generally, the tensile strength increase when the oven-dry temperature and starch concentration increases up to the center point, whereas both EA and WA increase when dry oven temperature decrease and increase starch content.





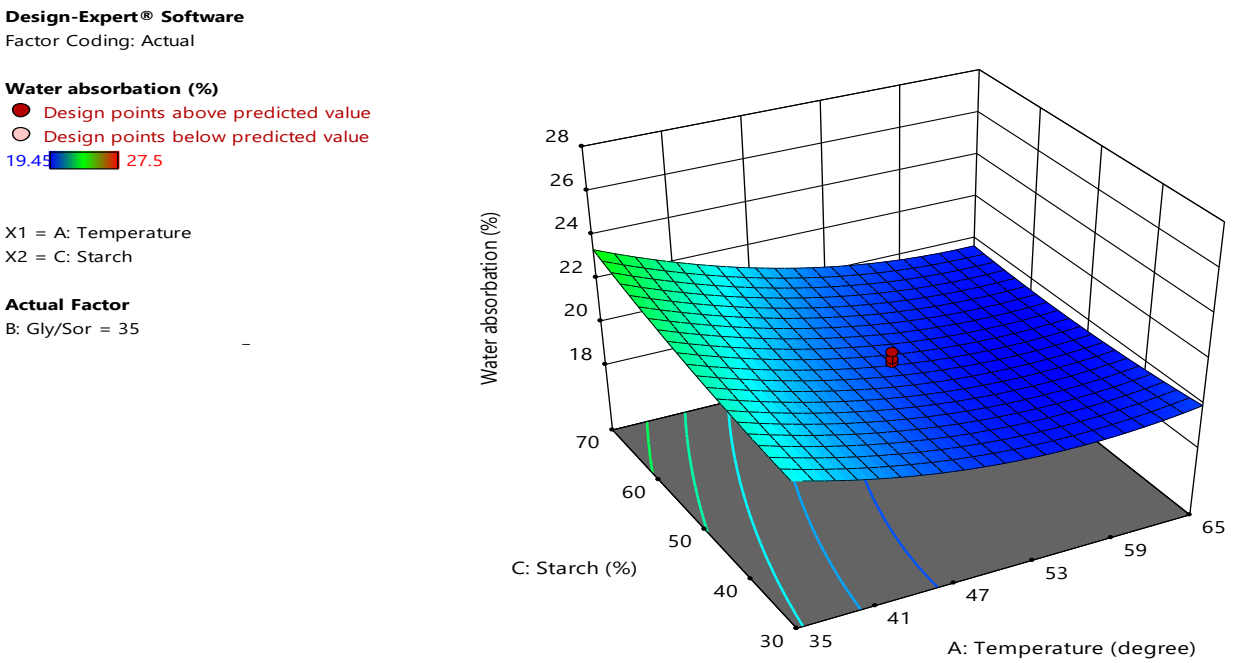


Figure 0.15 interaction effects of dry oven temperature and starch concentration on tensile strength, elongation and water absorption

3 Interaction effects of both plasticizer(glycerol and sorbitol) and starch on the tensile strength, elongation and water absorption

The Figure 4.17 of the 3D represents the effects of starch concentration with plasticizers and concentration and their mutual interaction on the elongation of the film. Varying starch concentration and plasticizer concentration had a significant effect on the tensile strength, elongation and water absorption. The tensile strength of the film is affected by both starch concentration and plasticizer concentration. The maximum tensile strength of the film happens at the minimum starch concentration (16.3641%) and at the center point of plasticizer concentration (35%). On the other hand, the minimum tensile strength occurs at a maximum starch concentration (83.6359%) and maximum plasticizer concentration (60.2269%). This happens because as the starch content increases, the hydrophobic nature of keratin destroyed by the hydrophilic nature of starch,The increment in starch concentration weakens the hydrophobic network found in protein, this similar to [41]. This is opposite to plasticizer concentration as the plasticizer concentration increase up to the center point (35%), the tensile strength of the film was an increase, this is because the fragile nature of keratin can improve by plasticizer which can give flexibility to the film, but as the concentration of plasticizer increases the tensile strength become decline because of the plasticizer can the break the bond between the polymer. This work also [14] was justified.

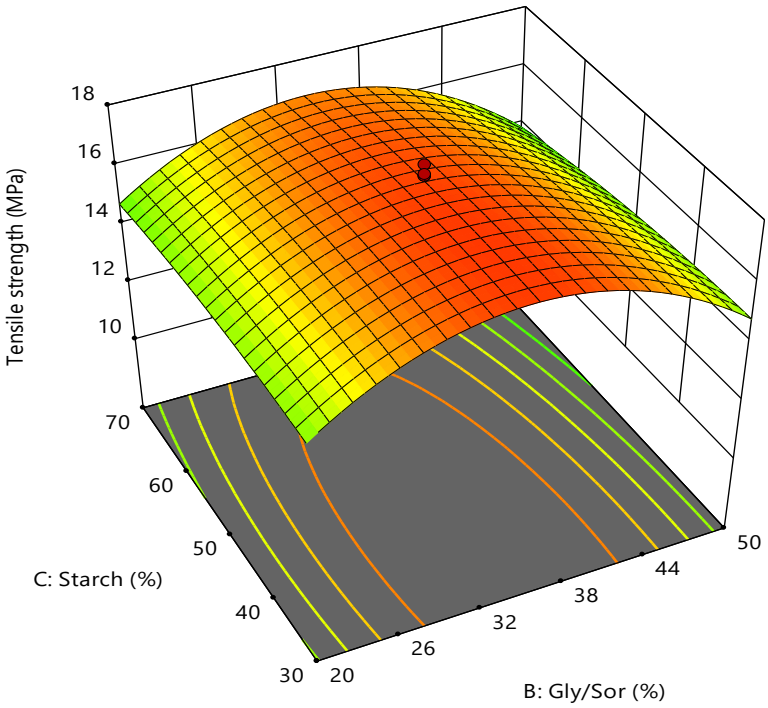
Both EA and WA are directly affected by the increase and the decrease of the tensile strength of the film. When the tensile strength of film increase, both EA and WA decrease. As show figure, the maximum EA and WA happen at 83.6359% starch concentration and 60.2269% plasticizer concentration. The starch concentration increase both EA and WA increase, and as plasticizer concentration increase both EA and WA decrease up to the center point (35%).

Design-Expert® Software
Factor Coding: Actual

Tensile strength (MPa)
Design points above predicted value
Design points below predicted value
10 17.86

X1 = B: Gly/Sor
X2 = C: Starch

Actual Factor
A: Temperature = 50

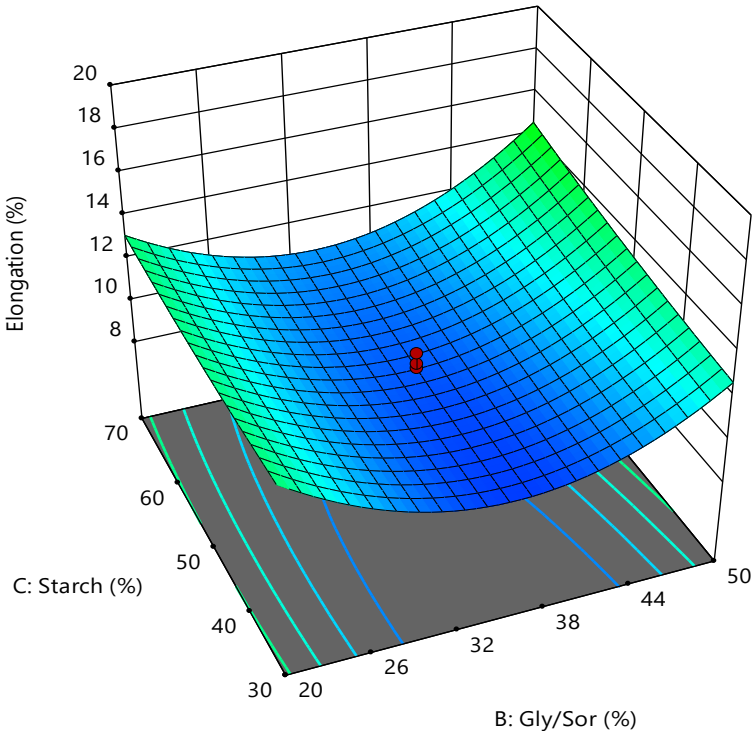


Design-Expert® Software
Factor Coding: Actual

Elongation (%)
Design points above predicted value
Design points below predicted value
8.9 20

X1 = B: Gly/Sor
X2 = C: Starch

Actual Factor
A: Temperature = 50



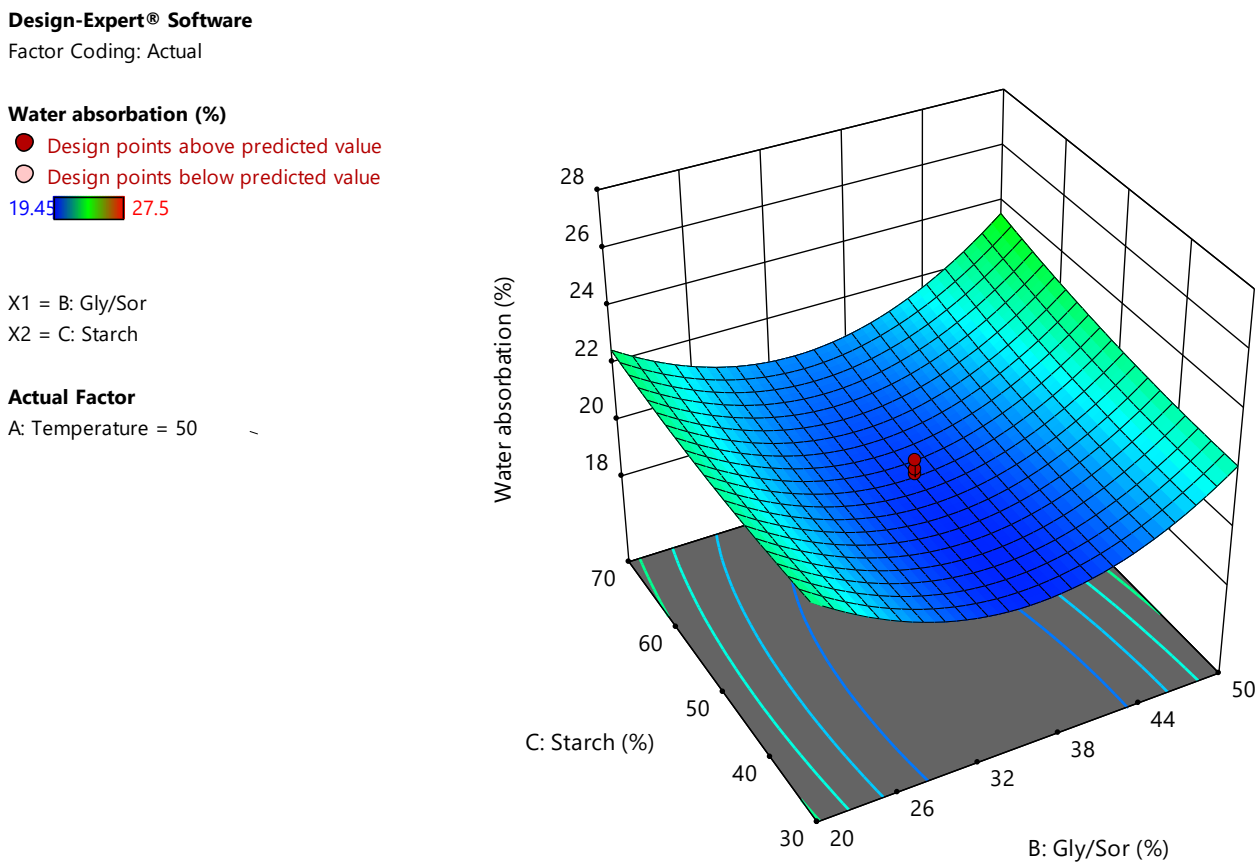


Figure 0.16 interaction effect of starch and plasticizer concentration on the tensile strength, elongation and water absorption

4.4.5 Process factors and response variables optimization

Optimization is the process of finding conditions that give maximum or minimum values of a specific function. Optimization is the act of getting the best possible result under given circumstances. In design, construction, maintenance, Engineers need to make decisions. The goal of all such decisions should be either to minimize effort or to maximize benefit. The effort or benefit is often usually expressed as a function of certain design variables. Hence, optimization is the process of finding the conditions that give the maximum or the minimum value of a function.

Optimization of bioplastic film production process factors and responses was carried out by a multiple response method called desirability (D) function to optimize different combinations of process parameters. The process factors oven-dry temperature, plasticizer concentration, and starch concentration and process responses are optimized based on the principles of optimization.

The principles of optimization tell us to maximize the economic benefit by minimizing process cost, the process variables need to set as much as possible at their minimum value, and the three response variables tensile strength, elongation at the break and water absorption were set to maximum, minimum and minimum respectively. Numerical optimization was used to optimize the process.

Table 0.12 summary of constraint responses and goals of optimizations

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:temperature	in range	35	65	1	1	3
B:Gly/sor conc	in range	20	50	1	1	3
C:starch conc	in range	30	70	1	1	3
tensile strength	maximize	10.21	18.11	1	1	5
Elongation	minimize	9.7739	17.5693	1	1	5
water absorption	minimize	19.75	28.5	1	1	5

Based on desirability analysis, a total of 4 optimum points via numerical optimization (appendix table) generated from this the more preferred one is selected based on the operating cost and the product quantity as well as quality. The desirability function was used to identify the optimum levels of factors and to get a maximum desirable response, and the optimized combinations of process variables were selected among the solutions generated with maximum combined desirability value, i.e., 0.978 show in table 4.13.

Table 0.13 Optimum operating point

D.oven temp	Gly/sor conc (%)	Starch conc (%)	TS	EA	WA	Desirability
49.9474	34.6097	49.9491	17.7604	8.98932	19.4477	0.978

In order to verify this prediction, experiments were conducted, and the results show that 17.86Mpa tensile strength, 8.99% elongation at the break and 19.45% water absorption was obtained, as shown in the table 4.9. Therefore, numerical optimization can be taken as an optimal value because the predicted value is close enough to experimental. The model capable of predicting value is closing enough with experimental.

The model capable of predicting the maximum tensile strength and the minimum elongation and water absorption value showed from the table that the optimum values of the process variables were 49.9474 dry oven temperature, 34.6097% plasticizer concentration, and 49.9491% starch concentration.

Table 0.14 Model validation

Number	Dry oven temp	Starch conc (%)	TS	EA	WA
Predicated	49.9474	34.6097	17.7604	8.98932	19.4477
experimental	50	50	17.86	8.99	19.45

The tensile strength of chicken feather keratin via mango seed starch-based bioplastic film was found to be 17.86, value obtained showed that tensile strength of keratin via starch bioplastic film was higher when compared with other species such as wheat Gliadin protein (1.6-11.3Mpa), soya protein starch(7Mpa), gluten protein starch(7-9Mpa), rapeseed protein starch(9.5-11Mpa), zein starch(10-12.5Mpa) [41]. The mechanical properties of different types of a protein depend on both their extracted resources and molecular weight. The tensile strength obtained in this study was highest than other studies.

This is because the plasticizer used here was a blend of glycerol and sorbitol. The molecular weight of material affects the mechanical properties of the produced film. Since the molecular weight of sorbitol higher than the molecular weight of glycerol, the tensile strength of the final film increases. The blending of glycerol and sorbitol gives intermediate tensile strength because the higher molecular weight of sorbitol can make the interspaced between protein less. The other research on the keratin starch bioplastic plasticized with only glycerol had less tensile strength (15Mpa) [6]than the tensile strength obtained in this work (17.9Mpa). The tensile strength

increase of produced film because the sorbitol had ring conformation, which may make it difficult to insert between protein-protein chains. This then gives it less able to destroy the protein network.

Elongation at the break result obtained under the optimal condition was determined as 9.89%.[42] Report as 4.33 % elongation for the gelatin-starch film. [6] Also, report as 9.10% elongation for the bioplastic developed from keratin and avocado seed starch plasticized by glycerol. The film uptake for the film at the optimal condition was determined as 19.45%. [43], report that the soya protein plasticized by sorbitol water absorption was 40%.

4.5 Application studies result of the bio-plastic film

1. **Water solubility test**: The film solubility in water was determined according to the method reported by [6]. Keratin, by its nature, insoluble in water. However, when it is blended with starch, the solubility of the film increase. Starch, being a hydrophilic polymer, shows a high affinity towards water. The solubility of the film is highly dependent on the plasticizer used. The improved properties of the film can be obtained by blending different plasticizers. The film produced by blending glycerol and sorbitol is less soluble in water when compared to the film produce by only glycerol. Since the molecular weight of glycerol is smaller than sorbitol, the film produced by glycerol had a high affinity towards water, but the film plasticized by both sorbitol and glycerol had less affinity towards water. This is because of the high molecular weight of sorbitol. The solubility of film produced by sorbitol, glycerol and both glycerol and sorbitol was 22.16%, 24.84% and 24%, respectively.
2. **Density test**: - The density of the keratin-based films was measured for both control and matrix. It was observed that the possibility to increase the density by blending starch.The density increment is due to the compatibility of plasticizer, keratin and starch. The obtained result for keratin vs. starch plasticized by glycerol, keratin vs. Starch plasticized by sorbitol and keratin vs. starch plasticized by mixing both sorbitol and glycerol was 1.72 g/ml, 1.65g/ml, and 1.685g/ml, respectively. The result obtained from this work, the difference in the density depended on the molecular weight of the plasticizer. If the molecular weight higher, the interaction between polymer and plasticizer decreases.
3. **Transparency of produced bioplastic: -** The transparency of the film is determined using a spectrophotometer (UV 700) for both control and for the film obtained at the optimum

conditions. The percentage transmittance of keratin VS starch plasticized by glycerol, keratin vs. starch plasticized by sorbitol and keratin vs. starch by mixing both glycerol and sorbitol film at a wavelength of 600nm was determined. The result shows that the films were yellowish in color.

The film plasticized with glycerol had a higher percentage of transmittance than both plasticized by sorbitol and the mixing of glycerol and sorbitol film. 195.53% for film plasticized with glycerol. 194.75% for film plasticized with sorbitol, and 189.01% for film plasticized by mixing both sorbitol and glycerol. Film plasticized by sorbitol had low transparency. This is happening because the molecular weight of sorbitol is high. This decreases the interspacing between keratin-keratin and keratin-starch. Plasticizer was used to increase the transparency and flexibility of film, so the film plasticized by glycerol had high transparency than both plasticized by sorbitol and mixing sorbitol and glycerol.

The decrease in transparency values was observed due to the sorbitol plasticized. Transparency of the film is of importance in some instances when used as packaging materials. The addition of sorbitol generally causes the film to reduce the films to reduce their transparency, but the small differences compared to control. A glycerol plasticized film was rather transparent.

4. **Moisture:** glycerol is more hydrophilic than sorbitol and has a greater plasticizer. The films produced in this work were made with glycerol had a moisture content of 18.9%, while those made with had a moisture content of 11%. The film plasticized by mixing both sorbitol and glycerol had an intermediate moisture content of 14.95%.
5. **The thickness of films:** A digital micrometer was used to measure the film thickness. Since 40ml hot solution was put into a petri dish for the keratin-starch glycerol, keratin-starch sorbitol and keratin-starch with glycerol-sorbitol, and to some amount of starch and might vaporize during gelatinization and oven drying the film, comparing the three samples the film synthesized from only. The film plasticized by sorbitol had a small thickness difference than plasticized with glycerol and both glycerol and sorbitol.

   The reason is molecular weight and boiling point of sorbitol higher than glycerol. The obtained thickness was 0.83nm, 0.86, and 0.84 for film plasticized by glycerol, sorbitol and both glycerol and sorbitol, respectively, by taking the average of the triplicates.

Table 0.15 Results on some physicochemical properties for keratin-starch plasticized by glycerol, sorbitol, and both glycerol and sorbitol.

Product	Thickness(mm)	Moisture (%)	Transparency (%)	Solubility (%)	Density(g/ml)
KS GLY	0.83	18.9	195.53	24.84	1.65
KS SOR	0.86	11	189.01	22.16	1.72
KS GLY-SOR	0.84	14.95	194.75	24	1.68

6. **FTIR analysis result of the film:-** The FT-IR spectra of keratin, starch, glycerol and sorbitol based film are shown in the Figure 4.18. The major absorption band C-O, C=O, O-H, N-H, C-H, amide IV, amide III, carbonyl group, C-C and S-S bonds. Keratin vs. Starch bioplastic absorption peaks at 3100-3600 cm^{-1}, 1660 cm^{-1}, 1150 cm^{-1}, 1250 cm^{-1}, and 900-1000 cm^{-1} relate to N-H stretch functional groups and O-H C-C, N-H, C-O stretching and saccharide. The three samples show in the figure 4.18 had absorption peaks about 1680 cm^{-1}, 1550 cm^{-1}, 1200 cm^{-1}, and 800 cm^{-1} indicate that the availability of amide I (C=O stretching), NH bending(II), amide III, and amide IV, respectively. The peak that appears in the 1700 cm^{-1} shows the presence of the carbonyl group. Due to the interaction of hydroxyl groups(-OH) in the starch molecule and the amino groups($-NH_2$) or carbonyl group(C=O) of the keratin protein, the keratin-starch blend films showed absorption peaks about 2900-3250 cm^{-1}. The peak near 2900 cm^{-1} suggested the presence of C-H stretching. The presence of broadband about 3000-3500 cm^{-1} was an indicator of OH and NH stretching. The shifts in the spectra due to the interaction between keratin-starch and plastic izer. Since glycerol is a highly hydrophilic material that contains more OH group than sorbitol and also sorbitol had high molecular weight, the film produces by glycerol only was shifts in the spectra. The film produced using plasticized both glycerol and sorbitol was appearing in the intermediate.

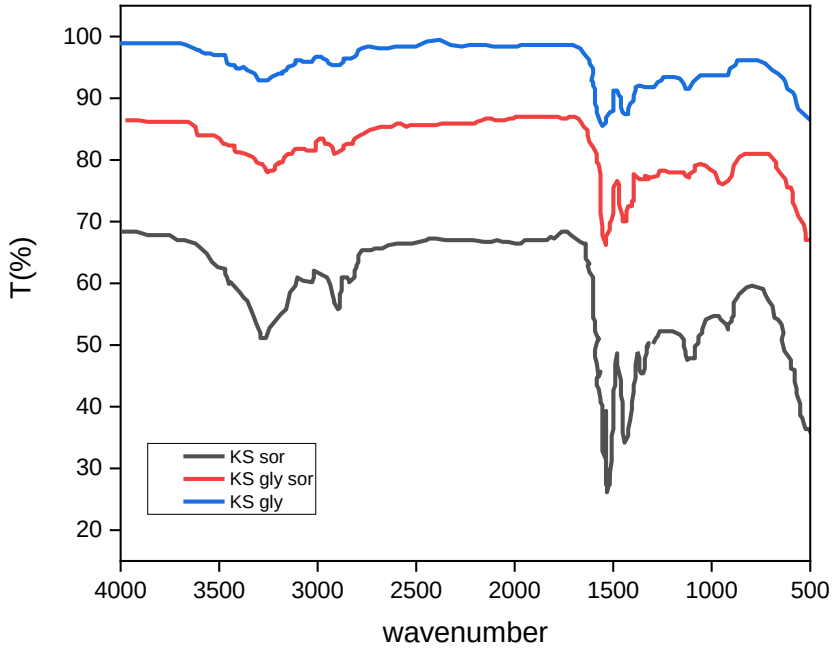


Figure 0.17 FTIR result of keratin-starch glycerol, keratin-starch sorbitol and keratin-starch glycerol vs. sorbitol

7. **Thermal analysis of the film: -** TGA may be a technique measuring the variation within the mass of a sample as a function of time or temperature when it undergoes temperature scanning in a controlled, inert or oxidative atmosphere. Overheating generally implies variation to the relative molecular mass (and molecular mass distribution) of the polymer, and typical property changes include minimize ductility and embrittlement, chalking, color deviations, cracking, a general reduction in most other desirable physical properties of the film. The most important mass loss was observed at a higher temperature.

   This is mainly because of the fact that at high temperatures, the components of the long-chain backbone of the film can begin to be broken (chain scission) and react with each other to vary their properties. The mass loss for three films (keratin starch plasticized with GLY, keratin starch plasticized with SOR, and keratin starch plasticized GLY and SOR). As shown in the Figure 4.19 compare to the three films, the film plasticized by glycerol only show quick weight loss.The degradation of every film resin consisted of three weight-loss steps. The primary gradual weight loss happen at 150^{o}C due to the evaporation of moisture, the

second weight loss happened between 150 and 300°C is thanks to the decomposition of quill and elimination of hydrogen groups, decomposition and depolymerization of starch carbon chains .the film plasticized by GLY showed mas loss than the film plasticized SOR, this happens because the film plasticized by GLY had high moisture content than SOR. An equivalent result was obtained for starch-based plastic plasticized by glycerol and sorbitol[36]. Comparing weight loss of keratin starch plasticized film, it is often seen SOR plasticized film is more thermally resistant than GLY, and GLY-SOR plasticized film at a temperature below 300°C.

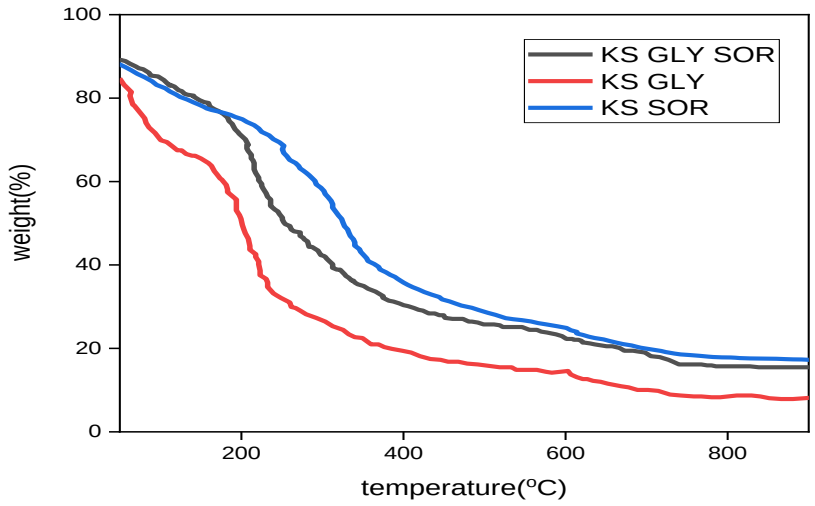


Figure 0.18 TGA analysis of the produced film

8. **XRD analysis of the film**: - to analyze the effect of the addition of keratin, starch, and plasticizer on the properties of the films. The Figure 4.20 shows the different diffraction patterns of three samples, which are keratin starch GLY, keratin starch SOR and keratin starch with GLY and SOR. All the three samples show broad diffraction peaks in the 2Ɵ values of 9 and 20°, which indicate that semi-crystalline materials. The sample which is plasticized by GLY only shifts to a higher angle indicated a decline in the subsequent interlayer spacing, which means that the mixed component had an arranged structure. Since the molecular weight of GLY plasticizer lower the space occupied between which mean interlayer spacing become decline. When the interlayer spacing decreases, the mixed

component had an arranged structure. The increase of d-spacing showed that the blend has a less arranged structure, thus lead to more difficult crystallization. Sorbitol has a higher molecular weight. When mixed with keratin and starch, it increases the interlayer spacing, which means that it had higher d-spacing, which means less ordered structure. This happens due to higher molecular weight components. It is difficult to disperse. From the crystalline peaks, the crystallinity indexes were calculated based on the equation, and the results of all the three sample were completely amorphous structure with crystalline index less than 20%.

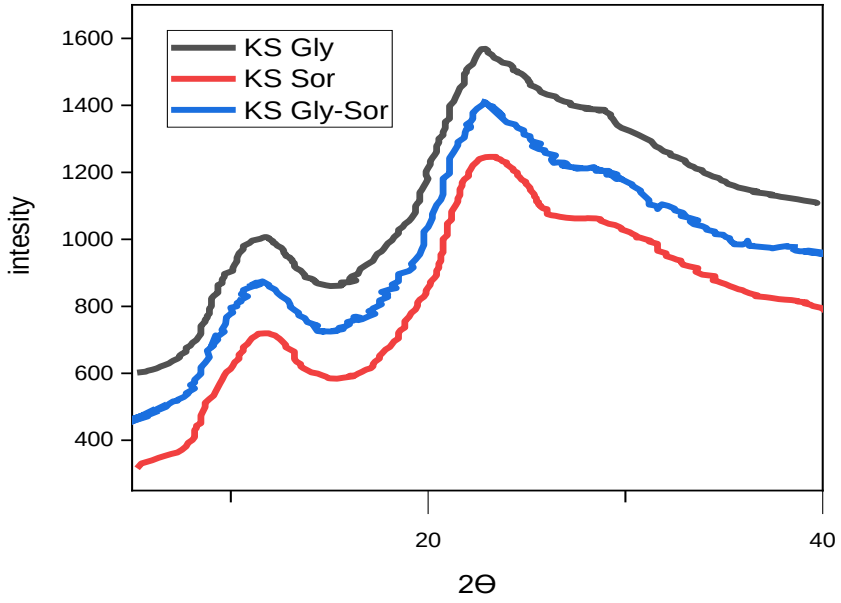


Figure 0.19 XRD analysis the produced film

CHAPTER FIVE

5 Conclusion and Recommendation

5.1 Conclusion

In this research, the development of bioplastic film from chicken feather keratin and mango seed starch plasticized with blending glycerol and sorbitol was investigated. This work also intended to study the proximate composition of a chicken feather, the influence of keratin extraction parameters: extraction time and extraction temperature on the keratin yield and characterization of keratin properties. On the basis of these results, it can be concluded that chicken feather keratin should be considered suitable for bioplastic synthesis because the proximate composition shows that values of 11.9% moisture, 1.45% total ash,0.79% crude lipid,89.5% crude protein, 2% crude fiber, this proximate compostion result tells the chicken fether can be used as a source of keratin. This raw material is suitable for bioplastic synthesis, mainly because of its relatively higher protein content, which is the main source of keratin. The extracted keratin was characterized by using FTIR for keratin and also for the raw feather. The result shows the extracted keratin had the same composition as the raw feather. In addition to FTIR, the crystallite of keratin and feather was identified by using XRD, which shows that both keratin and feather had a semi-crystalline structure. The thermal stability of keratin and feather identify by using TGA. The result shows that the mass loss of both keratin and feather had two stages. The mango seed starch was extracted, and characterization shows that values of 10.5% moisture, 8.5% total ash, 6.66% crude lipid, 2.17% crude protein, 34.37% crude fiber, and 37.8% nitrogen-free extract.

During the synthesis of bioplastic film, the effect of independent variables(dry oven temperature, plasticizer(GLY and SOR), and starch concentration on tensile strength(TS), elongation at the break(EA) and water absorption(WA) using CCD response surface experimental with K<6 was studied. From the conducted experiment, the recorded result shows the minimum EA and WA were obtained at medium drying temperature, plasticizer and starch concentration.

This result also indicates that maximum (WA, EA), and minimum TS were obtained at the possible combination of both minimum dry oven temperature and maximum starch and plasticizer (GLY and SOR) concentrations. Higher TS was obtained at the combination of all medium-dry oven temperatures, plasticizer and starch concentrations.

The interactions of both drying temperature and plasticizer had a significant effect on TS and EA. A significant effect has been observed on the TS and WA due to the interaction of drying temperature and starch concentrations. Both starch and plasticizer concentrations also significantly affect the EA of the film.

The optimized conditions that have been considered had high tensile strength, low elongation. Low water absorption is chosen using numerical optimization as a combination of 50^{o}c Dry oven temperature, 35%plasticizer(GLY and SOR) and 50% starch concentration to obtain a good quality plastic film 17.9Mpa TS,9.89% EA and 19.45% WA of the film). The film produced by blending GIY and SOR enhances the physiochemical properties of the film.

Using the optimized plastic film, the thickness, solubility, moisture, density, and transparency were determined for three keratin starch plasticized with glycerol and sorbitol, keratin starch plasticized with glycerol, and keratin starch plasticized with sorbitol. The plasticized with glycerol and sorbitol indicates that a better bioplastic film with a better physicochemical property when compared to the other two. The TGA results show that the mass loss of among the three samples the film plasticized by sorbitol only is less than the other two. The film plasticized by blending GLY and SOR had an intermediate-mass loss. The best plastic film synthesized at the optimum operating condition was also characterized by FTIR show as the keratin and starch well interact chemically. In addition to this, all three samples show semi-crystalline properties when they characterize using XRD. Finally, the overall results showed that the chicken feather keratin and mango seed starch has a promising potential to be used in combination with a plasticizer (GLY and SOR) in bioplastic film synthesis for dry good packaging applications.

5.2 Recommendations

1. In this research, only the effect of three independent variables was studied by holding the other variables constant for the as-synthesized film. One can also investigate the other factors' effect like mixing time of keratin and starch, mixing ratio plasticizer (because in this research ratio of plasticizer is 1:1 ratio), mixing temperature of keratin and starch.
2. In this research, the techniques' used was a solvent casting method. In future work, I recommend using other methods such as extrusion, hot-pressing.
3. It is advisable to incorporate the antimicrobial inhibitor to enhance the shelf life of the good to be packed.
4. In the future research I recommend to do biodegradable teste for final biofilm.

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Appendices

Appendix A: supporting pictures during the study

A. collect feather

B. washing feather

C. Dry the feather by sun

D. Feather powder

D. Dissolving feather

E. Centrifuge

F. Adjacent the pH

G. Keratin powder

Figure A_1: keratin extraction/ sample pictures

Mango seed kernels

Dry kernels

Mango seed flour

Dissolving

Figure A_2 starch extraction

A.Mixing of keratin starch

B.Labeling pertdish

C. casting the mixture on petridish

D. oven drying

E.Bioplastic film

Figure A_4: Bioplastic film synthesis/ sample pictures (A-E)

Appendix B: design expert data

Table B_1: analysis of variance [Partial sum of squares], for elongation at the break

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	119.74	13.30	99.49	< 0.0001	significant
A-temperature	19.36	19.36	144.79	< 0.0001	
B-Gly/sor conc	0.4919	0.4919	3.68	0.0841	
C-starch conc	1.88	1.88	14.03	0.0038	
AB	0.1596	0.1596	1.19	0.3002	
AC	2.87	2.87	21.45	0.0009	
BC	1.02	1.02	7.59	0.0203	
A^2	15.41	15.41	115.23	< 0.0001	
B^2	84.52	84.52	632.01	< 0.0001	
C^2	0.4320	0.4320	3.23	0.1025	
Residual	1.34	0.1337			
Lack of Fit	0.3130	0.0626	0.3056	0.8904	not significant
Pure Error	1.02	0.2049			
Cor Total	121.08				

Table B_2: model adequacy measures for elongation at the break

Std. Dev.	0.3657	R^2	0.9890
Mean	12.83	Adjusted R^2	0.9790
C.V. %	2.85	Predicted R^2	0.9681
		Adeq Precision	28.2408

Table B_3: analysis of variance [partial sum of squares], for water absorption

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	131.72	14.64	105.05	< 0.0001	Significant
A-temperature	24.23	24.23	173.88	< 0.0001	
B-Gly/sor conc	0.6251	0.6251	4.49	0.0602	
C-starch conc	2.24	2.24	16.06	0.0025	
AB	0.0800	0.0800	0.5742	0.4661	
AC	2.49	2.49	17.85	0.0018	
BC	0.7938	0.7938	5.70	0.0382	
A^2	14.11	14.11	101.29	< 0.0001	
B^2	93.35	93.35	670.07	< 0.0001	
C^2	1.15	1.15	8.25	0.0166	
Residual	1.39	0.1393			
Lack of Fit	0.5779	0.1156	0.7088	0.6426	not significant
Pure Error	0.8153	0.1631			
Cor Total	133.12				

Table B_4: Model adequacy measures for water absorption

Std. Dev.	0.3733	R^2	0.9895
Mean	22.50	Adjusted R^2	0.9801
C.V. %	1.66	Predicted R^2	0.9578
		Adeq Precision	28.6385

Table B_5: Fit summary for TS (1), EA (2) and WA (3)

Response 1: tensile strength

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.3202	0.0004	0.0400	-0.2502	
2FI	0.9106	0.0002	-0.1354	-0.6954	
Quadratic	**< 0.0001**	**0.9869**	**0.9805**	**0.9793**	**Suggested**
Cubic	0.9675	0.7792	0.9700	0.9506	Aliased

Response 2: elongation

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.3533	0.0003	0.0256	-0.2746	
2FI	0.9055	0.0002	-0.1504	-0.7609	
Quadratic	**< 0.0001**	**0.8904**	**0.9790**	**0.9681**	**Suggested**
Cubic	0.7699	0.8930	0.9731	0.9804	Aliased

Response 3: water absorption

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.2899	0.0001	0.0541	-0.2321	
2FI	0.9331	< 0.0001	-0.1272	-0.7851	
Quadratic	**< 0.0001**	**0.6426**	**0.9801**	**0.9578**	**Suggested**
Cubic	0.5522	0.5106	0.9787	0.8558	Aliased

Table B_6: sample sequential Model sum of squares for TS(1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	4415.20	1	4415.20			
Linear vs Mean	22.90	3	7.63	1.26	0.3202	
2FI vs Linear	3.78	3	1.26	0.1762	0.9106	
Quadratic vs 2FI	**91.62**	**3**	**30.54**	**248.68**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.0953	4	0.0238	0.1262	0.9675	Aliased
Residual	1.13	6	0.1888			
Total	4534.73	20	226.74			

Table B_7: Lack of fit tests for EA

Source	Sum of Squares	Mean Square	F-value	p-value	
Mean vs Total	3291.41	3291.41			
Linear vs Mean	21.73	7.24	1.17	0.3533	
2FI vs Linear	4.04	1.35	0.1838	0.9055	
Quadratic vs 2FI	**93.97**	**31.32**	**234.23**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.3089	0.0772	0.4506	0.7699	Aliased
Residual	1.03	0.1714			
Total	3412.49	170.62			

Table B_8: solutions found for numerical optimization during plastic synthesis.

Number	temperature	Gly/sor conc	starch conc	TS	EA	WA	Desirability	
1	**49.949**	**34.607**	**49.949**	**17.76**	**8.98**	**19.448**	**0.97**	**Selected**
2	49.949	34.607	49.949	17.76	8.98	19.448	0.97	
3	49.949	34.607	49.949	17.76	8.98	19.448	0.97	
4	49.949	34.607	49.949	17.76	8.98	19.448	0.97	
5	49.949	34.607	49.949	17.76	8.98	19.449	0.97	

Appendix C: Regression model equations

Final equations in terms of the codded factor for keratin yield

$$\text{yield} = +72.43 + 6.62 * \text{A} + 5.18 * \text{B} - 4.86\text{A} * \text{B} - 9.32 * \text{A}^2$$

Final equation in terms of Actual factors

$$\text{yield} = -371.40850 + 12.81501? * \text{temperature} + 0.57220 * \text{time} - 8.09696\text{E} - 003 * \text{temperature} * \text{time} - 0.093175 * \text{temperature}^2$$

Final equations in terms of the actual factor for tensile strength (TS) elongation at break (EA) and water absorption (WA).

TA = +17.32+1.22* temperature-0.2074* Gly/sor conc-0.3811* starch conc+0.1187 temperature * Gly/sor conc+0.6312 temperature * starch conc-0.2438Gly/sor conc * starch conc-1.01 temperature^2-2.40 Gly/sor conc^2-0.2051starch conc^2

EA =+10.35-1.19*temperature-0.1898Gly/sor conc+0.033479 starch conc-0.1413 temperature * Gly/sor conc-0.5988 temperature * starch conc+0.3562 Gly/sor conc *

starch conc+1.03temperature²+2.42 Gly/sor conc²+0.1731 starch conc²

WA =+19.89-1.33 temperature+0.2139 Gly/sor conc+0.4047starch conc-0.1000 temperature* Gly/sor conc-0.5575temperature* starch conc+0.3150 Gly/sor conc* starch conc+0.9895temperature²+0.2.55 Gly/sor conc²+0.2824 starch conc²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Final equation in terms of the coded factor for tensile strength, elongation at the break and water absorption.

TA =+17.32+1.22 A-0.2074 B-0.3811 C+0.1187 AB+0.6312 AC-0.2438 BC-1.01 A²-2.40 B²-0.2051 C²

EA =+10.35-1.19 A+0.1898 B+0.3707 C-0.1413 AB-0.5988 AC+0.3562 BC+1.03 A²+2.42 B²+0.1731 C²

WA =+19.89-1.33 A+0.2139 B+0.4047 C-0.1000 AB-0.5575 AC+0.3150 BC+0.9895 A²+2.55 B²+0.2824 C²

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Appendix E: 2D interaction graphs of each factor on the three responses

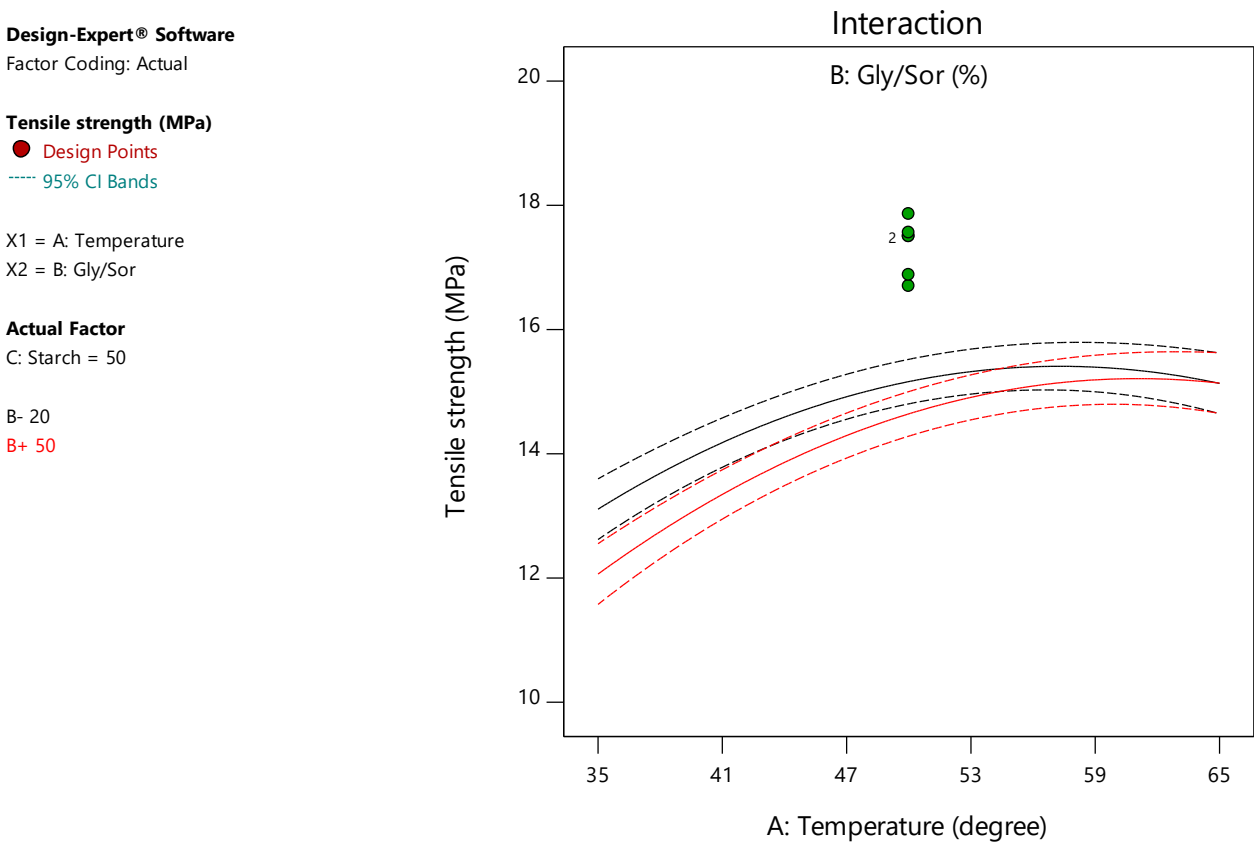


Figure E_1: The effect of dry oven temperature and GLY-SOR concentration on the tensile strength of the film.

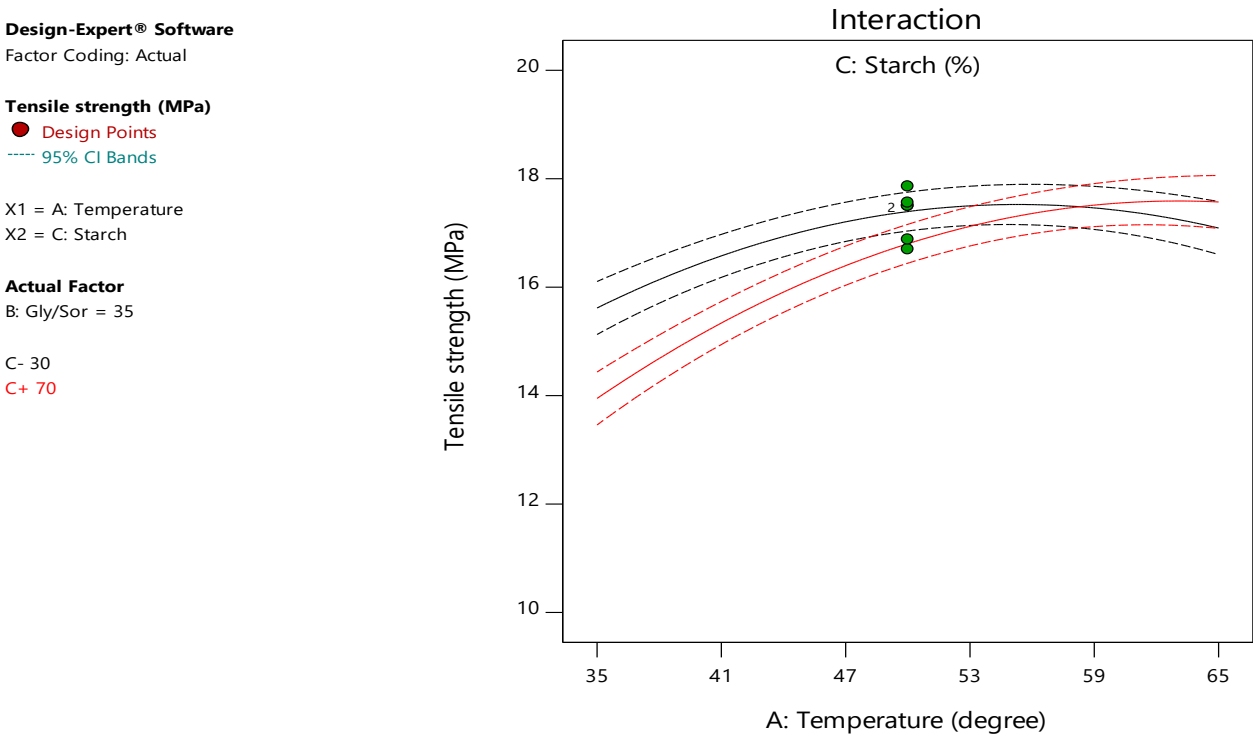


Figure E_3: the effect of dry oven temperature and starch concentration on the tensile strength of the film

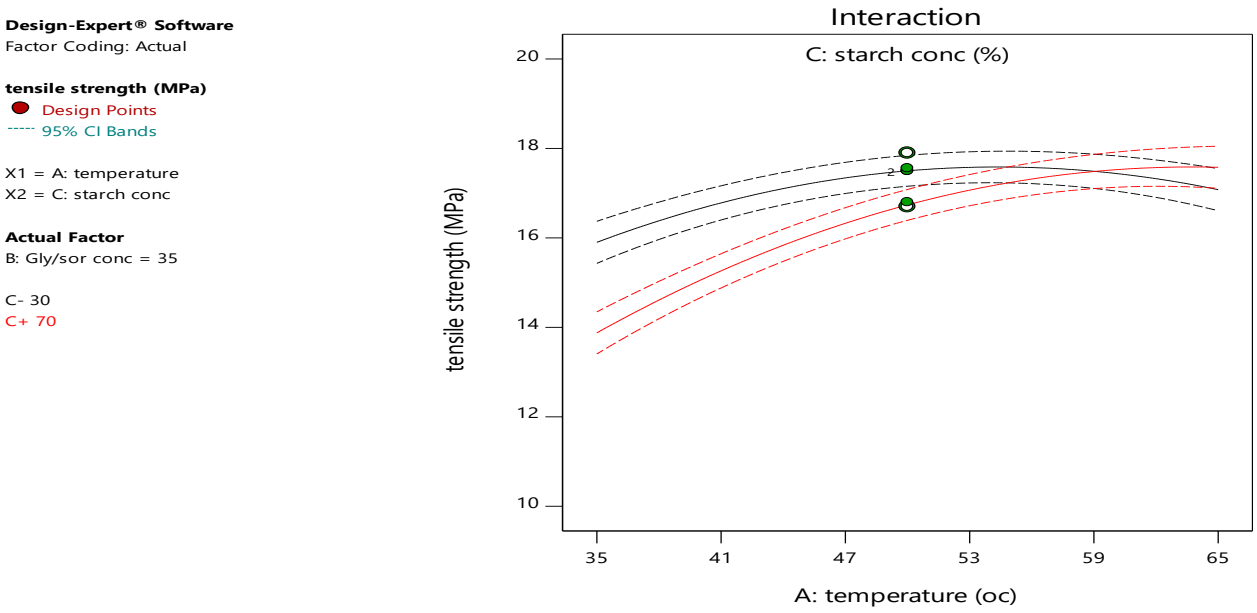


Figure E_4: The effect of dry oven temperature and starch concentration on elongation at the break of the film

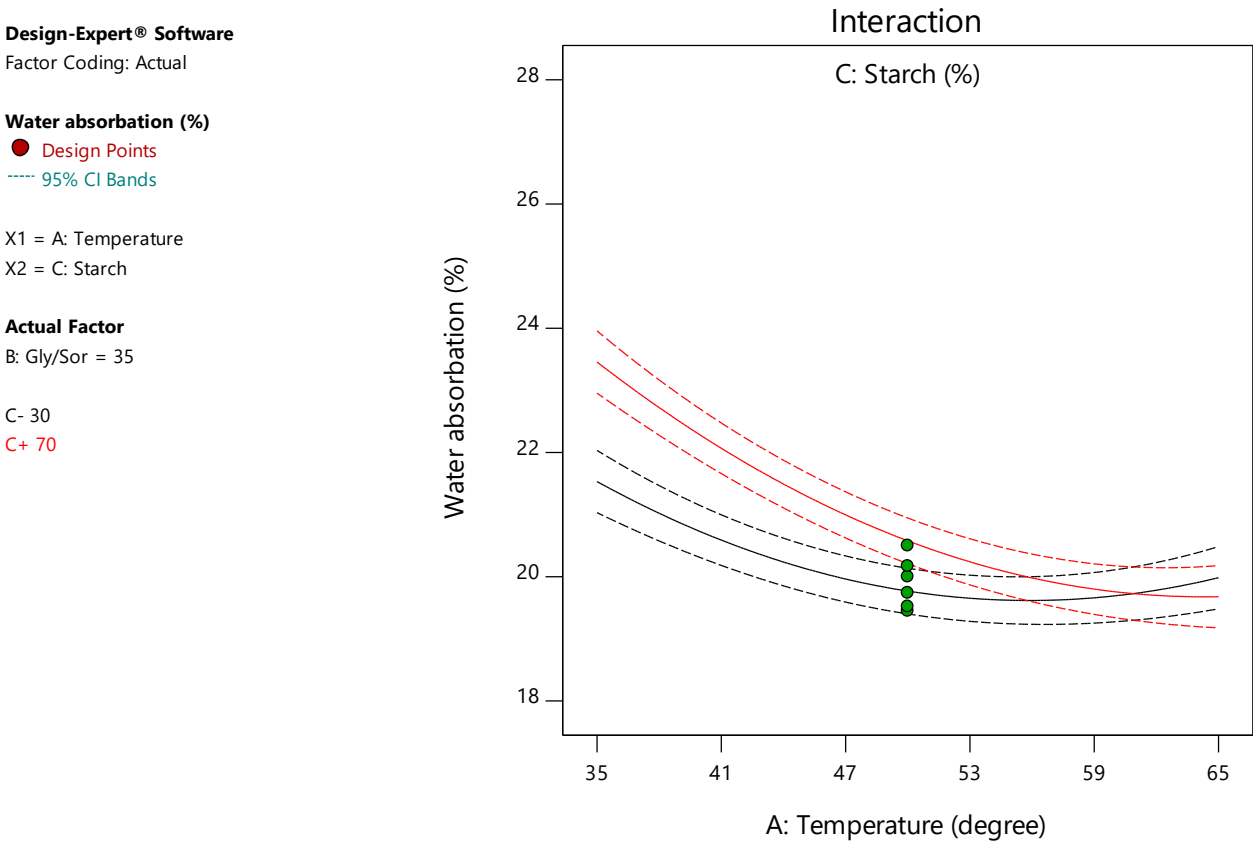
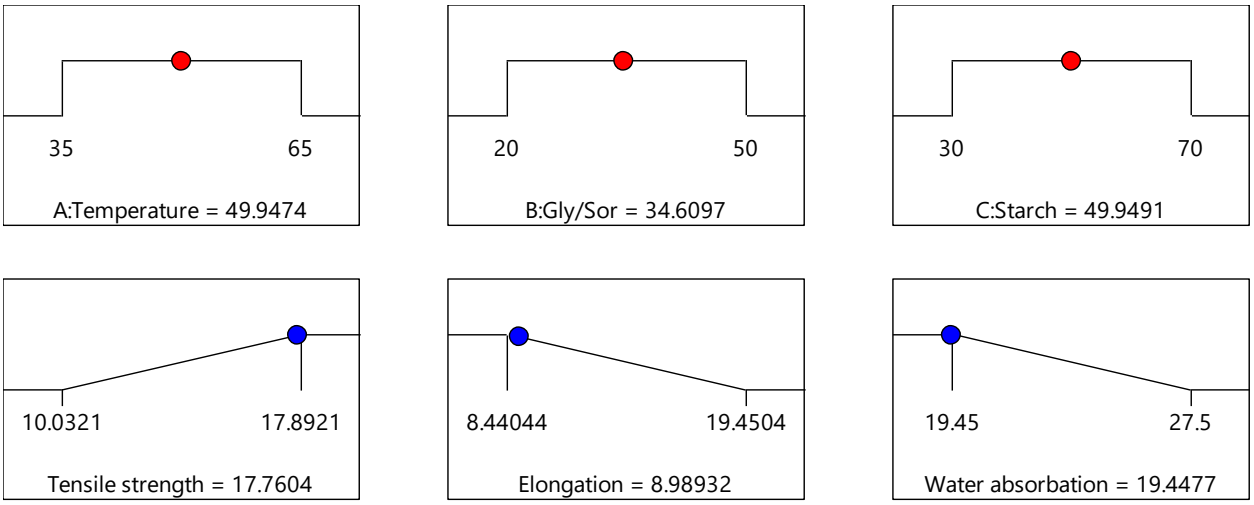


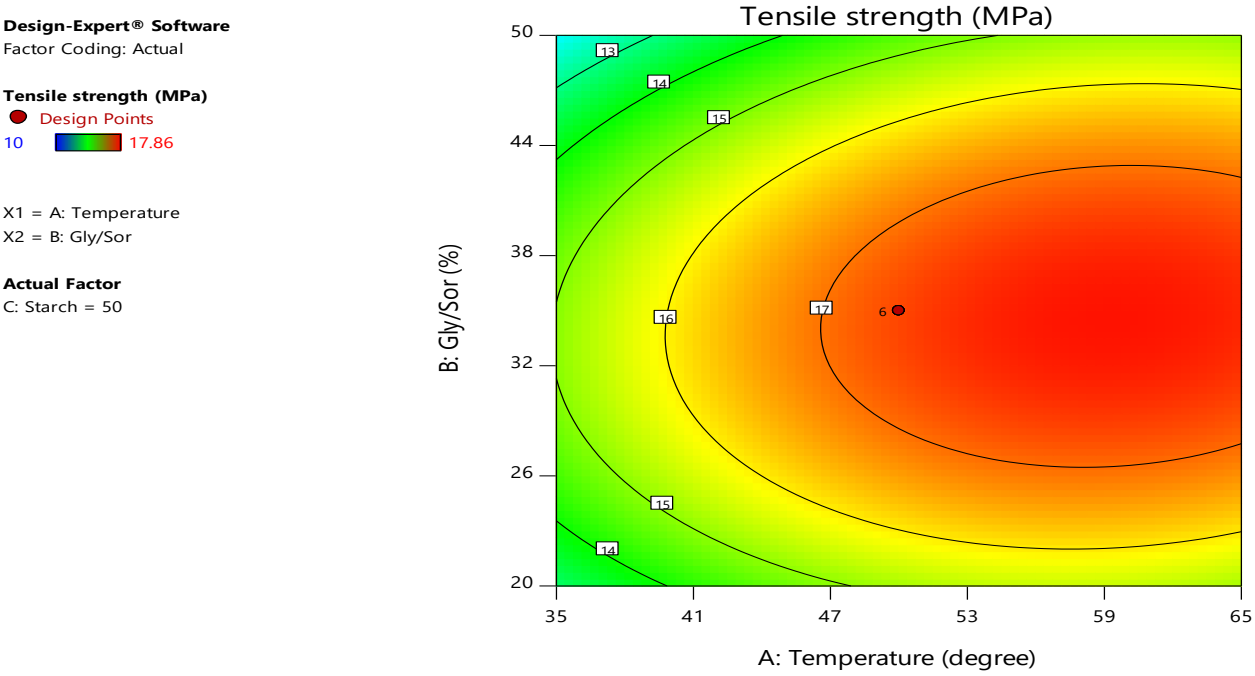
Figure E_5 the effect of dry oven temperature and starch concentration on water absorption of the film

Appendix F: Ramps numerical optimization

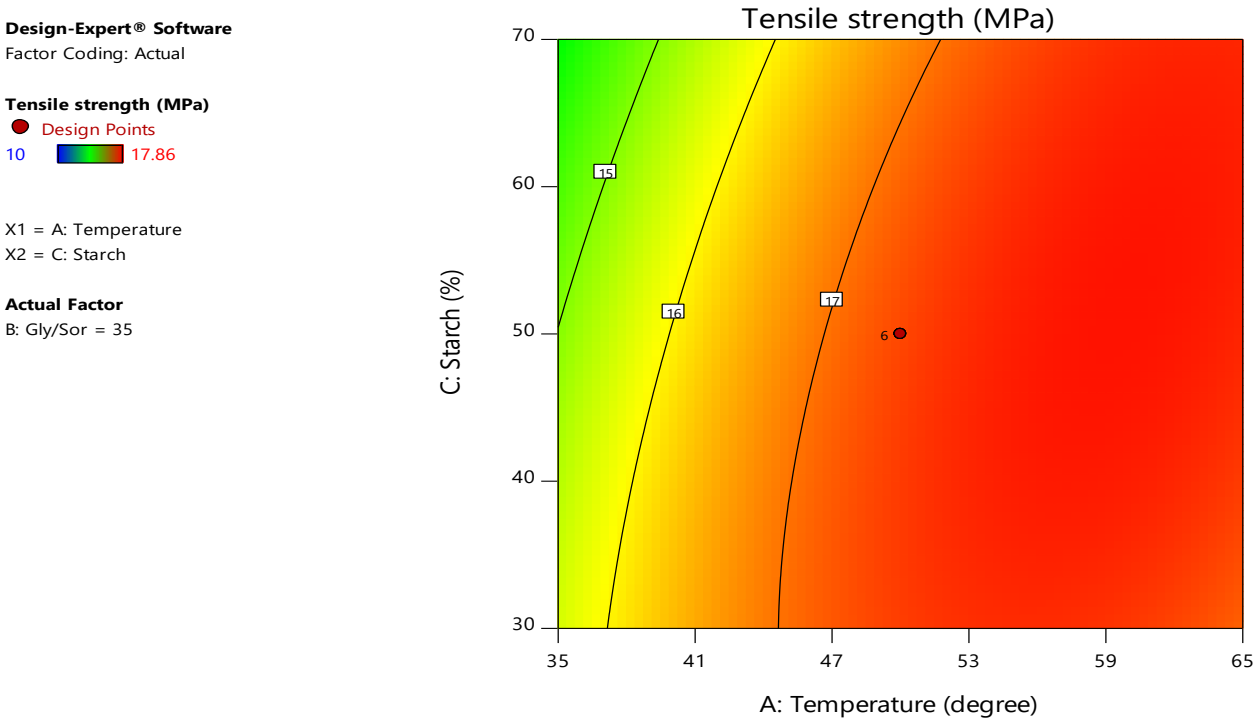


Desirability = 0.978
Solution 1 out of 6

Appendix g: sample contour plots for significant factors

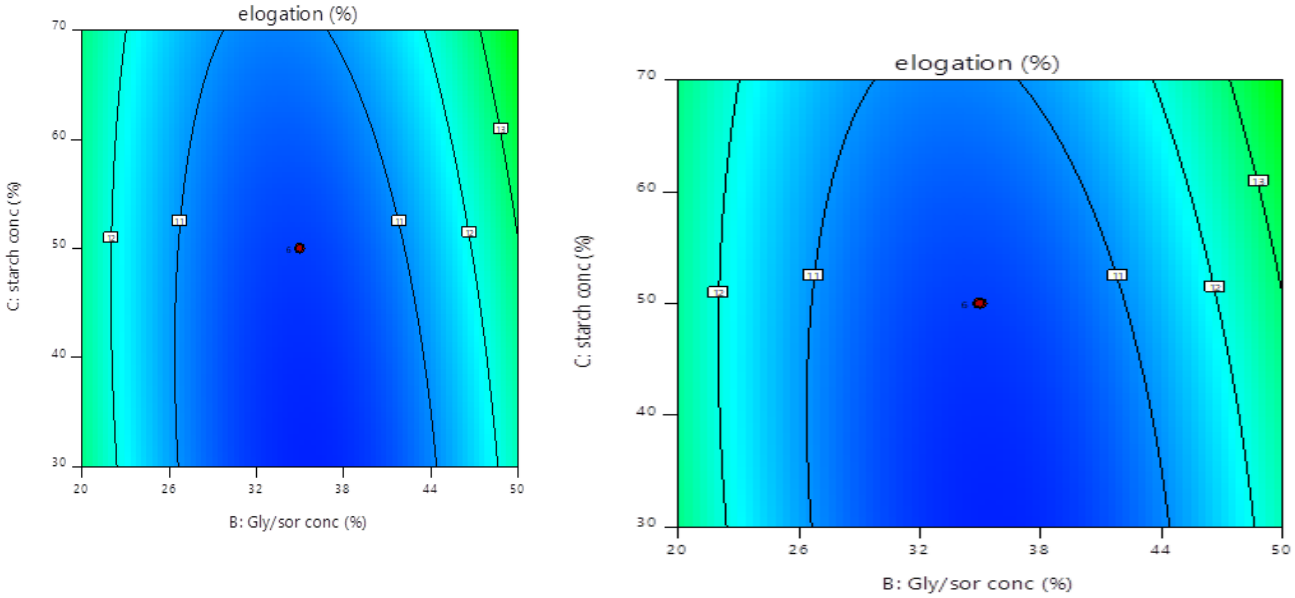


A



B

Figure g_1; interaction effect of drying temperature and GLY-SOR conc.on TS (A) and the effect of drying temperature and starch concentration on TS(B)



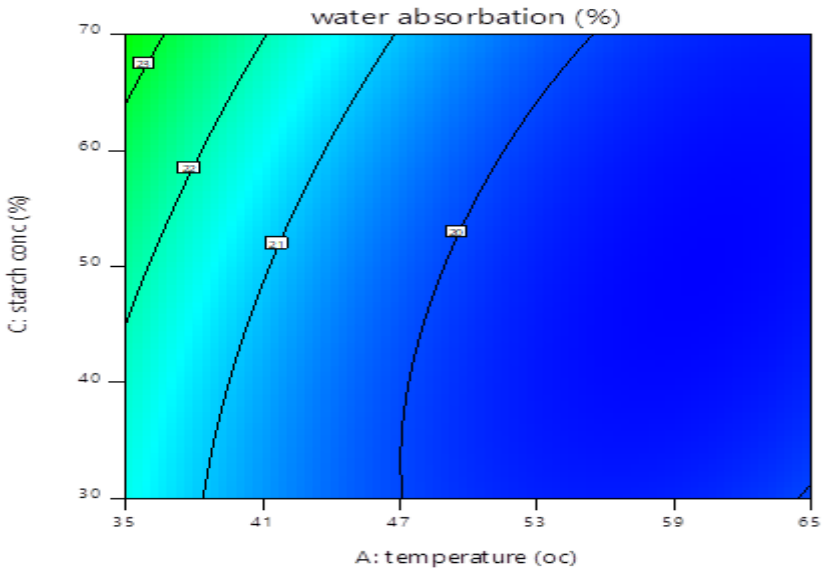
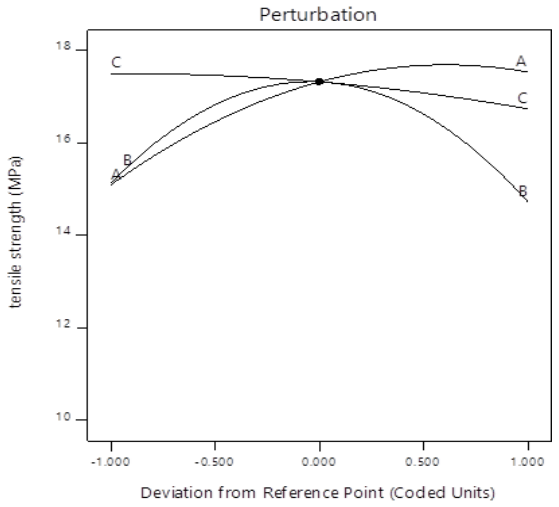
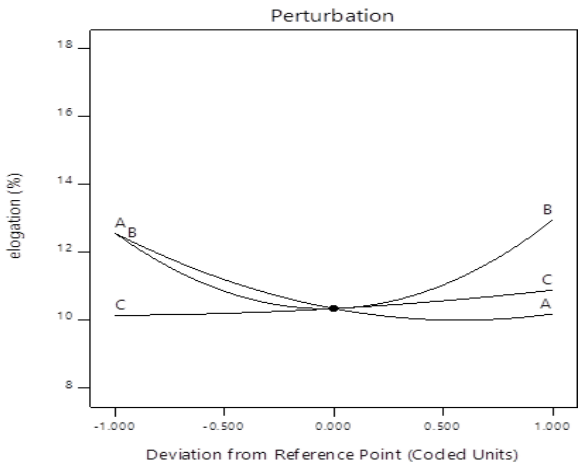


Figure K_2 effect of the dry oven with GLY-SOR and GLY-SOR with starch on EA and dry oven with starch on WA

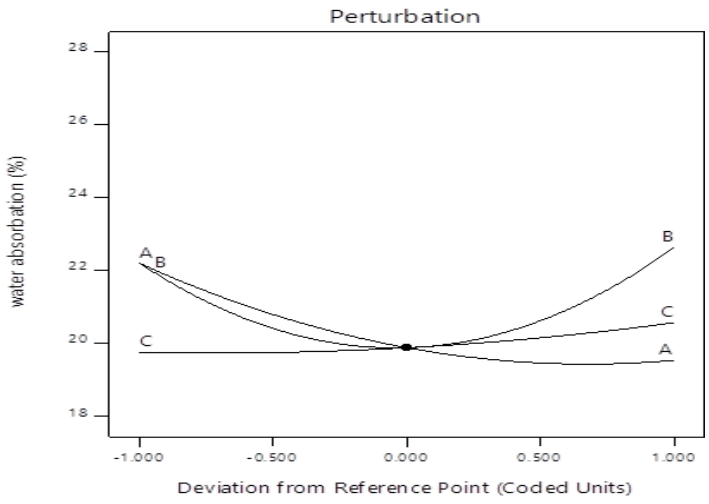
Appendix J, Perturbation graphs



A



B

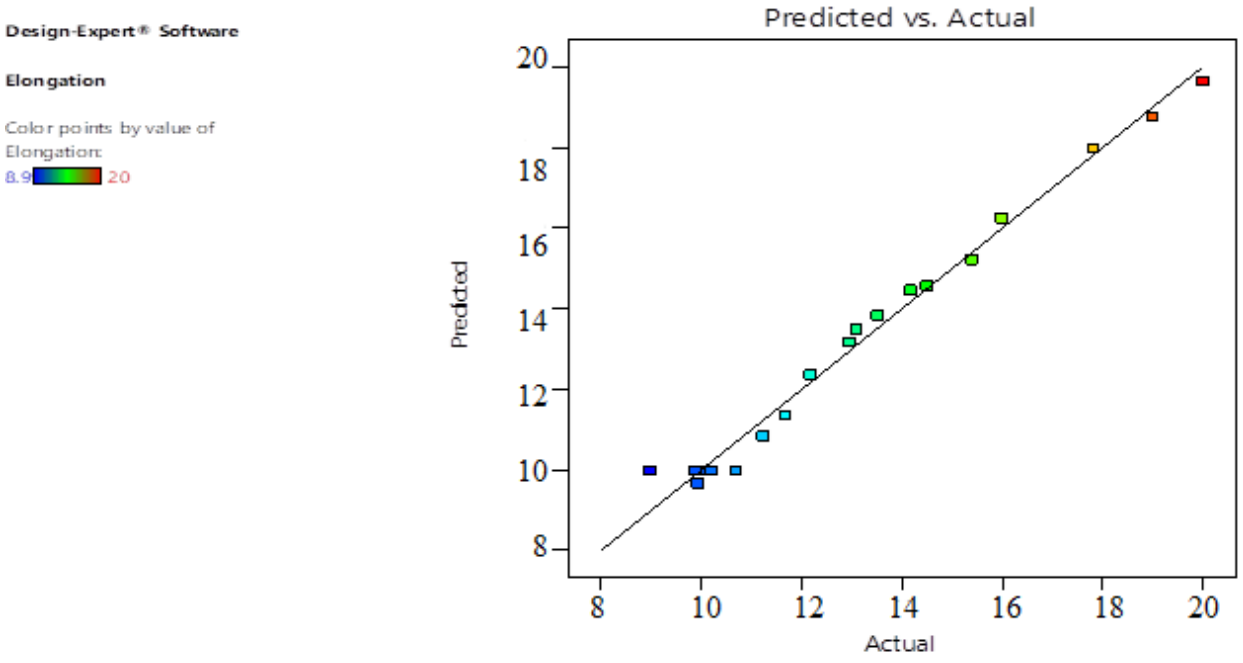


C

Figure H_1; Perturbation graphs for TS(A), EA(B), and WA(C)

Where: A=drying temperature, B=GLY-SOR and C= starch conc.

Appendix K, Predicated vs. Actual



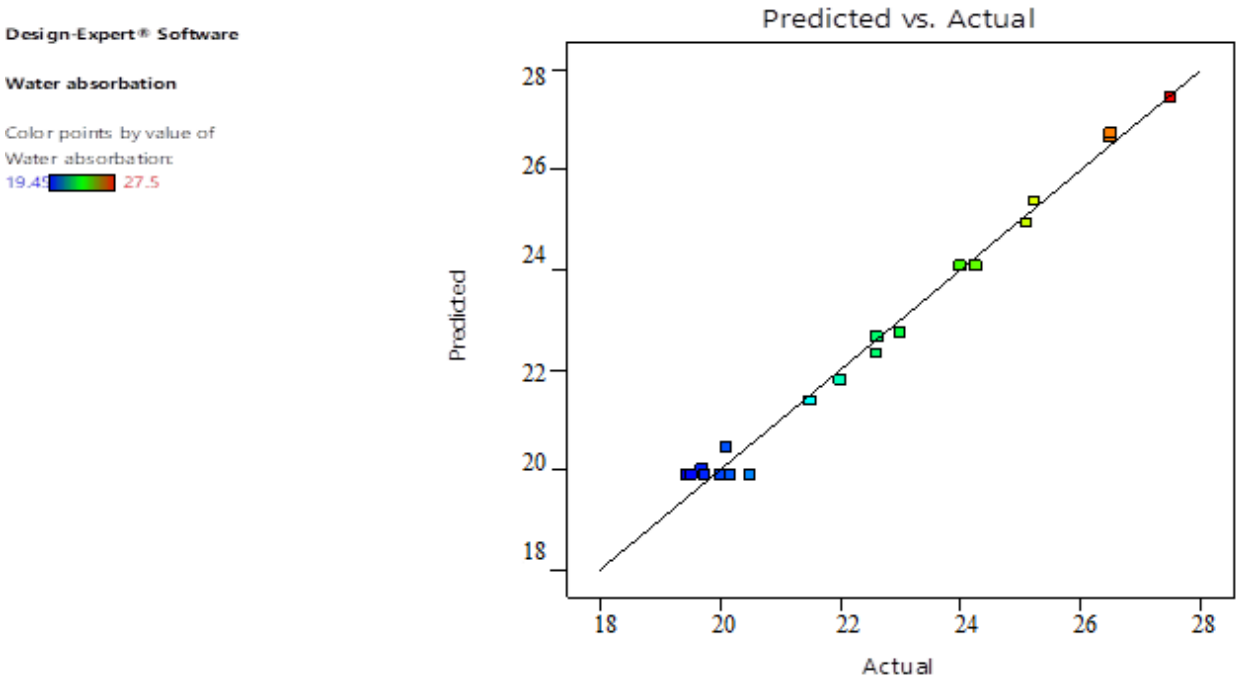
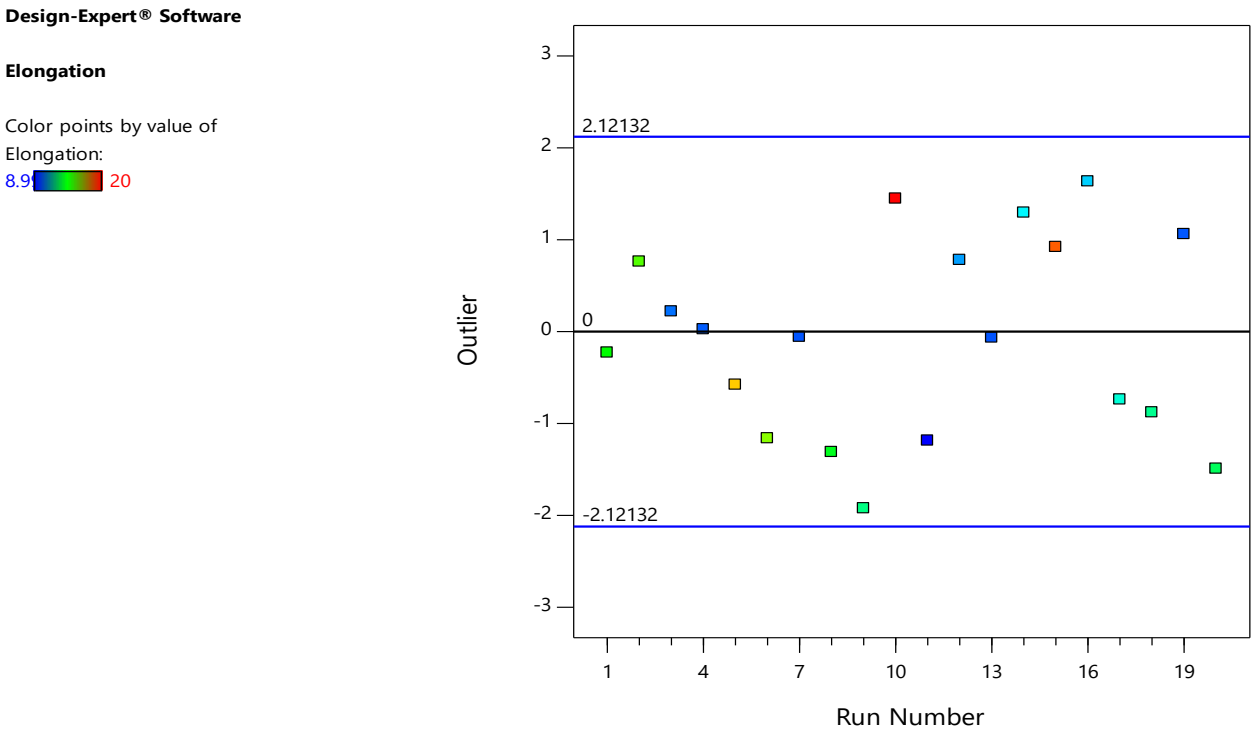


Figure I_1 predicated vs. actual graph of both elongation and water absorbation

Appendix L, Outiler T Run number



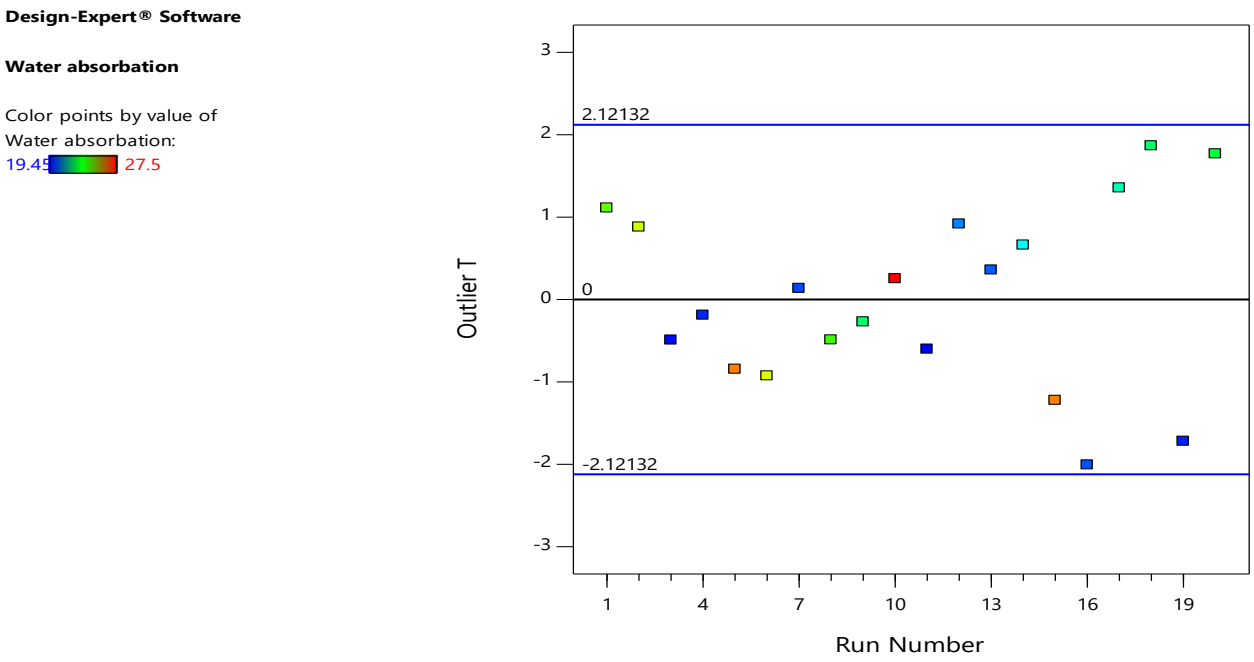


Figure M_1 Outlier T vs. Run number of both elongation and water absorbation