ADDIS ABABA UNIVERSITY

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SCHOOL OF GRADUATE STUDIES

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Preparation, Optimization and Characteristics of pharmaceutical Grade Corn Starch from local corn Using Acetylated Method

By Ameha Mikre

Advisor Dr. Ing. Belay Woldeyes

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A thesis submitted to school of graduate of Addis Ababa University in partial fulfillment of requirements for the degree of Masters of Science in Chemical Engineering (Process Engineering)

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This is to certify that the thesis prepared by Ameha Mikre, entitled Preparation, Optimization and Characteristics of pharmaceutical Grade Corn Starch from local corn Using Acetylated Method submitted in partial fulfillment of the requirements for the Degree of Master of Science in Process Engineering complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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ACRONOMYS

AAiT	Addis Ababa Institute of Technology
AC	Acetylated
AM	Amylos
ANOVA	Analysis of variance
AP	Amylopectin
BMIC	1-butyl-3- methylimidazolium chloride
BMIC	1-butyl-3- methylimidazolium chloride
BP	British pharmacopeia
CTA	3-chloro-2-hydroxypropyltrimethyl ammonium chloride
DMSO	Dimethyl sulphoxide
DS	Degree of substitution
EC	Ethiopian Calendar
ECH	Epichlorohydrin
EP	European pharmacopeia
ETA	2,3-epoxypropyltrimethylammonium chloride
FAME	Vinyl esters, fatty acid chlorides, carboxylic acids
FAO	Food and Agricultural Organization
ILs	Ionic liquids
INFPRI	International Food Policy Research Institute
$POCl_3$	Phosphoryl chloride

SP	Swelling power
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
USP	United State pharmacopeia

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Abstract

Corn starch was extracted from local corn (Melkasa-2) obtained from Melkasa Agricultural Research Center. And this extracted corn starch synthesized by the reaction of corn starch with acetic anhydride in the presence of acetic acid under varying reaction temperature and time. The result was with different value of degree of substitution (minimum DS= 0.26 and maximum DS=2.38) and different value acetyl percent (minimum Ac=6.45 and maximum Ac=43.29). The effects of temperature and time on acetylated modified corn starch were that as time and temperature increase the acetyl percentage and degree of substitute also increase. The Infrared spectrophotometer was demonstrated that the spectra of acetylated modified corn starch and the standard corn starch (commercially available pharmaceutical grade) had similar chromatogram or finger print. The swelling power and solubility of acetylated modified corn starch was higher than that of native starch. The moisture content (5.78%) was also in the range of commercially available(NMT 15%) and also other physicochemical property like sulphated ash (0.198%), bulk density of normal (0.725) and acetylated modified (0.726) corn starch was not significant, and also the water binding capacity (WBC) g/g of native starch is higher than of acetylated modified corn starches. All the above chemical and physicochemical property of acetylated modified corn starch parameter fulfilled the criteria of commercially available pharmaceutical grade starch. From the above result it is conclude that its possible to prepare pharmaceutical acetylated corn starch.

Key words: Acetylated modified corn starch

Chapter 1

1. Introduction

1.1 Background

Background

Corn (*Zea mays* L.) is the third important cereal crop globally after wheat and rice [FAO, 2011]. The crop occupies a pivotal role in the world economy and is traded widely. Corn demand is projected to increase by 50% worldwide and by 93% in sub-Saharan Africa between 1995 and 2020 [FAO, 2007]. In the past, much of the global use of corn has been for animal feed. However, corn is increasingly used for human consumption and accounts for 70% of the food consumed in sub-Saharan Africa [FAO, 2007]. The recent volatile food market and rising prices for most food crops may increase the importance of corn production. In addition, because of its productivity and wide adaptation, corn remains an important source of food with great potential to improve the livelihoods of most poor famers in developing countries [FAO, 2011].

Corn is the largest and most productive crop in Ethiopia. In 2007/08, corn production was 4.2 million tons, 40 percent higher than teff, 56 percent higher than sorghum, and 75 percent higher than wheat production. With an average yield of 1.74 tons per hectare (equal to 3.2 million tons grown over 1.8 million hectares) from 1995 to 2008, corn has been the leading cereal crop in Ethiopia since the mid-1990s in terms of both crop yield and production. Wheat and sorghum yields have averaged 1.39 and 1.36 tons per hectare, respectively [INFPRI ifpri@cgiar.org].

In addition to the highest total production per annum and the highest per-hectare yield, corn is also the single most important crop in terms of number of farmers engaged in cultivation. The vast majority of Ethiopian farmers are small-scale producers – estimates show about 94 percent of Ethiopian farmers rely on less than 5 hectares of land, of which 55 percent cultivate less than 2 hectares. Eight million smallholders were involved in maize production during 2008/09 production season, compared to 5.8 million for teff and 4.5 million for sorghum, the second and third most cultivated crops in Ethiopia [INFPRI ifpri@cgiar.org].

If Ethiopia had this much amount of resource annually. Why the country imports pharmaceutical grade corn starch? And also last year in our company (East African Pharmaceutical PLC) the veterinary line was closed for three months due to the shortages of this starch. This resource and the problem we face in our company initiates me to prepare pharmaceutical grade corn starch from local corn.

In this research, preparation of pharmaceutical grade corn starch using acetylation method is through the reaction of corn starch with acetic anhydride in the presences of glacial acetic acid and methanesulfonic (methyl sulfonic acid). Modification of starch is carried out to enhance the positive attributes and to eliminate the shortcomings of the native starches.

1.2 Statement of the problem

Due to the very high growth in the population and economy of the country finding an alternative source for producing valuable products becomes crucial especially for materials that are easily produced such as starch. In Ethiopia there are no any large scale production of starch and modified starch processing industry. According to Central Statistical Agency of Ethiopian 106,350.1 tons of modified maize starch was imported from China and India in the year 2004 EC at around 1,383,565.4 birr (79,000 dollar). The consumption of starch in developing country like Ethiopia is expected to increase. Due to government transition policy plan from Agricultural to Industry, it is estimated a 50% increase in starch consumption will occur in the coming some year. The expected increase in the growth of additional industries (such as textile, paper, food, and pharmaceutical) that are using modified corn starch will increase marketing demand [Ethiopia Custom Office Statistical centre database file system. Addis Ababa, Ethiopia.2004 EC].

Operating conditions such as temperature, reaction time, pH and different concentration of solvent have effect on the degree of substitution (DS), acetyl (%) and quality of modified corn starch or yield (%). So this thesis aim is to find optimal operating conditions that will maximize the DS and yield for the production of modified acetylated corn starch from known verity (Melkasa 2) of corn.

1.3 Objectives

1.3.1 General objective

To synthesized pharmaceutical grade corn starch from local corn and estimation of starch production plant in Ethiopia.

1.3.2 Specific objectives

The specific objective of this study includes the following:

- Extraction of normal corn starch.
- To modify the extracted corn starch using acetylating method.
- Characterization and Physicochemical property of native and modified starch
- To study Characteristics of modified corn starch
- To compare the physico-chemical properties of the acetylated modified corn starch with that of commercially available modified corn starch (pharmaceutical).
- Estimation of starch production plant.

Chapter 2

2.Literature review

The corn plant (*Zea mays*) is a high-capacity, factory for efficiently converting large amounts of radiant energy from the sun into stable chemical energy. This energy is stored as cellulose, oil and starch in the corn plant and in the corn kernel. The corn plant is also one of nature's greatest multipliers. Approximately four months after planting it reached to use to produced starch. By careful genetic control, corn has been developed which can grow in the temperate and semi-tropical areas throughout the world.

2.1 The different kinds of corns

Corns, also named maize, is a cereal grain and is known for being a high-yielding variety of cereal grains. There are multiple types of maize which are used for various purposes like [International starch institute, science park Aarhus, Denmark 2006].

Corn, Zea Mays, is grown in most countries throughout the world. It requires, however, warmer climates than found in the temperate zones to grow to maturity.

Dent corn, the scientific name of which **Zea**, **Mays Indentata** is called “field” corn. It is a corn variety with kernels that contain both hard and soft starch and become indented at maturity. It is major crop used to make food, animal feed, and industrial products. This is the variety to be considered for corn starch manufacturing.

Flint corn, known by the scientific name Zea Mays indurata, is a variety of corn having hard, homy, rounded, or short and flat kernels with the soft and starchy endosperm completely enclosed enclosed by a hard outer layer. It is similar to dent and is used for the same purposes. Most of it is grown in South America.

Waxy corn is a corn variety with grains that have a waxy appearance when cut, and that contains only branched-chain starch. It is grown to make special starches for thickening foods.

Sweets or "green" corn is eaten fresh, canned, or frozen. It is a type of corn that is grown in many horticultural varieties. It is occasionally considered a distinct species (Zea saccharata or

Zea rugosa), a subspecies (Zea Mays rugosa), or a specific mutation of dent corn. It is distinguished by kernels containing a high percentage of sugar in the milk stage when they are suitable for table use.

Popcorn is a variety of corn, Zea Mays everta, which has small ears and small pointed or rounded kernels with very hard corneous endosperm that, on exposure to dry heat, are popped or everted by the expulsion of the contained moisture, and form a white starchy mass many times the size of the original kernel.

Indian corn has white, red, purple, brown, or multicoloured kernels. It was the original corn grown by the Indians, and is known by the scientific name Zea Mays. It is many times seen in harvest time and Halloween decorations.

Flour corn, also called "soft" corn or "squaw" corn, has kernels shaped like those of flint corn and is composed almost entirely of soft starch. It is known by the scientific name Zea Mays amylacea. The USA grows small amounts of blue flour corn to make tortillas, chips, and baked goods. In South America this corn is grown in various colours to make food and beer (Aarhus, Denmark, 2006).

2.2 Corn producer in the world and Africa

The United States of America ranks as the world's largest grower of corn with 392.25 million tones annually. From this total annual production, around 40% of corn is processed in the USA at industry level to produce starch and different maize sweeteners. Corn is widely used in the production of animal feed, organic fertilizers, different household utensils, adhesives, textile manufacturing, cosmetics, and pharmaceuticals and as a component of many food products [Corn Refiners Association, 2006]

Ethiopia is among the major corn producers in Africa and ranked fourth next to South Africa, Nigeria and Egypt. The crop is widely cultivated in Ethiopia at altitudes ranging from 1500–2200 meters above sea level of Western, Southwestern, and Southern parts of the country. Corn production takes significant share of cereals and grain in any production year. In the past five years from 2003/04 - 2007/08 maize productions averagely reached 3,160,022.3 metric tones.

This recent progress is a result of increase in both yield and area cultivation. Three regional states including Oromia, Amhara and SNNP contribute to 94% of the total annual production. Oromia region alone contributes to 60% of the country's corn production. West Gojam, East Showa, Jimma, East welega and West welega zones are major producing areas and together contribute to 60% of total production.

2.3 Starch

Starch is, after cellulose, one of the most abundant polymers found on earth. It is a major component of crops and an important raw material for food industrial processes. Starch serve as a kind of energy storage for plant and is mostly found in seeds and roots in the form of granules. It consists of two main components of amylose and amylopectin. These glucose units are linked together by α-(1,4) or α-(1,6) glycosides bonds to form one huge molecule of starch. In general, common starches comprise of 15%-25% of amylose and 75%- 85% of amylopectin. These two fractions occur in different proportions in starches from different sources and contribute to their differece in physical and chemical properties. In a case of special breed, or waxy starches no amylose or a very low amount of amylose is present. Modification of starch is carried out to enhance the positive attributes and to eliminate the shortcomings of the native starches [Corn Refiners Association, 2006].

The industrial utilization of native starches is limited because of inherent imperfect nature, such as water insolubility and theirtendency to easily retrograde and undergo syneresis and therefore form unstable pastes and gels. Starch modification does not only decrease retrogradation, gelling tendencies of pastes and gel syneresis but also improves paste clarity and sheen, paste, and gel texture, film formation and adhesion.

Another aim of starch modification is to stabilize starch granules during processing and make starch suitable for many food and industrial applications. The functionality of starch can be modified through physical, chemical, enzymatic, biotechnological, or their combinations.

2.4 Production of starch

Corn kernels have three main parts: the seed coat or pericarp, the starchy endosperm, and the embryo, commonly called the germ. The pericarp is the outer skin or hull of the kernel which serves to protect the seed. The endosperm, the main energy reserve, makes up about 80% of the total weight of the kernel. It is about 90% starch and 7% gluten protein, with the remainder consisting of small amounts of oil, minerals and trace constituents. The embryonic germ contains a miniature plant made up of a root-like portion and five or six embryonic leaves. In addition, large quantities of high energy oil are present to feed the tiny plant when it starts to grow, as along with many substances required during germination and early development [Corn Refiners Association, 2006].

2.4.1 Starch production process from corn

The corn wet milling process is illustrated in Figure 2.1, in which the kernel is separated into its component parts, and those parts are then further subdivided, processed and steeps as follow.

Cleaning

The raw material for wet milling is shelled dent corn delivered in bulk. The corn is inspected and cob, dust, chaff and foreign material are removed. The cleaning is normally done twice before wet processing. After cleaning the corn is transported to the steeps.

Steeping

A proper steeping is essential for high yields and high starch quality. The steeping is carried out in a continuous counter-current process. The cleaned corn is filled into a battery of large steeping tanks (steeps), where the corn is soaked in hot water 30 to 48 hours to begin breaking the starch and protein bonds. The gluten bonds within the corn begin to loosen and release the starch. The steeping is actually a controlled fermentation. Sulphur dioxide improves the fermentation by enhancing growth of favourable micro-organisms, preferably lactobacillus, while suppressing detrimental bacteria, moulds, fungi and yeast. Solubles are extracted and the kernel softens. The

2.1 Flow charts

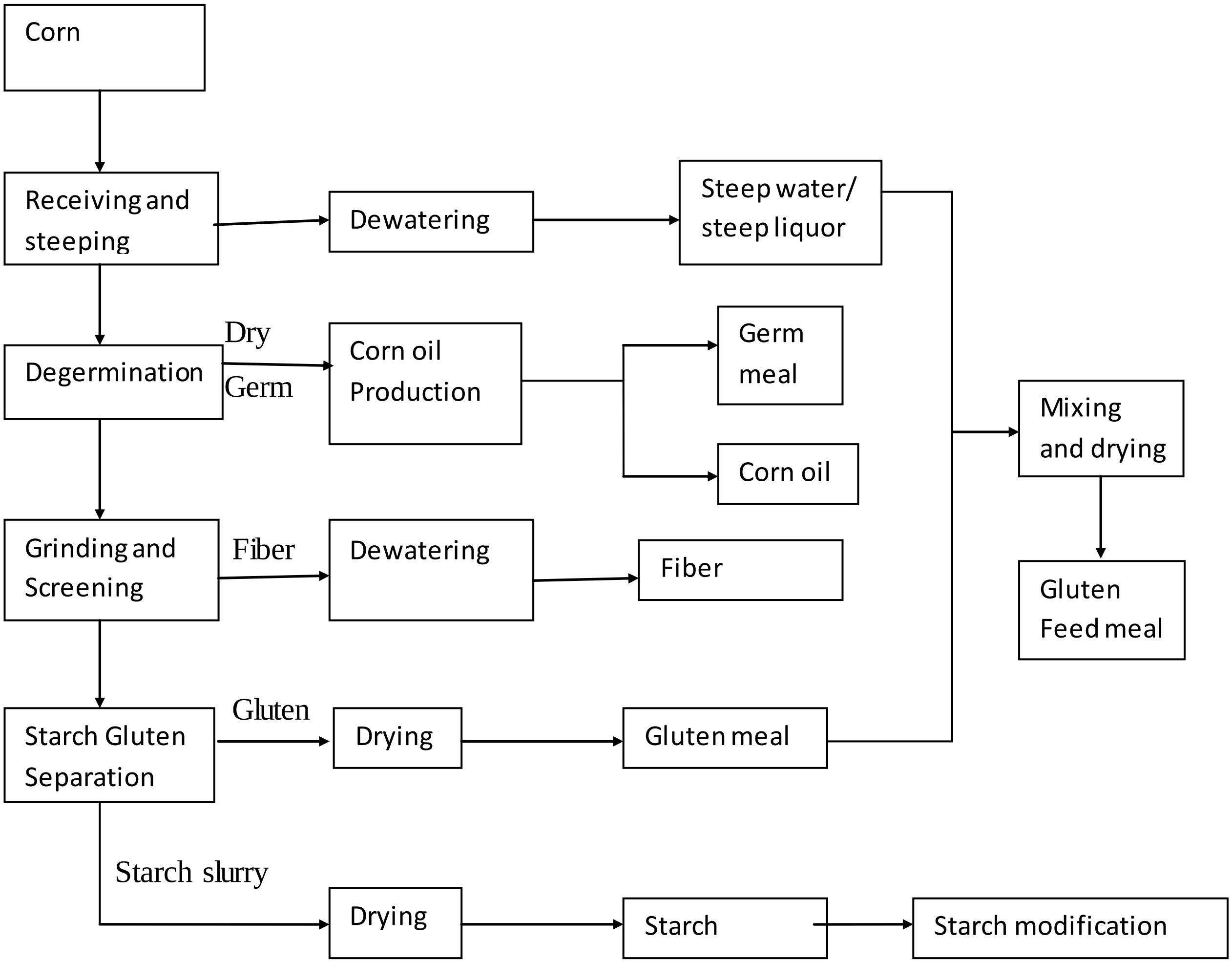


Figure 2.1 Flowchart of starch production process from corn

kernel swells to more than double its size and increases its moisture content from about 15% to 45%.

Steep water evaporation

The steep water containing approximately 10% dry substance is drained from the kernels and condensed on a multi-stage evaporator. Most organic acids formed during the fermentation are volatile and evaporate with the water. The condensate from the first evaporator stage will therefore be discharged after the heat is recovered by preheating the entering steepwater. The steepwater is condensed to an auto-sterile product - a valuable nutrient in the fermentation industry - or concentrated to approximate 48% dry matter and mixed and dried with the fibre fraction.

Germ separation

The soften kernels are broken up in attrition mills to loosen the hull and break the bonds between germ and endosperm. Water is added to assist the wet milling. A good steeping ensures that the germ will be freely released intact from the kernel by a gentle milling operation with no free oil. Oil constitutes half the weight of the germ at this stage and the germ is easy to separate bcentrifugal force. The lightweight germs are separated from the ground slurry by hydrocyclones in a two step separation with regrinding in between. The germs are washed repeatedly counter-currently on a three-stage screen to remove starch. Process wash water is added at the last stage.

Germ drying

Surface water is removed from germs by a tapered screw press. The dewatered and clean germs are fed to a rotary steam tube bundle dryer and dried to approximate 4% moisture. Low moisture content improves shelf life. The germs are cooled and pneumatically transported to a germ silo ready for bagging or oil extraction.

Corn oil

Mechanical presses and/or solvent extraction are used to extract the crude oil from the germ. The crude oil is refined and filtered. A typical yield per ton corn is 27 kg corn oil. During refining free fatty acids and phospholipids are removed. The finished corn oil finds uses as food and cooking oil or as raw material for margarine. Refined corn oil has a pleasant taste and does not develop off-flavours in cooking and frying. The high content of polyunsaturated fats is a nutritional advantage. The residual extracted corn germ meal is used in animal feeds or combined with the gluten feed.

Fine grinding and screening

After germ separation the mill flow is finely ground in impact or attrition mills to release starch and gluten from the endosperm cell walls (fibers). The degerminated mill starch leaving the fine mill is pumped to the first stage of a fibre washing system, where starch and gluten is screened off. The overs, hull and larger fibres, are washed free from adhering starch and gluten (insoluble protein) on screens in counter-current with process wash water added at the last stage. The last fibre washing stage has a slightly courser screen for pre-dewatering the fibre prior to a tapered screw press.

Fiber drying

The dewatered fibers from the dewatering press may be mixed with concentrated steep water and cakes from the oil press and dried to approximate 12% moisture. The dried fiber are pelletized to reduce bulkiness and pneumatically transported to silo ready for shipping. The fiber fraction is a valuable constituent of animal feeds.

Primary separation

Crude starch milk from the dewatering screen ahead of the fine mill and from the first stage fiber washing is combined. The crude starch milk contains starch, gluten and soluble. A primary separator splits by gravity the mill stream in two fractions:

- Gluten Overflow
- Starch Underflow

A mill stream thickener may be applied on the separator feed.

Gluten recovery

The gluten fraction from the primary separator is traditionally concentrated on a nozzle type continuous centrifugal separator- a gluten thickener.

The gluten separator splits the gluten fraction in two streams:

- Process water Overflow
- Gluten Underflow

Multi-stage hydrocyclone unit

The underflow, which is mainly protein and a small amount of starch, is discharge to the gluten dewatering section.

Gluten dewatering

The gluten slurry is dewatered on a vacuum belt filter or decanter. The decanter removes more water, but requires strict pH-adjustment to the iso-electrical point of the gluten. Dewatering splits the gluten stream in:

- Process water
- Gluten (moist)

The dewatered gluten is dried in a rotary steam tube bundle dryer to approximately 10% moisture and disintegrated in a hammer mill. Drying is facilitated by powder recycling. The dried gluten is sold as corn gluten meal with 60% protein. It is a valuable source of methionine. The high xanthophyll content - typically 500 ppm - makes it an efficient pigmenting ingredient in poultry feeds.

Starch refining

Washing with fresh clean water refines the crude starch milk. With hydrocyclones it is feasible to reduce fibre and solubles including soluble protein to low levels with a minimum of fresh water. To save water the wash is done counter currently, i.e. the incoming fresh water is used on the very last step and the overflow is reused for dilution on the previous step, and so on. By using multi stage hydrocyclones all soluble materials and fine cell residues are removed in a water saving process. The refined starch milk contains an almost 100% pure starch slurred in pure water.With a middling separator the overflow from the starch refining hydrocyclones may be refined into:

- Process water Overflow
- Starch Underflow

In the strong gravitational fields of a hydrocyclones and a centrifuge, the starch settles quickly. Refining is based on the differences in weight density between water, fibres and starch: Although some impurities go with the starch in the underflow, there is - by means of a sieve - a last chance to remove the larger particles. Impurities not removed this way are not removable by any known technique.

Centrifuges and hydrocyclone

The centrifuge obtains its gravitational force by spinning the bowl. The hydrocyclone has no moving parts and the separation is totally dependent on the pressure difference over the cyclone. The technological development and quality demands have forced centrifuges to give way for hydrocyclones in corn starch refining, but centrifuges still hold a defensive position in gluten concentration. Starch is among the most pure of all agricultural products. Actually, purity is the most important parameter in being competitive.

Starch dewatering

The purified starch milk is discharged to a peeler centrifuge for dewatering. The peeler filtrate is recycled to starch refining. The dewatered starch is batch-wise peeled off and discharged by gravity to the moist starch hopper.

Starch Drying

From the moist starch hopper the starch is fed by a metering screw conveyor into a flash dryer and dried in hot air. The inlet air temperature is moderate. The dried starch is pneumatically transported to a starch silo ready for screening and bagging. The moisture of cornstarch after drying is normally 12-13 %. Before delivery the starch is screened on a fine sieve in order to remove any scale formed in screw conveyors etc. [International Starch Institute, Science Park Aarhus, Denmark 2006].

2.5 Modification of starches

Starch modifications are means of altering the structure and affecting the hydrogen bonding in a controllable manner to enhance and extend their application. The alterations take place at the molecular level, with little or no change taking place in the superficial appearance of the granule [Kaur *et al.*, 2012], the utilization of starch in its native form is often limited by some undesirable characteristics such as poor solubility, low mechanical properties, and instability at high temperature and pH during processing [Xie *et al.*, 2011].

Native starches when cooked can easily retrograde and there is a gelling tendency of pastes besides easily undergoing syneresis. Therefore starch modification not only decreases retrogradation, gelling tendencies of pastes and gel syneresis but also improves paste clarity and sheen, paste and gel texture, film formation and adhesion. Modification of starches has brought about an evolution of new processing technologies and market trends. These highly functional derivatives have been tailored to create competitive advantage in a new product, improve product aesthetics, simplify product proclamation, lower recipe/ production costs, increase product all through, eliminate batch rejects, ensure product consistency and extend shelf-life whilst clearly making starch relevant in all stages of a product's lifecycle. Basically there are four broad based kinds of modifications: chemical, physical, enzymatical and genetical [Kaur *et al.*, 2012].

2.5.1 Physical Modification

The physical modification of starch is mainly applied to change its granular structure and convert native starch into cold water soluble starch or small-crystallite starch [Lewandowicz and S´mietana, 2004; Wu *et al.*, 2010]. Starch can be modified physically by treating native starch granules under different moisture/temperature conditions, by applying pressure or shear or by mechanical attrition. Physical modification of starches can be applied alone or in combination with chemical modification. Physical modification comprises pregelatinization, heat-moisture treatment, extrusion, or annealing [Bergthaller and Hollmann, 2007].

Although native starch has been used for food processing, the physical properties of these starches and the colloidal sols produced from them are limited in terms of the commercial applications for which they are suited [Laovachirasuwan, P., Peeerapattana, J., Srijesdaruk, V., Chitropas, P., Otsuka, M]. Most native starches are limited in their applications due to their instability under various temperatures, shears, and pH conditions. Additionally, most starch

granules are inert, insoluble at ambient temperature, highly resistant to enzymatic hydrolysis and therefore lack specific functional properties. Native starches are often modified to develop specific properties such as solubility, texture, adhesion, and heat tolerance, so as to be suitable for industrial applications [Anderson, A. K., Guraya, H. S., James, C., Salvaggio, L11–16].
Physical modification is simple, cheap, and safe because it requires no chemicals or biological agents. Classification of starches from different botanical sources based on physical modifications depends on whether the molecular integrity of the starches are destroyed or preserved after the modification. The former encompasses all the pre-gelatinization processes (drum drying, spray drying, and extrusion cooking) where starch granular order is loss together with partial depolymerization of starch components and the latter are the hydrothermal processes, e.g., ANN and HMT where the molecular integrity of the starches are preserved. Recently new methods of physical modifications of starches from different botanical sources have been investigated and are as follows: superheated starch; iterated syneresis; thermally inhibited treatment; osmotic pressure treatment; multiple deep freezing and thawing; instantaneous controlled pressure drop (DIC) process; mechanical activation with stirring ball mill; micronization in vacuum ball mill; pulsed electric fields (PEF) treatment; corona electrical discharges [Adeleke Omodunbi Ashogbon1 and Emmanuel Temitope Akintayo, 2014].

2.5.2 Chemical Modification

Chemical modification involves the introduction of functional groups into the starch molecules, resulting in markedly altered physicochemical properties. Such modification of native granular starches profoundly changes the proximate compositions, gelatinization, retrogradation, and pasting characteristics. Chemical modification is intended to facilitate intra- and inter-molecular bonds at random locations in the starch granule for their stabilization. The chemical and functional properties achieved by modified starches depend, inter alia, on starch source, reaction conditions (reactant concentration, pH, reaction time, and the present of catalyst), type of substituent, degree of substitution (DS), and the distribution of the substituents in the starch molecule. Mainly, there are three ways of chemically modifying starches. Modification of starches can be carried out in aqueous solution or slurry. After completion of the reaction, starch is filtered off, washed, and dried. A second method is heating the starch until gelatinization sets in and then adding the chemicals in a small volume of a solvent. After completion of the reaction starch is dried. A third possibility is to moisturize the dry starch with chemicals, dry it, and heat the starch until the reaction is finished [Bergthaller and Hollmann, 2007]. The most popular chemical modification methods include acid hydrolysis, oxidation, etherification, esterification, cross-linking, and dual modification [Fornal *et al.*, 2012].

Modification is generally achieved through derivatization, such as acetylation, cationization, oxidation, acid hydrolysis, and cross-linking. These techniques are however limited due to issues concerning consumers' safety and the environment. There is an evolving new trend called dual modification, which involves the combination of physical and chemical agents, e.g., microwave-assisted acetylation or HHP-assisted phosphorylation [Adeleke Omodunbi Ashogbon1 and Emmanuel Temitope Akintayo, 2014].

2.5.2.1 Acetylated (Ac) modifiction

Acetylation is one of the common chemical method of starch modification, it is achieved by esterification of native starch with either acetic anhydride or vinyl acetate in the presence of alkaline catalyst, e.g., NaOH, KOH, $Ca(OH)_2$,Na_2Co_3. The acetylation reactions are typically carried out in water, though organic solvents also have been shown to be effective. Ac starch is a starch ester that has been extensively studied over the last two decades [Wang, Y.-J., Wang, and L.2002].

Introduction of acetyl groups reduces the bond strength between starch molecules causing an increase in the swelling power and solubility of the modified starch. Hydrogen bonding is restricted in Ac starches as a result of inter- and intra-molecular electrostatic repulsion in the starch molecule. Ac starches act as RS and have been observed to increase large bowel SCFAs. In Ac starch, parts of the hydroxyl groups in anhydroglucose units have been converted to acetyl groups. A low degree of substitution DS with 0.01–0.2 acetylated starch has been applied in many areas, such as film forming, binding, adhesion, thickening, stabilizing, and texturing. Ac starch with low DS is commonly obtained by esterification of native starch with acetic anhydride in an aqueous medium in the presence of an alkaline catalyst. However, high DS Ac starches have received much attention in recent years for their solubility in acetone and chloroform and for their thermoplasticity. Furthermore, high DS Ac starches can have very different properties such as hydrophobicity, melt processibilty and a number of non-food applications, such as tablet binders, hot melt adhesion, coating, cigarette filters, biodegradable packaging materials and pharmaceutical aspects have been suggested [Shogren]. Preparations of a series of starch esters, such as Ac starches by high temperature and pressure reaction. And also prepared Ac starches with different DS by the reaction of the native corn starch with acetic anhydride in the pressure of acetic acid under varying reaction temperatures [Chi et al.]. They found the modified corn starches possessed better hydrophobic performance than the parent starches. Cloudiness and syneresis of aqueous dispersions of waxy starches could also be minimized through acetylation of AP outer branches. Acetylation reduces retrogradation tendency of starches, however, level of DS of acetyl groups is a major determinant in imparting desirable properties to the starch. Although many studies on the esterification of starches have been conducted recent focus is on the application of green chemistry that is environmentally friendly. Generally, high fatty acid esters of starches could be prepared by reacting native starch with fatty acid vinyl esters, fatty acid chlorides, carboxylic acids, or FAME as reactants in organic solvents such as toluene, pyridine or DMSO and basic salts such as K_2CO_3,CH_3COONa, NaH_2PO_4, and CH_3OK as catalyst. However, these organic solvents are poisonous and exorbitant and therefore have higher environmental impact than water. Hence, there is a strong incentive to develop green, environmentally friendly solvents for starch esterification. Apossible solution is the use of densified CO_2. Super-critical or densified CO_2 is nonflammable, non-toxic, chemically inert and a “green” solvent. It has been shown to be an excellent solvent and attractive for polymer processing. Furthermore, CO_2 can be easily separated from the reaction mixture by depressurization. Recent works have described the production of fatty acid starch esters using a fatty acid vinyl or methyl esters or a fatty acid anhydride with supercritical CO_2 as a solvent. These methods have produced products with broad range of DS values (0.01–0.31). Recently, other desirable green solvent, ionic liquids (ILs) at ambient temperature were considered for wide range of synthesis and catalysis because of their possession of low melting points, wide liquid ranges, and low or negligible vapor pressure. Of interest is the ILs bearing the imidazolium structure, have been known to be non derivatizing medium capable of dissolving polysaccharides, such as starch and cellulose. Using 1-butyl-3- methylimidazolium chloride (BMIC), starch could dissolve in it up to 15% w/w concentration at 80°C. Synthesized high fatty acid esters of corn starch by reacting the starch with FAME using BMIC-IL as the reaction medium [Xie and Wang]. They found that the hydroxyl groups in the starch molecules were converted into ester groups, additionally, the morphology and crystallinity of the modified starch ester granules were largely disrupted or altered.

2.5.2.2 Cationic starch

Cationic starch is produced from the reaction of starch with reagents containing amino, imino, ammonium, sulphonium, or phosphonium groups. The free hydroxyls of the native starch is commonly altered by using cationic monomer such as 2,3-epoxypropyltrimethylammonium chloride (ETA) or 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CTA) under wet or dry processes or a process in betweenthe two processes. In the dry-cationization process (absence of a liquid phase) reagent is sprayed to the dry starch during extrusion; in the semi-dry method, reagent is sprayed onto the starch and the mixtures are thermally treated. Both methods above reduced environmental pollution. However, by-products may be produced along with the desired products, it might also be costly separating the net product from the by-products. In the wet-cationization method, reactions are processed homogeneously in dimethyl sulphoxide (DMSO) or heterogeneously in an aqueous alkaline suspension. The latter researchers reported that high-DS cationic derivative were prepared in aqueous alkaline solutions, homogeneously with DMSO, and heterogeneously with ethanol–water. Usually, the high-DS cationic starch is prepared by an aqueous procedure, as water favors the diffusing of the cationic agent into the starch granule.

However, hydrolysis or other side reactions may compete for the etherification of starch in the presence of water. Thus, it is necessary to choose appropriate media in which water is partially replaced in the reaction mixture by an active solvent.

A further investigation, making use of corn starch, cationic reagent (ETA) and dioxane–THF–water (dioxane–THF–water) medium revealed that the granular morphology and crystallinity of the modified corn starch granules were destroyed [Wang et al.]. The damaged morphology of the cationic corn starch granules were attributed to the penetration of its interior by cationic reagent resulting in subsequent enlargement and disintegration of the granules. The same work adduced the loss of crystallinity to the effect of the aqueous alkaline environment during the modification. In their own contribution, indicated that the changes in the pasting and thermal properties of cationic corn starches correlated well with the increase of DS, together with the characteristics of granular morphology [Kuo and Lai]. They further reported the gradual reduction of the structural and molecular integrity of cationic starches with raising DS up to 0.04311, while evident physicochemical and morphological changes took place when DS was further increased to 0.1215. The cationic substitution by aqueous methods is believed to occur mainly at the amorphous region, as evidenced by the unchanged patterns and persistent birefringence under polarized light microscopy. Also treated mixtures of starches and semicarbazide hydrochloride with either conventional or microwave heating and obtained a product that contained cationic groups [Siemion et al.]. .

Generally, physicochemical properties as well as granular morphology of starch were always altered after cationization, especially those involving high DS. Furthermore, lower pasting temperature, higher peak viscosity, and various changes in setback were observed in the RVA of different kinds of starches. Additional facts are the smaller DH and lower transition temperature of cationic starches when compared to native starches as analyzed by DSC. There are many different applications of cationic starch; it is used for water treatment as flocculants and as additive in textile and cosmetic products. Furthermore, some researchers have found cationic starch to be useful in dynamic coating additive for protein analysis [Adeleke Omodunbi Ashogbon1 and Emmanuel Temitope Akintayo, 2014].

2.5.2.3Cross-linked starch

Cross-linking has been used to modify native starch utilizing various cross-linking agents such as sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (ECH), and phosphoryl chloride ($POCl_3$). Other cross-linking agents are adipic–acetic mixed anhydride, and mixture of STMP and STPP. ECH is no longer used for food grade manufacturing in the U. S. because the chlorohydrins are carcinogens. The cross-linking of starch to be affected by many factors, such as starch source, cross-linking reagent concentration and composition, the extent of substitution, pH, reaction time and temperature [Chung et al].

The type of cross-linking agent determines the changes in functional properties of a treated starch, because the molecular structures of the cross-linked systems produced by different cross-linking agents are different. Therefore, based on the reagent used for cross-linking, the final product is generally classified into three types: the first type is a mono-starch phosphate which is produced by esterification of starch with ortho-phosphoric acid, sodium or potassium ortho-phosphate, or STPP. The second classification is a distarch phosphate which is produced when a native starch is reacted with STMP or $POCl_3$. Final type of cross-linked starch is a phosphated di-starch phosphate which resulted from combined treatments of mono-starch phosphate and distarch phosphate. The botanical sources of starch and conditions and methods used for its modification have great influence on the properties of the final product.

Cross-linked corn starch with STMP/STPP (99:1 w/w) and found that cross-linking decreased the SF, solubility and paste clarity of corn starch. And also reported lower water solubility and swelling power (SP) when cross-linked wheat starch is compared to its native counterpart. Identical results were realized [Gunarantne, Corke and Mirmoghtadaei et al.] on reduction in solubility and SP of various cross-linked starch systems. The decreased paste clarity is consistent with results obtained and the cross linked starches showed lower paste clarity than their counterpart native starches. These decreased paste clarity of cross-linked starches were attributed to their being more compact when compared to native starches. Furthermore, it has been indicated that the reduced swelling of cross-linked starches might be partly responsible for their decreased paste clarity. The decreased SF with increasing level of cross linking concurs with the work of [Mirmoghtadaie et al.]. It is an established fact that cross-linking strengthens the bonding between starch chains, thus resulting in resistant to swelling [Koo et al.]. The solubility of cross-linked corn starch decreased with increasing degree of cross-linking at all temperatures when compared to native corn starch, identical patterns was reported [Kaur et al.] For potato starches modified with ECH and $POCl_3$ at different concentrations. The above phenomenon could possibly be due to increased density of cross-links in the starch structure that prevent disintegration of starch granules during the attainment of gelatinization temperature by various starches.

In furtherance of their works on cross-linked corn starch found no difference in the crystallinity of the modified corn starch compared to the native starch. Identical crystalline pattern was also observed in the treatment of wheat starch with $POCl_3$ [Koo et al.]. This re-echoed the accepted fact that cross-linking mainly take place in the amorphous regions of the starch granules and did not alter the crystalline patterns of starches. The SEM of native and cross-linked starch granules differ significantly. The native corn starch granules were polygonally shaped with well-pronounced edges, whereas cross-linked starch granules possessed slightly rough surface and black zone on the granular surface. Similar results were reported [Carmona-Garcia et al.]. On crosslinked banana starch obtained by identical treatment of native banana starch with STMP/STPP. Identical morphological changes were also reported when native wheat starch was cross-linked with $POCl_3$. The black zones on the starch granule surfaces indicate slight fragmentation and the formation of deep grooves in the starch granules.

Hydroxypropylation and acetylation of starches also increase granule surface area and granule porosity. The cross-linked wheat starch with large granules (A-types) possessed more spots compared to smaller ones (B-types). Similarly, [Hung et al.] had previously indicated that A-granules are affected more than B-granules by cross-linking agents. It has been observed that the granule structures were severely altered with increase in the concentration of the cross-linking reagents. The botanical source of the starch may also affect the morphological properties as a result of chemical modification. In contrast, no changes were observed in the size and shape of potato starch granules when cross linked by $POCl_3$ [Majzoobi et al.].

Generally, cross-linking reduces the moisture, protein, and fat contents of the native starches that were modified irrespective of the botanical sources of the starches and the cross-linking reagents involved, although the level of reduction differ. When they treated banana starch with different cross-linking agents. On a broader note, also reported reduction in proximate compositions and AM content when cocoyam starches were separately oxidized, acetylated, and acid-thinned [Lawal].

2.5.3 Enzymatic Modification

Enzymatic modification is modification of starch using enzymes that are found in Eukarya, bacteria and archaea representatives, mainly hydrolyzing enzymes. The enzyme used has to be free of enzymatic components that can cause undesirable damage to the starch molecule [Kaur *et al*, 2012]. There are basically four groups of starch-converting enzymes: (i) end amylases; (ii) exoamylases; (iii) debranching enzymes; and (iv) transferases .The enzymatic method has many advantages over other methods as it is safer for both the environment and consumers and the reaction can be more specifically controlled under the mild conditions thus consequently may result in fewer by-products [Tri Le *et al.*, 2009].

Chapter 3

3. Materials and methods

The experimental work has been done in the laboratories of East African Pharmaceutical PLC and School of Chemical and Bio Engineering, Addis Ababa Institute of Technology; Addis Ababa University.

3.1 Method and extraction of normal corn starch

3.1.1 Materials

The varietiy Melkasa 2, of corn were obtained from Melkasa Agricultural Resarch Institute center. The chemicals acetic anhydride, sodium hydroxide, hydrochloric acid, sodium metabisulfite, ethanol (96 per cent), glycerol, sodium thiosulphate, glacial acetic acid, potassium iodide, starch solution, ammonia, ammonium iron (III) sulphate, citric acid, thioglycollic acid, was purchased from RFCL Limited New Delhi, BDH chemicals Ltd poole England and Abron Exports-133001 India. And all chemicals used in the study were of analytical grade.

3.1.2 Methods of Extraction

Raw material obtained from Melkasa Agricultural Research Center, Adama/Nazret. The unwanted impurities were separated and cleaned manually. The Corn starch was isolated according to the method of Takeda et al. (1988) as follows: corn kernels will be soaked/steeped in 0.5% Sodium metabisulphite solution, pH 4–5 at 50–52 ˚C for 2 days. Then the germ will be manually separated from local corn. The softened kernels was homogenized with cold water (10^0C) for 10 min and then, the homogenate will be squeezed through a 100 mesh sieve. The starch was washed three times with cold water followed by centrifugation at 1000rpm for 15 min and impurities like protein and fiberous materials were separated from starch then, starch was dried in an air dried oven at 40^0C for 12 h.

3.1.2.1 Determination of percent yield of corn starch

Percentage yields of dry basis extracted corn starch and acetylated modified corn starch was determined according to the method described by the Association of Official Analytical Chemists with slight modification, [AOAC, 2000].

3.2 Modification of native corn starch using acetylated method

3.2.1 Material

The extracted normal corn starch was used in this method to modification.

3.2.1.2 Preparation of acetylated corn starch at different concentration

Modification of starch is necessary because of normal starch shortcomings. Modification of starch carried out to enhance the positive attributes and to eliminate the shortcomings of the native starches [Corn Refiners Association, 2006].

Various methods have been developed to produce a range of modified starches, physical, chemical, enzymatic and genetic, with a variety of characteristics and applications. Physically modified starches are simple and inexpensive because they can be produced without chemicals or even biological agents. In contrast, chemical modification is possible due to ubiquitous hydroxyl groups in starches that have been exploited for over a century, principally in the preparation of starch esters and ethers, but also in more subtle alterations, e.g., in order to tune the structure of starches for specific applications. All these techniques tend to alter the highly flexible starch polymer with changed physicochemical properties and modified structural attributes of high technological value for the food and non-food industries. Modification of starch is an ever evolving industry with numerous possibilities to generate a novel starch which includes new function and value added properties as demanded by the industry [perez, S., Bertoft, E., starch 2010, 62, 389-420]. This study was focused on acetylated chemical method of modification and all the raw materials were used the extracted Melkasa 2 corn starch.

Procedure: Acetylated starches were prepared as described by Phillips, Liu, Pan, and Corke (1999). Starch (100 g) was dispersed in 225 ml of distilled water and stirred for 60 min at 25 ˚C. The suspension was adjusted to pH 8.0 with 3.0% NaOH. Acetic anhydride (2, 4, 6, 8, 10, 12 g) was added drop-wise to the stirred slurry, while maintaining the pH within the range 8.0–8.4 using 3.0% NaOH solution. The reaction was allowed to proceed for 10 minutes after the completion of acetic anhydride addition. The slurry was then adjusted to pH 4.5 with 0.5 N HCl. After sedimentation, it was washed free of acid, twice with distilled water and once with 95% ethanol, and then oven-dried at 40 ˚C. Yield (%) of modified starch was calculated on starch dry weight basis.

3.2.1.3 Preparation of acetylated corn starch at different time and temperature

The starch was dried at 50 ˚C for 24 h before reaction to avoid the interference of moisture. Ten grams corn starch (0.06 mol anhydroglucose unit) and 0.36 mol glacial acetic acid were placed in a 250 mL three-neck flask. After stirring for 5 min, 0.32 mol cooled acetic anhydride was added to the mixture. After 15 min, 0.0035 mol of methanesulphonic (methyl sulfonic acid) acid was subsequently added to the reactor. The reaction was stirred by changing the reaction time at 1, 2 and 3 h at 200 rpm/min. By changing the reaction temperature at 50 ˚C, 62.5 ˚C, 75 ˚C, it was possible to prepare esters with three different DS. The reaction was terminated by pouring into distilled water. The mixture was washed with excess distilled water. The solid was dried at 50 ˚C for 12 h in vacuum oven [Linpin Isotemp 202–00 A, Linpin Scientific, Shanghai, China].

3.2.1.4 Yield percentage of modified starch

$$\text{Yield (\%) of modified a starch} = \frac{\text{(weight of modified starch)}}{\text{(Weight of unmodified starch)}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

3.2.1.5 Determination of acetyl content (%)

$$\omega = \frac{(v2 - v1) \times N \times 43)}{(1000 \times m)} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Where: ω acetyl content

v_1- the volume of 0.5 N HCl in mL used for titration of 0.50 g native starch;

v_2- the volume of 0.5 N HCl in mL used for titration of 0.50 g sample

N- the normality of HCl solution

m -the weight of the sample;

43 the molecular weight of the acetyl group;

162 the molecular weight of anhydroglucose unit

3.2.1.6 Degree of substitution (DS)

Degree of substitution is the average number of hydroxyl groups substituted per anhydroglucose unit. The maximum possible DS is 3.0 Determination of DS of acetylated starch by titration involved complete basic hydrolysis of the ester linkages and titration of the excess alkali. Native and acetylated starches (0.50 g) were weighted accurately and added into the sodium hydroxide solution (25 mL, 0.5 N). The mixture was stirred for 72 h at 50 rpm/min, at room temperature. After indicator (3 to 5 drops of 1% thymolphthalein) was added the solution was immediately titrated with 0.5 N hydrochloric acid to the thymolphthalein endpoint. Reference sample was treated in a similar way.

$$DS = \frac{(162 \times \text{Acetyl } (\%))}{(43-42 \times \text{Acetyl } \%)} \quad \ldots\ldots\ldots\ldots 3.3$$

Where: 43 the molecular weight of the acetyl group;

162 the molecular weight of anhydroglucose unit

3.2.1.7 Experimental design data analysis and modeling

The experimental design for this experiment was Design Expert 6.0.8 Portable software. The response surface methodology involves in the design of experiments, selection of levels of variables in experimental runs, fitting mathematical models and finally selecting variables' levels by optimizing the response [Khuri and Cornell 1987].

A two variable (three levels of each variable) central composite face centered experimental design was employed [Montogomery, 2001]. The parameters are temperature and time. The independent variables were temperature (50, 62.5 and 70 ˚C) and time (1, 2 and 3 hourse.) The response Variables were acetyl %, degree of substitute and yield %.

3.2.1.8 Data analysis

Statistical analysis and optimization Responses obtained as a result of the proposed experimental design were subjected to regression analysis in order to assess the effects of temperature and time. Second order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 6.0.8 [Stat-Ease Inc, Minneapolis, MN, USA].

Data was analyzed by multiple regression analysis and statistical significance of the terms was examined by analysis of variance (ANOVA) for each response. The adequacy of regression model was checked by correlation coefficients. The lack of fit test was used to judge the adequacy of model fit. To aid visualization of variation in responses with respect to processing variables, series of contour plots were drawn.

3.3 Characterization and Physicochemical property of native and modified starch

3.3.1 Materials

The material used in this experiment was from normal corn starch that was extracted from Melkasa 2 and the acetylated modified corn starch prepared as describe above acetylated method using different temperature and time.

3.3.2 Methods

3.321 Infrared spectroscopic analysis

Infrared spectroscopy (IR) provides information about chemical groups containing highly polar bonds, or bonds whose dipole moment changes during vibration, [Moralesa *et al*., 2012]. Infrared red spectroscopic analysis was performed using a Buck scientific Infrared spectrometer (Model 500, USA). The native and acetylated starch samples were collected using the KBr pellet method. IR spectra were recorded at a resolution of 4 cm^{-1} and wave number range between 600 and 4000 cm^{-1} . The native and acetylated starches were equilibrated at 50 ˚C for 24 h prior to Infrared red spectroscopic analysis. Take a sample weight of 2mg and 300mg of KBr and prepared a pellet and run using Infrared spectrophotometer with KBr pellet using as a blank and measure the transmitant or absorbance[BP 2007].

Preparation of disc: Triturate 1mg to 2mg of the substance being examined with 300mg to 400mg of finely powdered and dried potassium bromide or potassium chloride. Carefully grind the mixture, spread it uniformly in a suitable die, and submit it in vacuo to a pressure of about 800Mpa.

Procedure: prepare the substance being examined and the reference substance by the same procedure and record the spectra between 4000cm^{-1} and 670cm^{-1}(2.5µm to 15µm)under the same operational condition.

3.3.2.2 Structure and shape

Starch granules vary in size and shape, characteristic of specific plant sources. Starch molecules are oriented within granules in specific crystalline patterns and view in aqueous suspension under polarized light. Examined under a microscope using a mixture of equal volumes of glycerol and distilled water not more than traces of matter other than starch granules are present [BP 2007].

3.3.2.3 Bulk density and pH determination

Bulk density of native and modified corn starches was determined according to the method of Adeleke and Odedeji (2010) as follows: starch sample (50 g) was put into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume. Bulk density (g/ cm3) was calculated by dividing the weight of sample on its volume after tapping. The pH of the starch samples was measured according to the method of Adeleke and Odedeji (2010). pH meter (previously calibrated) with reference buffer solutions (pH 4.0 and pH 7.0 buffers)

Procedure:

Shake 5.0 g with 25.0 mL of carbon dioxide-free distilled water for 60 s. Allow to stand for 15 min.

3.3.2..4 Water binding capacity

Distilled water (15 ml) was added to 1 g of the corn starch sample in a weighed 25 ml centrifuge tube. The tube was agitated on a vortex mixer for 2 min, and then centrifuged at 1250g for 20 min. The clear supernatant was decanted and discarded.The adhering drops of water was removed and reweighed.[Adeleke and Odedeji, 2010].

3.3.2.5 Swelling power and solubility

Swelling power and solubility determination were carried out at 50, 70 and 90 ^{0}C using the method of Leach et al. (1959). A 1% aqueous suspension of starch (100 ml) was heated in a water bath at 90^{0}C for 1 h with constant stirring. The suspension was cooled for half an hour at 30 C. Samples were then poured into preweighed centrifuge tubes, centrifuged at 3000g for 10 min and weight of sediments was determined. For the measurement of solubility, the supernatants were poured into aluminum dishes and evaporated at 110 0C for 12 h and weight of dry solids was determined.

3.4 Characteristics of modified corn starch

3.4.1 Materials

The materials used in this experiment was from the acetylated corn starch that prepared as describe above acetylated method using different temperature and time.

3.4.2 Methods

3.4.2.1 Determination of Solubility

Isolated starch is typically a dry, soft, white powder. It is insoluble in cold water, alcohol, ether and most organic solvents. Starch, if kept dry, is stable in storage for indefinite periods Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a solid [Corn Refiners Association 1701 Pennsylvania Avenue, N.W. Washington, D.C. 20006-5805 202-331-1634 ,www.corn.org]

.**Procedure:** Observe the solubility of the powder in cold water and in ethanol (96 per cent) [BP 2007].

3.4.2.2 Determination of pH

Control of acidity and / or alkalinity provides a useful method of ensuring identity, stability and freedom from contamination. Water-siluble substances which are naturally acidic or alkaline usually have limits of acidity or alkalinity which is expressed as a ph rang for solution of specified concentrations .thus**,** the limits of acidity for adrenaline acid tartrate (Epinephrine bitratrate) ensure that it is essentially the acid tartrate. Likewise, limits of alkalinity for aminophylline lijection help to control the amount of ethylenediamine which may be present.

Water for injections, which should have ph 7.0,has strict limits on both acidity or alkalinity which are quantified by titration with standard sodium hydroxide and hydrochloric acid, using phenol rd indicator(Practical pharmaceutical chemistry fourth edition-part one, page 30-31) PH is the negative log of the hydrogen ion concentration. The pH value of a sample solution is determined by measuring the potential difference between two immersed electrodes. pH meter (previously calibrated) with reference buffer solutions (pH 4.0 and pH 7.0 buffers) using a pH meter (DBK DIGITAL PH METER (ATC)).

Procedure: Shake 5.0 g with 25.0 mL of carbon dioxide-free distilled water for 60 s. Allow to stand for 15 minutes [BP 2007].

3.4.2.3 Determination of Foreign Matter

In pharmaceutical grade starch to check whether foreign matter is there or not is a very important crucial step and has limit. Because this starches are used to prepare a medicine for human and veterinary. Must be pure from foreign matter or present in a small quantity not more than the limit [BP 2007].

Procedure:

Examined under a microscope using a mixture of equal volumes of glycerol and distilled water not more than traces of matter other than starch granules are present.

3.4.2.4 Determination of oxidizing substances

Some pharmaceutical compounds are affected significantly by oxidation and reduction. These include ascorbic acid, riboflavin (vitamin B_2), vitamin K, vitamin E. Oxidation can have deleterious effects on the cells and tissues of the human body [pharmaceutical analytical chemistry 2004].

Procedure:

Transfer 4.0 g to a glass-stoppered, 125 ml conical flask and add 50.0 ml of distilled water. Insert the stopper and swirl for 5 min. Transfer to a glass-stoppered 50 ml centrifuge tube and centrifuge. Transfer 30.0 ml of the clear supernatant liquid to a glass-stoppered 125 ml conical flask. Add 1 ml of glacial acetic acid and 0.5 g to 1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25 min to 30 min in the dark. Add 1 ml of starch solution R and titrate with 0.002 M sodium thiosulphate until the starch-iodine color disappears. Carry out a blank determination. Not more than 1.4 ml of 0.002 M sodium thiosulphate is required (0.002 per cent, calculated as H_2O_2) [BP 2007].

34.2.5 Determination of Sulphur Dioxide

Procedure: Mix 20 g with 200 ml of water until a smooth suspension is obtained, and filter. To 100 ml of the clear filtrate add 3 ml of starch TS, and titrate with 0.01N Iodine VS to the first permanent blue colour [BP 2007].

3.4.2.6 Determination of Iron

Considerable emphasis is placed on the limitation of physiologically harmful impurities. Special attention is given to control of such toxic elements as arsenic and antimony, as heavy metals lead. cadmium and mercury, which are potent nerve poisons.cintamination by arsenic and,particularly,bu lead is still widespread in industrialized countries as a result of atmospheric pollution.In consequence qualitative testes for heavy metals and arsenic are applied particularly stringently to all compounds,such as,for example, ant rheumatics,heart stimulants and antihypertensives, which are used for long-term administration .

Two tests are widely used.One based on the formation of red ferric thiocyanate after treatment with bromine or ammounium peroxydisulphate to ensure that the iron is oxidized to the ferric (iron iii) state, is official in the USP. The alternative test based on the formation of purple color by the reaction of iron with mercaptoacetic acid in the solution buffered with ammonium citrate and comparison of colour produced with a standard color containing a known amount of iron. The purple color is due to the formation of the coordination compound,ferrous mercaptoacetate, ferric iron being reduced to the ferrous state by the reagent.

Procedure: Shake 1.5 g with 15 ml of dilute hydrochloric acid R. Filter.

To 10 ml of the above solution add 2 ml of a 200 g/l solution of citric acid R and 0.1 ml of thioglycollic acid R. Mix make alkaline with ammonia R and dilute to 20 ml with water R. Prepare a standard in the same manner, using 10 ml of iron standard solution (1 ppm Fe) R [BP 2007].

3.4.2.7 Determination of Moisture content

Moisture in a weighed sample is removed by heating in an oven under specified conditions of time, temperature and vacuum and the residual dry substance weighed. The weight loss is calculated as moisture. Loss on drying is the loss of weight expressed as % w/w. Place 1.000 g of the substance to be examined in a weighing bottle previously dried under the conditions prescribed for the substance to be examined. Dry the substance to constant mass at 130 °C for 90 min [Adapted from Food Chemicals Codex, Current Edition, United States Pharmacopeia (USP), and Rockville, MD].

$$\textbf{\% of moisture} = \frac{\textbf{((Sample wt. (g)) – Dry Sample wt. (g))/}}{\textbf{(Sample wt. (g))}} \textbf{ x 100} \ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

3.4.2.8 Determination of Sulphated ash

The determination of sulphated ash is used in the case of unorganized drugs such as colophony, podophyllum resin, wool alcohols and wool fat. it is applied very widely to control the extend of contamination by non-volatile inorganic substance, as aspirin, benzyl benzoate, cyclizine hydrochloride and lignocaine hydrochloride (lidocaine hydrochloride).The test is also used to control traces of alkali metals in chloroform in the presence of potassium hydroxide.The substance is ignited with concentrated sulphuric acid,with decomposed and oxidized organic matter,leaving a residue of inorganic sulphates.reproduciable result are more Readily obtained in this determination than in a total ash determination because,in general,metal sulphates are stable unless heated very strongly.

Procedure: Ignite a suitable crucible (for example, silica, platinum, porcelain or quartz) at 600 ± 50 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant and weigh. Place on 1.0 g of the substance to be examined in the crucible and weigh. Moisten the substance to be examined with a small amount of sulphuric acid R (usually 1 ml) and heat gently at as low a temperature as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of sulphuric acid R (usually 1 ml), heat gently until white fumes are no longer evolved and ignite at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue. If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with sulphuric acid R and ignition, as previously, for 30 min periods until 2 consecutive weightings do not differ by more than 0.5 mg or until the percentage of residue complies with the prescribed limit. (Adapted from Food Chemicals Codex, Current Edition, United States Pharmacopeia (USP), Rockville, MD)

$$\% \text{ Ash} = \frac{\text{Ash Wt. (g)}}{\text{Sample wt. (g)}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.5 Compare the characteristics of acetylated corn starch with commercially available pharmaceutical grade corn starch

Comparing was done by using the above method of characteristics of modified corn starch.

Chapter 4

4. Result and Discussions

4.1 Chemical Compositions of the extracted corn starch

The proximate composition of the raw material result of extracted starch from Melkasa 2 Varity of corn as shown below Table 4.1. All the samples were on the dry state when they were analyzed and the results presented in the table are as g/Kg.

Table 4.1 Proximate composition of the raw material

Corn Variety	Starch(g/Kg)	Gluten(g/Kg)	Germ(g/Kg)	Fiber(g/Kg)
Melkasa 2	550.0	270.0	100.0	80.0

4.2 Modification of corn starch

4.2.1 Preparation of acetylated corn starch at different concentration

Acetylated starch was modified by the reaction of normal extracted corn starch with different concentration of acetic anhydride in the presence of sodium hydroxide in distilled water at constant pH, temperature and reaction time. The acetyl content (%) increased progressively, up to 10% level of addition of acetic anhydride, in cornstarch but further increase caused a decrease of a degree of substitution.This might have occurred due to the difference in the granular structure of starch, which in turn affected the introduction of acetyl group in to the starch molecules [Singh et al., 2002]. The degree of substitution result from table 4.2 was with in the range between 0.01 and 0.20. It is a low degree of substitution (DS) acetylated corn starch. From the above reason the effect of different concentration acetic anhydride was not significant at constan pH, temperature and time in basic medium. This low degree of substitute acetylated modified starch (product) is used in many, thickening, stabilizing and texturing [Boutboul, Giampaoli, Feigenbaum, & Ducruet, 2002; Matti et al., 2004] not pharmaceutical purpose.

Table 4.2The value of Ds, Acetyl % and Yield % of modified corn starch using different concentration of acetic anhydride at constant pH, reaction temperature and reaction time

Acetic anhydride (g/100g starch)	Acetyl (%)	Degree of substitution (DS)	Yield (%)
Native Starch	0.0	0.000	
2	3.425	0.134	95.4
4	3.575	0.139	93.5
6	3.750	0.147	96.5
8	3.975	0.156	96.7
10	4.675	0.185	96.2
12	3.825	0.149	94.5

Acetylated starch with low DS is commonly obtained by esterification of native starch with acetic anhydride in an aqueous medium in the presence of an alkaline catalyst. So it is not used for pharmaceutical purpose because of the low degree of substitution.

4.2.2 Preparation of acetylated corn starch with different reaction temperature and time

Acetylated starch was synthesized by the reaction of starch with acetic anhydride in the presence of methyl sulfuric acid (MSA). Modification was done at different reaction temperature (55-75 ˚C) and time (1-3 hours).

Table4.3Preparation of acetylated corn starch and result using Design Expert standard method

Std	Run	Block	Factor 1 A Temp.	Factor 2 B Time	Response 1 Acetyl %	Response 2 DS %	Response 3 Yield %
8	1	Block 1	62.50	3.00	30.39	1.63	94.7
1	2	Block 1	50.00	1.00	6.45	0.26	95.07
12	3	Block 1	62.50	2.00	20.21	0.95	93.74
4	4	Block 1	50.00	2.00	12.04	0.51	95.6
13	5	Block 1	62.50	2.00	19.87	0.89	93.87
10	6	Block 1	62.50	2.00	20.07	0.91	93.48
6	7	Block 1	75.00	2.00	26.66	1.35	94.00
2	8	Block 1	62.50	1.00	8.6	0.35	93.00
9	**9**	**Block 1**	**75.00**	**3.00**	**43.29**	**2.83**	**93.42**
3	10	Block 1	75.00	1.00	13.76	0.6	94.14
5	11	Block 1	62.50	2.00	21.1	0.86	94.1
11	12	Block 1	62.50	2.00	20.07	0.93	93.79
7	13	Block 1	50.00	3.00	17.77	0.81	95.21

4.2.3 Experimental design and modeling

The experimental design and model summary are shown Table 4.4

Table 4.4 Shows Design Summery

Study Type	Response Surface	Experimental	13
Initial Design	3 Level Factorial	Blocks	No Blocks

Design Model Quadratic							
Response	Name	Units	Obs	Minimum	Maximum	Trans	Model
Y_1	Acetyl	%	13	6.45	43.29	None	Quadratic
Y_2	DS	%	13	0.26	2.83	None	Quadratic
Y_3	Yield	%	13	93.00	95.60	None	Quadratic
Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	Temp	˚C	Numeric	50.00	75.00	-1.00	1.00
B	time	hr	Numeric	1.00	3.00	-1.00	1.00

4.2.4 The effect of temperature and time on acetyl percent

From the ANOVA for response surface quadratic model for acetyl %, the Model F-value of 330.78 implies the model was significant. There is only a 0.01% chance that a "Model F- Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. . In this case Temperature, Time, both are significant model terms. Values greater than 0.1000 indicate the model terms were found to be insignificant as shown Table 4.4.

Table 4.5 ANOVA for Response Surface Quadratic Model of Acetyl percent

Sourse	Sum of Squares	DS	Mean Square	F Value	Prob>F
Model	1112.13	5	222.43	330.78	<0.0001 Significant
A	375.25	1	375.25	558.05	<0.0001
B	653.96	1	653.96	972.53	<0.0001
A^2	0.016	1	0.016	0.024	0.8824
B^2	0.013	1	0.013	0.020	0.8922
AB	82.90	1	82.90	123.29	<0.0001
Residual	4.71	7	0.67	-	-
Lack of Fit	3.77	3	1.26	5.40	0.0685 not significant
Pure Error	0.93	4	0.23	-	-
Cor Total	1116.84	12	-	-	-

Table 4.6 Summary of statistics executed for expansion index

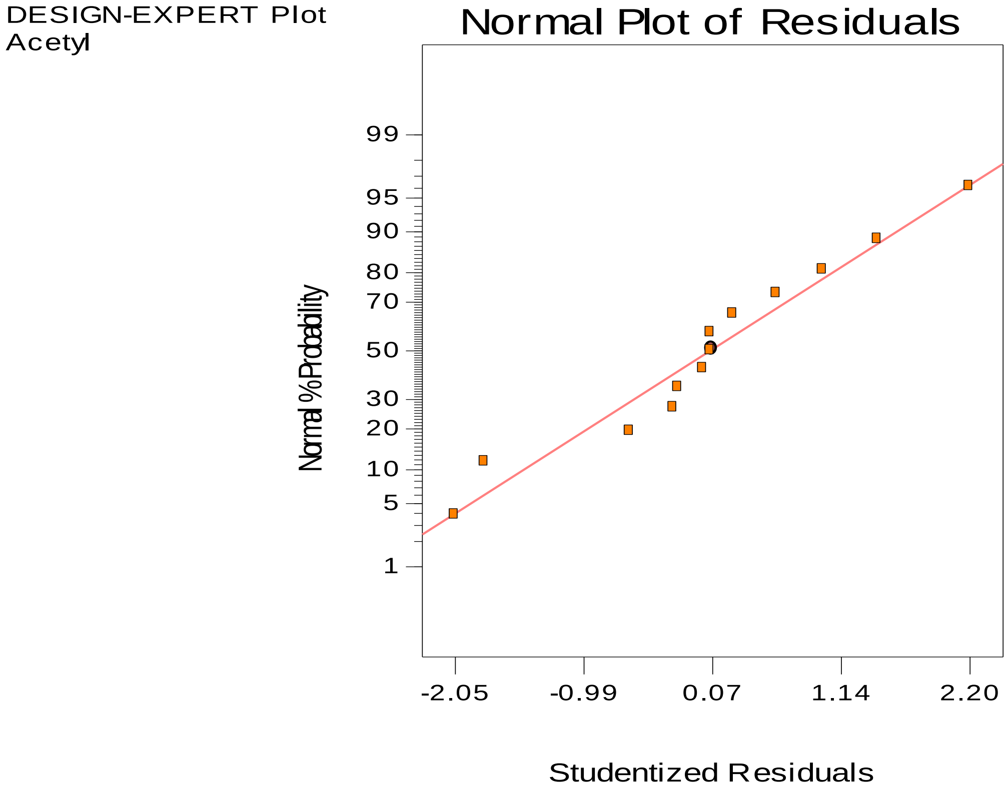
Std. Dev.	0.82	R-Squared	0.9958
Mean	20.02	Adj R-Squared	0.9928
C.V.	4.10	Pred R-Squared	0.9706
PRESS	32.79	Adeq Precision	65.872

The adequacy of the regression model was checked by R2, Adequate Precision and Fischer's F-test (Montgomery, 2001). A model is adequate in describing the response if the lack-of fit is insignificant. The R2 is defined the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon 1977). If the R2 value for a model is greater than 80 % then the model can be considered for further analysis (Filmore et al., 1976). Coefficient of variation indicates relative dispersion of experimental points from the model prediction and is desirable to have less than 10% (Nath and Chattopadhyaya 2007). The effect of variables at linear, quadratic and interactive level on the response or significance of F-value was described using significance at 95% confidence level (P< (0.05). The "Lack of Fit F-value" of 0.0685 implies the Lack of Fit is not significant relative to the pure error. It is desireble that the model lack of fit value to be non-significant we want the model to fit.

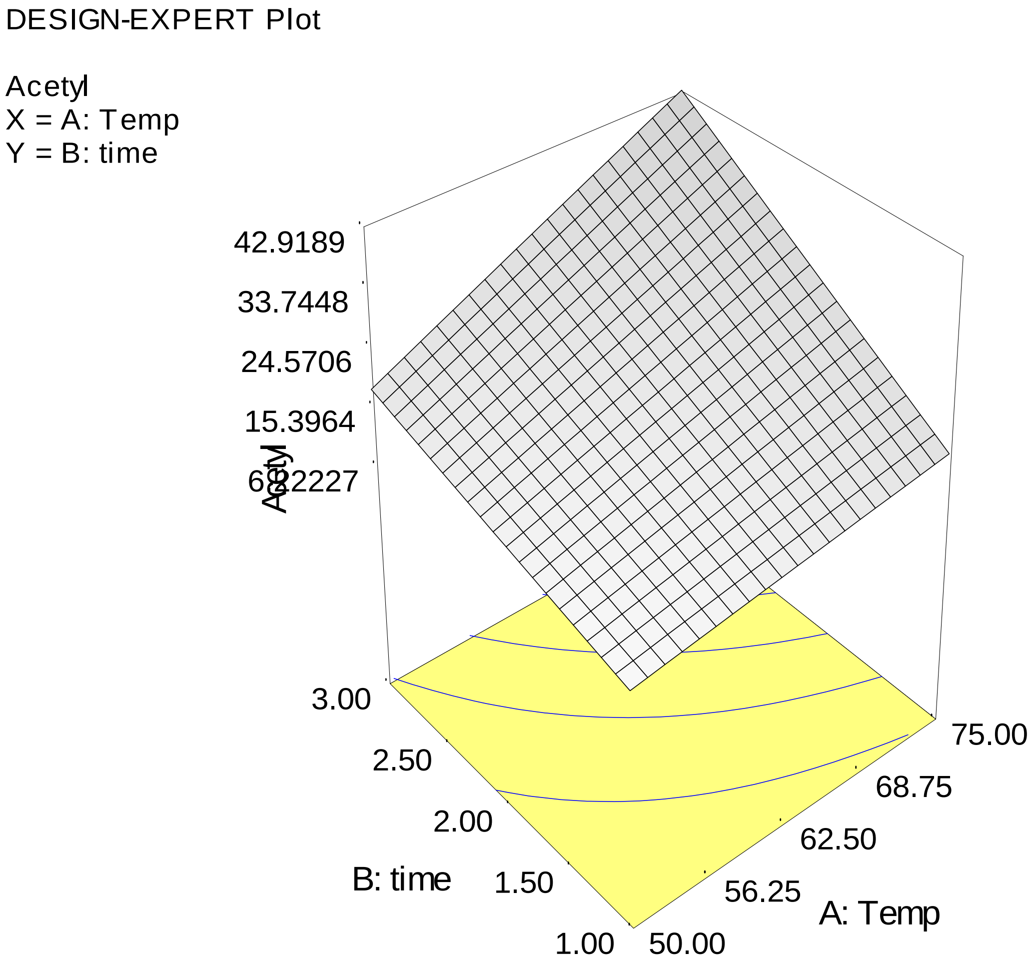
The "Predicted R-Squared" of 0.9958 is in reasonable agreement with the "Adjusted R-Squared" of 0.9928. "Adeq Precision" measures the signal to noise ratio as shown Table 4.4. A ratio greater than 4 is desirable. A ratio of 65.872 indicates an adequate signal. This model can be used to navigate the design space and prediction purpose. Considering these criteria, following response model was selected and the model equation in terms of coded variables were presented in the equation (4.1) for representing the variation of acetyl percent for further analysis.

Final Equation in Terms of Actual Factors:

Acetyl =+3.51282-0.035182* Temp-12.59974* time-4.84414E-004 * $Temp^{2}$+0.069310* $time^{2}$+0.36420* Temp * time………………………………………………………..………4.1


DESIGN-EXPERT Plot
Acetyl
Normal Plot of Residuals
Normal % Probability
99
95
90
80
70
50
30
20
10
5
1
-2.05
-0.99
0.07
1.14
2.20
Studentized Residuals

(a)



(b)

Fig 4.2 Effect of temperature and time on acetyl percent

4.2.5 Effect of temperature and time on degree of substitution

From the ANOVA for response surface quadratic model for degree of substitute, the Model F-value of 131.02 implies the model was significant. There is only a 0.01% chance that a "Model F- Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case temperature, time, squred and a combination of time and temperature are significant model terms. Values greater than 0.1000 indicate the model terms were found to be insignificant as shown Table 4.7

Table 4.7 ANOVA for Response Surface Quadratic Model of DS

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	5.26	5	1.05	131.02	< 0.0001 significant
A	1.71	1	1.71	212.37	< 0.0001
B	2.75	1	2.75	341.86	< 0.0001
A^2	0.016	1	0.016	2.01	0.1988
B^2	0.051	1	0.051	6.41	0.0391
AB	0.71	1	0.71	87.80	< 0.0001
Residual	0.056	7	8.036E-003	-	-
Lack of fit	0.051	3	0.017	14.04	0.0137 significant
Pure Error	4.880E-003	4	1.220E-003	-	-
Cot Total	5.32	12	-	-	-

Table 4.8 Summary of statistics executed for expansion index

Std. Dev.	0.090	R-Squared	0.9894
Mean	0.99	Adj R-Squared	0.9819
C.V.	9.05	Pred R-Squared	0.9059
PRESS	0.50	Adeq Precision	39.736

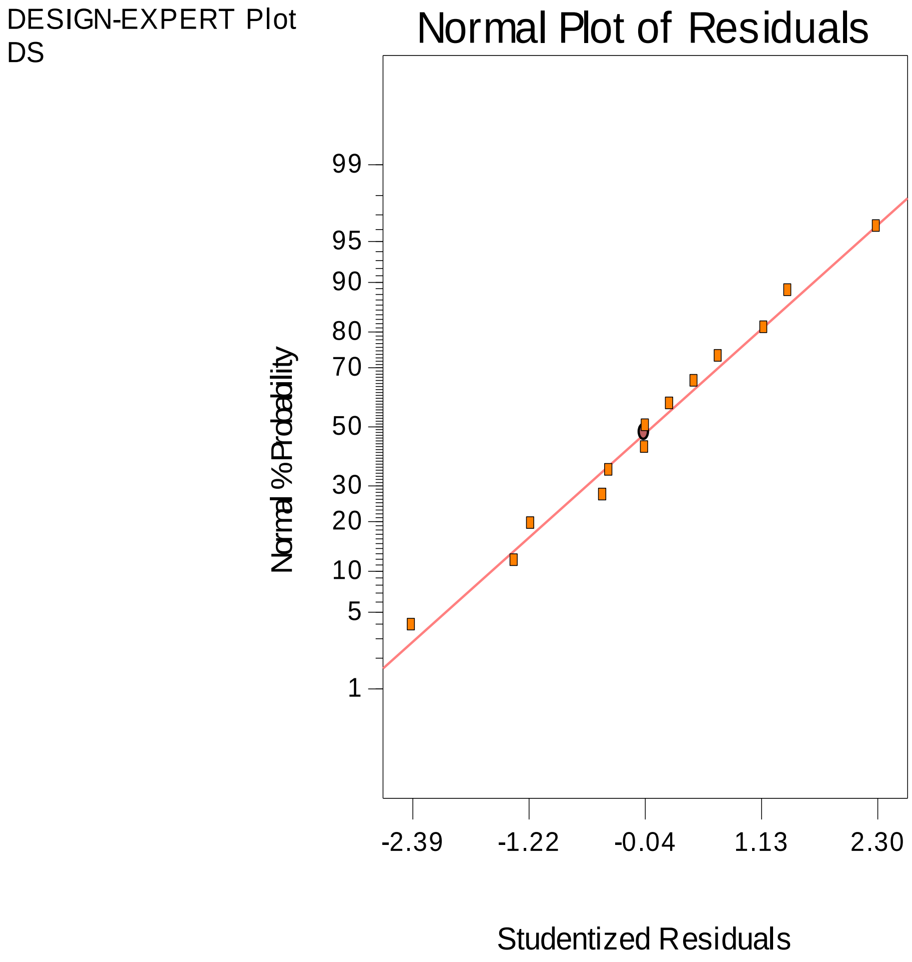
Where: Std.Dev = Standard Deviation , PRESS = Pridicted Error Square, C.V. % = Coefficient of Variation, a => The model is significant , b => lack of fit P-value is insignifican

The "Lack of Fit F-value" of 14.04 implies the Lack of Fit is significant. There is only a 1.37% chance that a "Lack of Fit F-value" this large could occur due to noise. We want the model to fit.

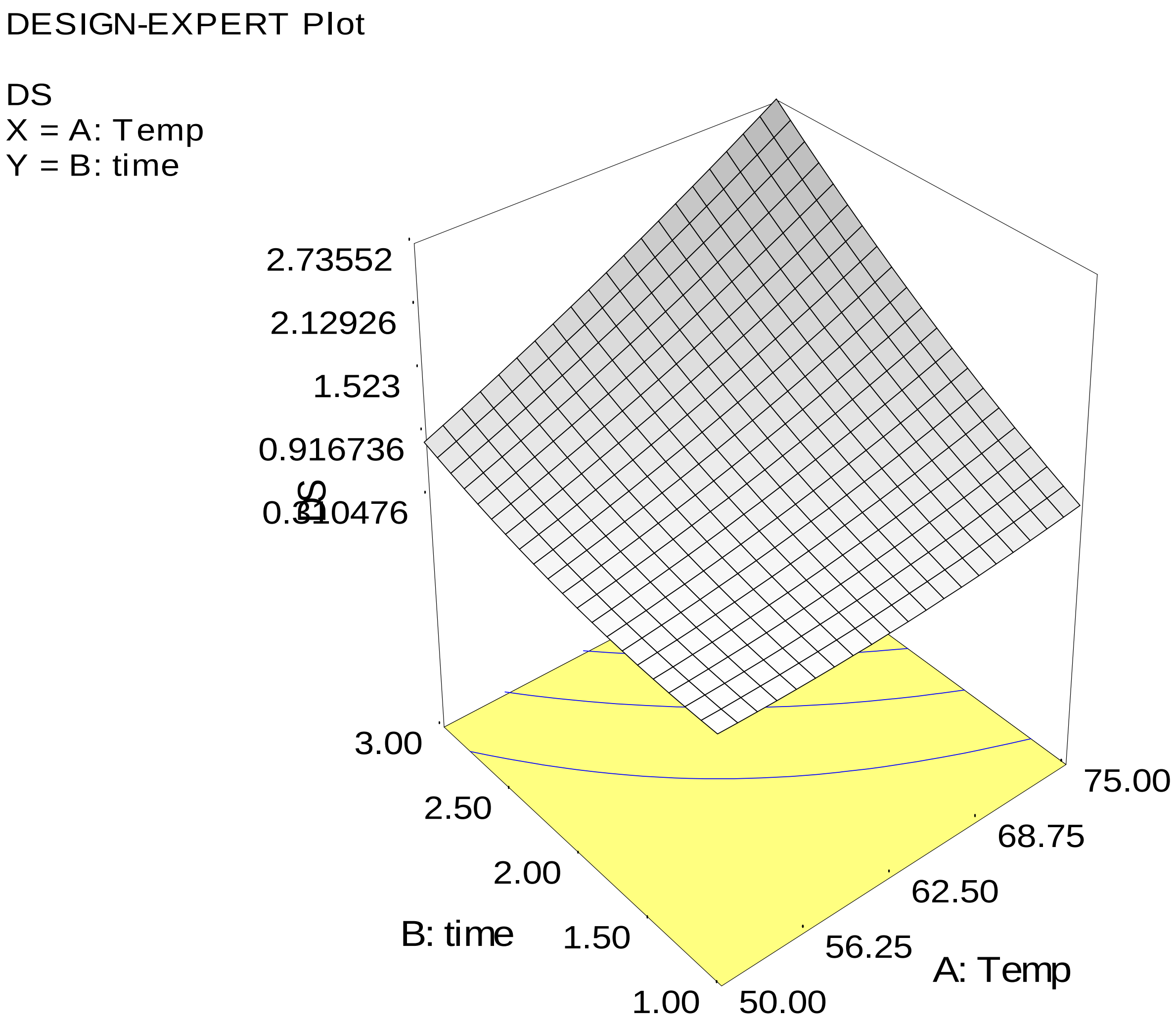
The "Predicted R-Squared" of 0.9894 is in reasonable agreement with the "Adjusted R-Squared" of 0.9819. "Adeq Precision" measures the signal to noise ratio table (4.4.). A ratio greater than 4 is desirable. A ratio of 39.736 indicates an adequate signal. This model can be used to navigate the design space and prediction purpose. Considering these criteria, following response model was selected and the model equation in terms of coded variables were presented in the equation (4.2) for representing the variation of degree of substitution for further analysis.

Final Equation in Terms of Actual Factors:

DS =+3.53241-0.085775* Temp-1.96954* time+4.89931E-004* Temp^2+0.13655* time^2+0.033600* Temp * time…………………………………………………………..……..4.2



(a)



(b)

Fig 4.3 Effect of temperature and time on degree of substitution

4.2.6 Effect of variables on yied percentage

From the ANOVA for response surface quadratic model for yield %, the Model F-value of 4.62 implies the model was significant. There is only a 3.50% chance that a "Model F- Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. . In this case Temperature and temperature squre are significant model terms. Values greater than 0.1000 indicate the model terms were found to be insignificant as shown Table 4.9

Table 4.9 ANOVA for Response Surface Quadratic Model of yield percentage

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	5.43	5	1.09	4.62	0.0350 significant
A	3.11	1	3.11	13.22	0.0083
B	0.21	1	0.21	0.89	0.3773
A^2	1.83	1	1.83	7.77	0.0270
B^2	0.051	1	0.051	0.22	0.6549
AB	0.18	1	0.18	0.79	0.4048
Residual	1.65	7	0.24	-	-
Lack of Fit	1.45	3	0.48	9.60	0.0267 significant
Pure Error	0.20	4	0.050	-	-
Cor Total	7.08	12	-	-	-

Table 4.10 Summary of statistics executed for expansion index

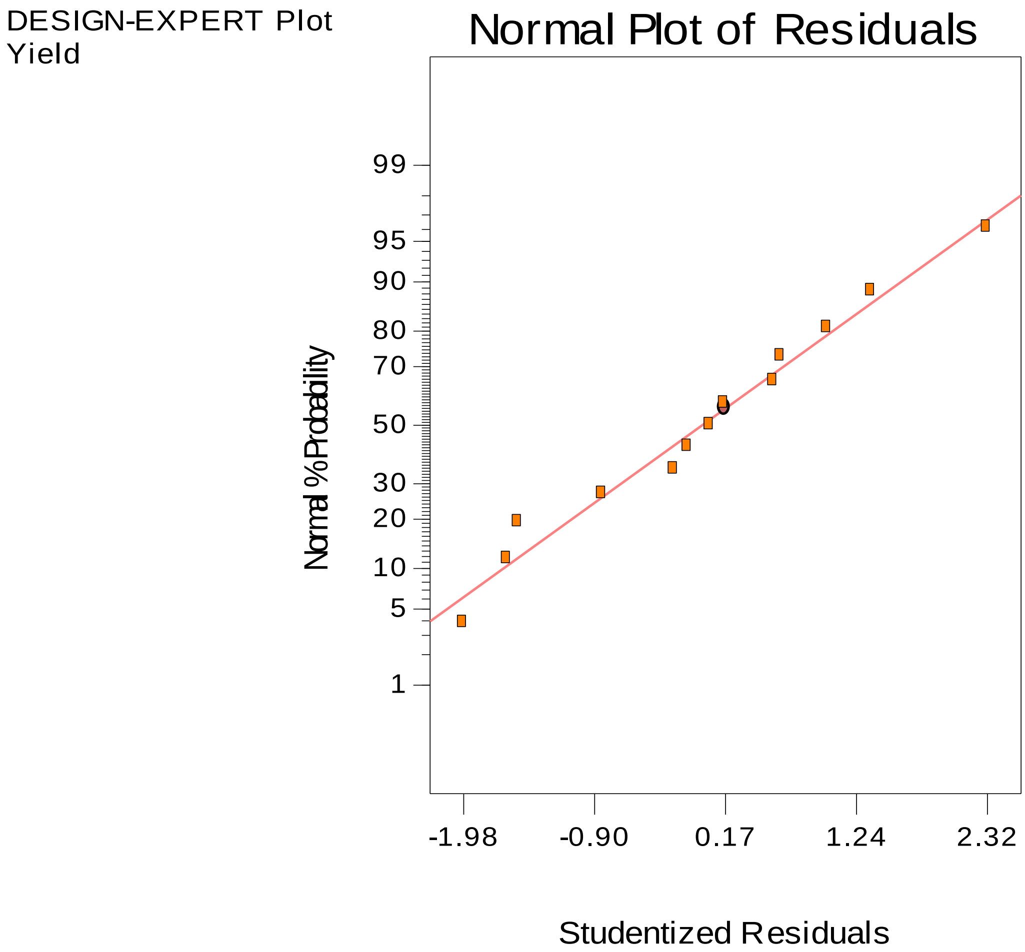
Std. Dev.	0.49	R-Squared	0.7673
Mean	94.16	Adj R-Squared	0.6011
C.V.	0.52	Pred R-Squared	-1.0709
PRESS	14.66	Adeq Precision	6.439

The "Lack of Fit F-value" of 9.60 implies the Lack of Fit is significant The "Lack of Fit F-value" of 9.60 implies the Lack of Fit is significant. There is only a 2.67% chance that a "Lack of Fit F-value" this large could occur due to noise. A negative "Pred R-Squared" implies that the overall mean is a better predictor of your response than the current model.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. This ratio of 6.439 indicates an adequate signal. This model can be used to navigate the design space. A negative “Predicted R-Squared" of -1.0709 implies that the overall mean is beeter predicted of the model response than the current model.

Final Equation in Terms of Actual Factors:

$$\text{Yield} = +114.72701 - 0.67423 * \text{Temp} + 1.80649 * \text{time} + 5.20828\text{E-}003 * \text{Temp}^2 - 0.13621 * \text{time}^2 0.017200 * \text{Temp} * \text{time} \ldots\ldots 4.3$$



(a)

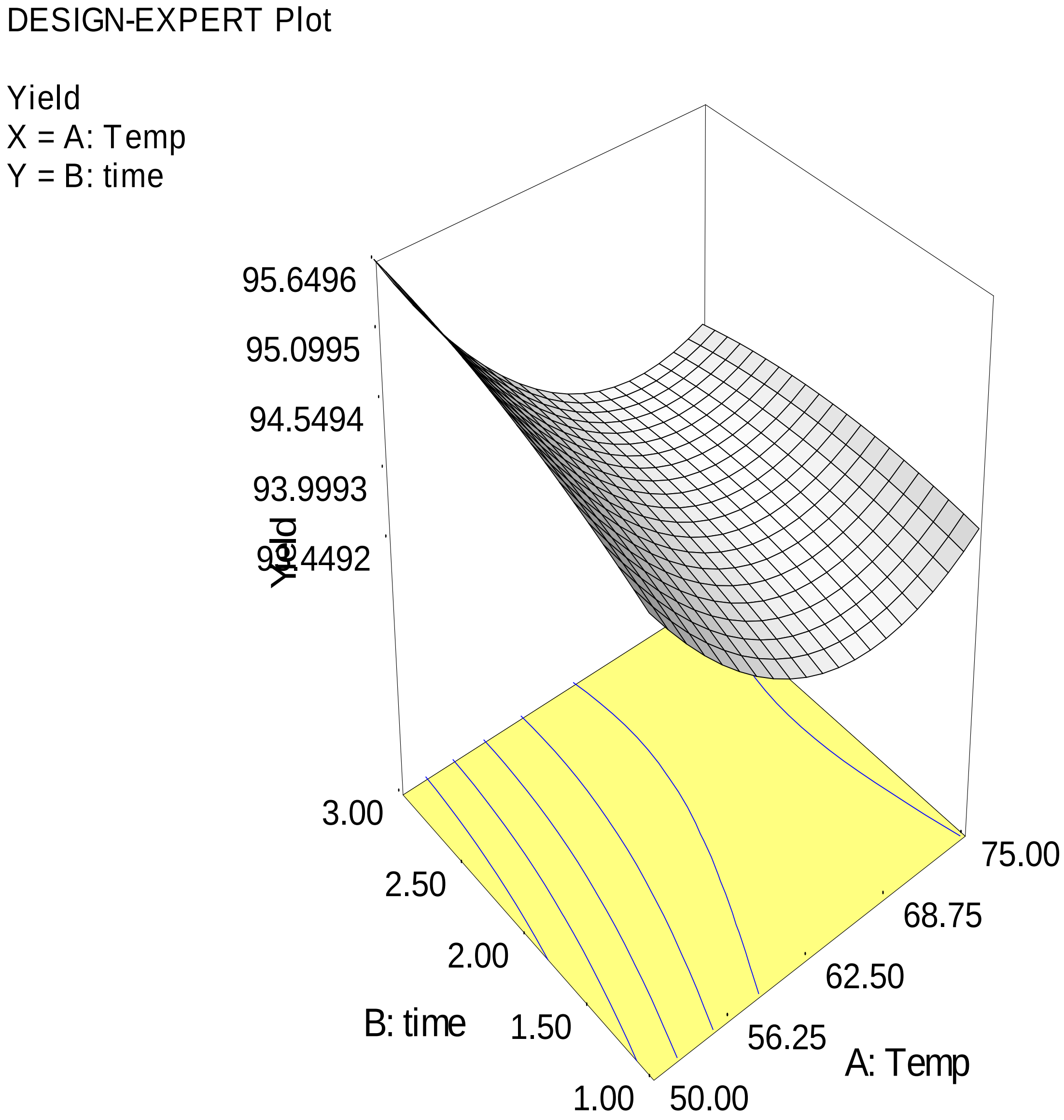


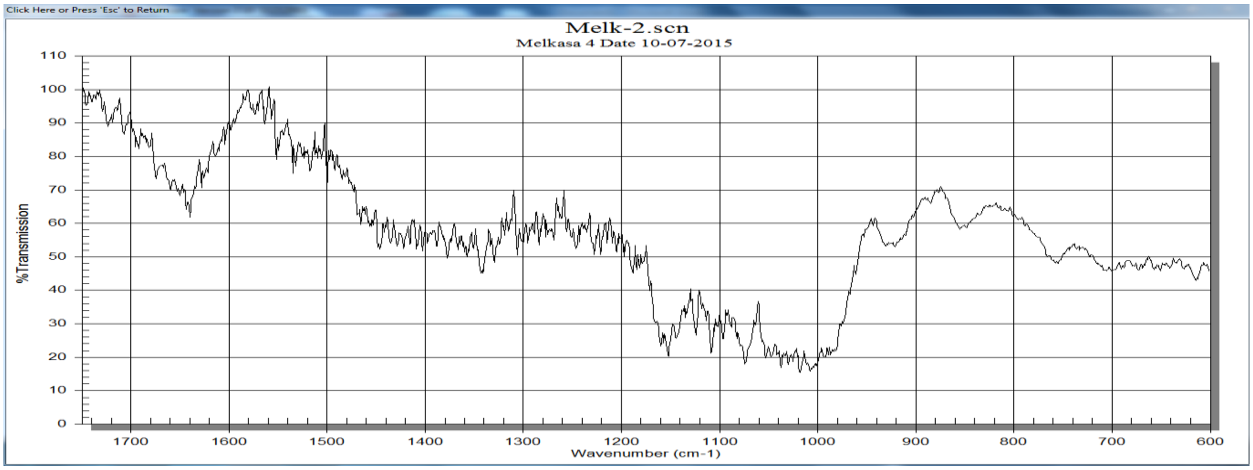
Fig 4. 4 Effect of temperature and time on yield percentage

The optimum temperature and time was on the maximum value of acetyl (42.9189), DS(2.73551) and yield (93.7796) . At this condition we get the optimum yields of product.

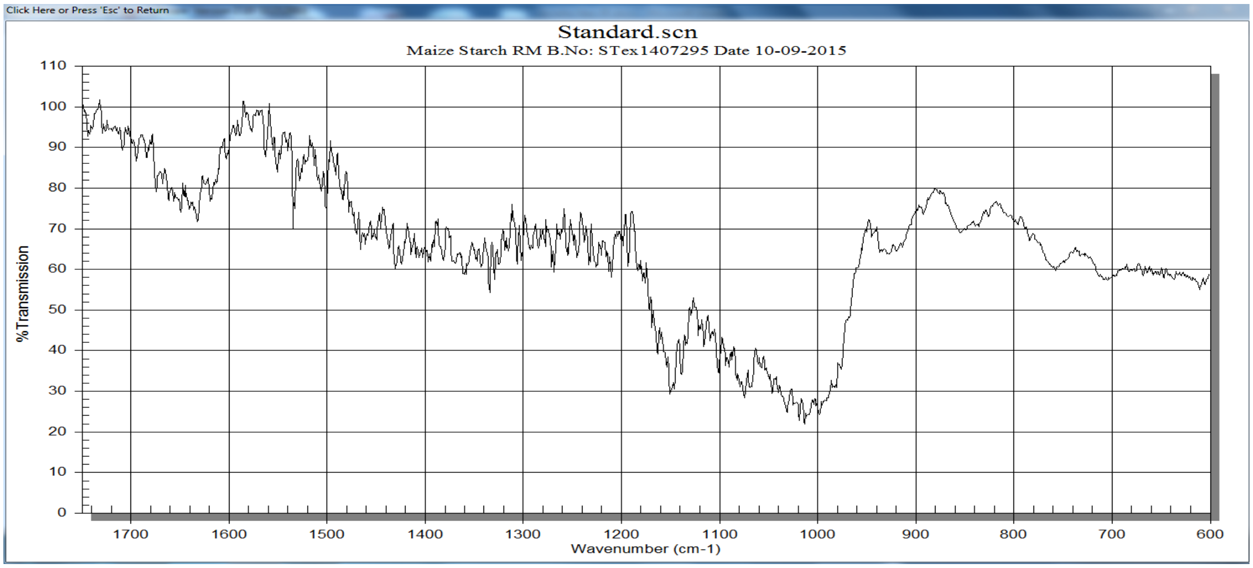
4.3 Characterization and Physicochemical property of native and modified starch

4.3.1 Infra red spectrometer analysis

The infra red of acetylated modified corn starch and working standard of pharmaceutical grade starch spectrums was similar as shown fig 4.5



(a)



(b)

Fig 4.5 Infra red spectrum of acetylated corn starch (a) and pharmaceutical grade corn starch (b)

The transmission minima (absorption maxima) in the spectrum obtained with the acetylated modified corn starch being examined corresponds in position and relative size to those in the spectrum optained with the pharmaceutical grade corn starch.

4.3.2 The granule structure and shape

The granular structures of corn showed no significant variations in shape and but in size. The size of corn starch granules ranged from 5 to 7 µm for small and 15 to 18 µm for large granules. The sizes and shapes of the acetylated corn starches were slight variations from native. The acetylation treatment brought granule fusion in both starches. The fusion of starch granules after acetylation could be attributed to the introduction of hydrophilic groups to the starch molecules, which resulted in increase of hydrogen bonding. Therefore, starch molecules coalescing together resulted in fusion of starch granules. These differences may be attributed to the differences in the granular structure of the starches. Corn starch granules have been reported to be more rigid and show more resistance towards modification [Singh et al., 2003].

4.3.3 Bulk density and water binding capacity

There is no significant difference could be recorded for bulk density between native and acetylated modified corn starch. However, the water binding capacity (WBC) of native starch is higher than of acetylated modified corn starches. The low WBC of acetylated modified starch samples may be due to the reduction of amorphous region in the starch granules. This reduces the number of available binding sites for water in the starch granules (Lawal, 2004).

Table 4.11 Result of bulk density, water binding capacity and pH

Starch Samples	Bulk density (g/cm^3)	WBC (g/g)	pH
Native Starch	0.725	10.50	5.47
Acetylated Modified Starch	0.726	8.45	5.89

Where: WBC: water binding capacity

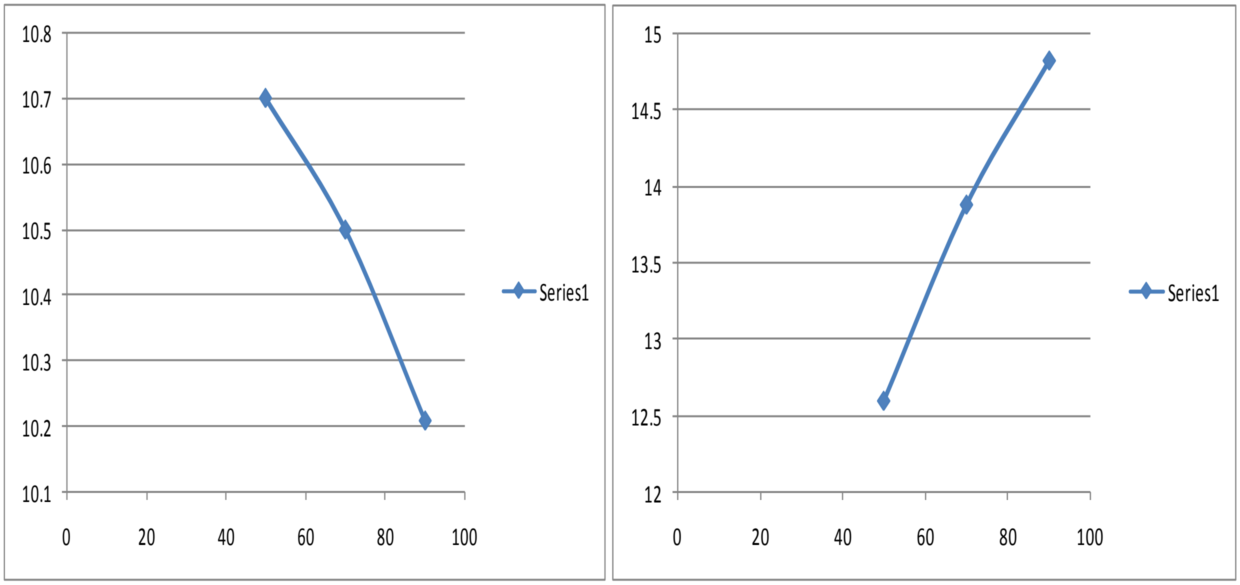
The pH values ranged between 5.0 and 7.0 of acetylated modified corn starch this indicate that it specifies the limit of pharmaceutical grade starch (BP, 2010).

4.3.4 Swelling power and solubility of native and modified corn starch

The low swelling power of native corn starch may be attributed to the presence of lipids in cornstarch (Galliard & Bowler, 1987). The acetylation treatment increased the swelling power of the native corn starch. This might be due to the presence of hydrophilic substituting groups that retained water (Betancur, Chel, & Canizares1997). Acetylation also increased the solubility of starches. The swelling power and solubility of native and acetylated modified corn starches at different temperatures 50, 70 and 90 °C are shown below and the effect graphically on fig 4.6 and 4.12

Table 4.12 shows swelling property and solubility

	Swelling power(g/g)			Solubility(g/g)		
Temperature	50°c	70°c	90°c	50°c	70°c	90°c
Native starch	10.7	10.50	10.21	0.215	0.251	0.29
Acetylated modified corn starch	12.6	13.88	14.82	1.42	1.29	1.98



(a) (b)

Fig 4.6 (a) and (b) shows temperature(X) vs swelling property(Y) of native and acetylated corn starch respectively

4.4 Characteristics of modified corn starch

4.4.1 Solubility

The modified acetylated corn starch was practically insoluble in cold water and in ethanol (96 per cent).

4.4.2pH

The pH was 5.89 within the limit from 4.00 to 7.00.

4.4.3 Foreign matter

It appears as either angular polyhedral granule of irregular sizes with diameters ranging from about 2 µm to about 23 µm or as rounded or spheroidal granules of irregular sizes with diameters ranging from about 25 µm to about 35 µm. The central hilum consists of a distinct cavity or 2- to 5-rayed cleft and there are no concentric striations. Between orthogonally orientated polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum (USP 2010).

4.4.4 Moisture content

The moisture content of analyzed samples ranged between 4.67% and 6.20%, these obtained values were less than 20% and were acceptable (Wolfgang et al., 1999) because it is allowed commercially up to 20% of moisture in starch as raw material. The low moisture content is usually reflects the high stability during storage, the starches are protected from mold growth and give a high yield of dry weight.

4.4.5 Sulphated ash

The determination of sulphated ash is used to know the presence of inorganic compound and sulphat compound in organic compounds of raw material. The presence of this impurity in human body they react with other substance or human tissue they form toxic compound for our body. . In the case of unorganized drugs such as colophony,podophyllum resin, wool alcohols and wool fat. it is applied very widely to control the extend of contamination by non-volatile inorganic substance, as aspirin, benzyl benzoate,i cyclizine hydrochloride and lignocaine hydrochloride (lidocaine hydrochloride).The test is also used to control traces of alkali metals in chloroform in the presence of potassium hydroxide. The substance is ignited with concentrated sulphuric acid,with decomposed and oxidized organic matter,leaving a residue of inorganic sulphates.reproduciable result are more Readily obtained in this determination than in a total ash determination because,in general,metal sulphates are stable unless heated very strongly.

So the concentration of the inorganic and sulphate compound must be known and have limit. The result was between 0.11 up to 0.43 percent. It is not greater than the limit 0.60 percent [BP 2007] as shown Table 4.13.

Table 4.13 Results of LOD, Shlphated ash, Oxidizable Substance and Sulpher dioxide

Modified starch	Moisture Content	Sulphated Ash (%)	Oxidizable substance	Sulpher dioxide
1	4.68 %	0.11	0.8ml	1.2ml
2	5.61 %	0.24	1.1ml	1.5ml
3	6.20 %	0.09	0.6ml	1.1ml
4	5.96 %	0.12	0.8ml	1.6ml
5	4.67 %	0.43	0.6ml	1.5ml

4.4.6 Oxidizable substance and Sulpher dioxide

The oxidizable substance and sulpher dioxide was also in the limit. The titration was not taking more than 1.4 ml of 0.002 M sodium thiosulphate is required (0.002 per cent, calculated as H_2O_2) less than the limit 0.78ml. In the case of sulpher dioxide it consumed 1.38ml of 0.01N Iodine less than the limit of 2.7ml.

4.4.7 Iron

Iron is an essential trace element primarily derived from dietary source that is necessary for the formation of red blood cells. Iron deficiency will lead to anemia (low levels of red blood cells), While persistently high levels can be toxic to the body organs. The iron test was complies show pink color in the test solution is not more intense than that in the standard.

4.5 Compare acetylated corn starch with commercially available pharmaceutical grade corn starch

By comparing the result of acetylated corn starch and pharmaceutical grade corn starch as shown below Table 4.14 it was conclude as follow.

Table 4.14The characteristics result of Acetylated and Pharmaceutical grade starch

Test parameter	Acetylated Starch	Pharmaceutical grade starch
Solubility	Complies	Complies
pH	5.89	4.38
Foreign matter	complies	complies
Moisture content	5.424%	9.23%
Sulphated ash	0.378%	0.30%
Oxidizable Substance	0.78 ml	1.2 ml
Sulpher dioxide	1.38 ml	2.2ml
Iron	Complies	Complies

From the above result as shown Table 4.14 the acetylated modified corn starch and the pharmaceutical grade starch commercially (Batch No STEX1407295 imported from India Supplier Maize product) available result was similar. The synthesized acetylated corn starch is used in pharmaceutical industry for tablet formulation as a binder of active and inactive ingredient.

Chapter 5

5. Estimation of starch production plant

A feasibility study can be defined as a controlled process for identifying problems and opportunities, determining objectives, describing situation, defining outcomes and assessing the range of cost and benefits associated with several alternatives for solving the problem (Thompson, 2003). Prior to establishing a new modified starch processing from local corn in order to utilize the potential from the sector, it is essential to carry out detail feasibility study to determine the potential of the business. A feasibility study for establishment of modified starch from corn processing plant consists of: Availability of raw material and seasonal variation, Market availability for finished products and semi processed products, Quality of raw material in the varieties needed for the various types of finished products, Processing capacity related to raw material availability ,quantity and seasonal variation, Processing equipments capacity for the mentioned points , Availability of workforce in the area and resources for training them to the required level, Availability of utilities: (electricity , running potable water, adequate waste disposal), Transport access to raw material fields and to products market.

5.1 The starch production

The corn wet milling process begins with the production of starch slurry. This slurry can be further processed to produced starch, modified starch, dextrin, corn sweeteners, and ethanol.

5.1.1 Production of starch slurry

First, shelled and cleaned kernels are placed in steep tanks and soak in water containing small quantity of sulfur dioxide or Sodium Meta bisulfate for 24 to 48 hours at a temperature of 50 ˚C. This process allows for extraction of soluble materials from the kernel. The sulfur dioxide or Sodium Meta bisulfate prevent fermentation and helps to separate the starch and protein. After steeping is completed, steep water is drained from the kernels and concentrated. This concentrated steep water is primarily used in production animal feed products.

Next, the kernels are ground in attrition mills to loosen the hull. Water is added to the mills, creating a mixture of macerated slurry and whole germ. This slurry is placed in hydro cyclone separators, which remove the lighter germ. The germ is then dried and either sold as is or further processed into corn oil and germ meal. The corn oil can be either refined to make a salad oil or cooking oil or a raw material input to margarine. The germ meal is used in the production of animal feed.

The remainder of the kernel, including the hull, gluten, and starch components, is sent through an additional series of grinding and screening process. After passing through a grinding mill, the hull particles are caught on screens, while the gluten and starch particles pass through. The hulls are later used to make animal feed or refined corn fiber.

The remaining slurry of gluten and starch, or mill starch, is then separated by centrifugation. The gluten is dried and either sold as corn gluten meal (60% protein) or used in producing corn gluten feed (21 % protein). The starch slurry is then washed and dewatered using filters or centrifuges. At this stage, the starch slurry can go through a number of processes that can yield starch, modified starch, dextrin or corn sweeteners and ethanol.

5.2 Market study and Plant capacity

5.2.1 Market study

The country's requirement of modified corn starch for pharmaceutical purpose and starch for other industry are mainly met through import. Import of corn starch in the past twelve years is provided in Table 5.1

Table 5.1 Imported starches

Year(GC)	Customs value (Birr)	Net Weight (tons)
2003	1,855,216	436.7
2004	1,243,253	253.85
2005	855,533	177.69
2006	1,655,321	324.64
2007	1,609,544	235.15
2008	141,251,858	23,751.47
2009	2,833,164	362.71
2010	4,762,674	458.04
2011	3,877,737	321.02
2012	8,435,780	544.99
2013	16,580,269	888.02
2014	14,039,169	671.24

Source: - Central statistical agency

The imported starch quantity varies from year to year sometimes increased and sometimes decreased but its demand is an increasing one. As shown the above table (Table 5, 1) the price increase from year to year per Kg of corn starch without including other charges and duty. The demand will be increase more than 50% due to government transition from agriculture to industrial.

5.3 Plant capacity and production program

5.3.1 Plant Capacity

The plant is envisaged to produce 800 ton/year, in 300 working days and operating 16 hrs per day.

The major buildings and civil works include:

- Buildings for production, offices, workshops and warehouses. The total area required is about 1000 m^2.

- Total land requirement including sewers, storage, open spaces etc. is estimated to be 1,500 m^2.

5.3.2 Production program

The production program is shown in Table 5.1. The production program is set by considering 300 working days per annum.

Table 5.2 Production program

Year	1	2	3	4
Capacity utilization (%)	70	80	90	100
Production Program	560	640	720	800

5.4 Material and Energy Balance

Material and energy balance are fundamental in the engineering approach to design a new processes or analyzing the result of in plant tests, play an important role in process streamlining, economic analysis of alternative technologies to deliver safe and nutritious food products meeting all quality specifications, waste reduction, and selecting process modifications to increase yield (Brian and Daniel).

5.4.1 Material Balance

Corn, also named maize, is a cereal grain and is known for being a high-yielding variety of cereal grains. There are multiple types of corn which are used for various purposes like grain, cattle, seed, or cobs for consumption. In this section, only the wet milling of the industrially processed dent corn is described. Dent corn, getting its name from the dent in the crown of the seed, is grown more than any other type of corn. Millions of tons of grain are produced from dent corn, and is used for human and industrial use, and for livestock feed. The starch reaches the summit of the seed, and the sides are also starchy. The denting is caused by the drying and shrinking of the starch.

Balance of a material for a process gain better control of process, energy efficiency, water conservation, optimization of throughput yield maximization, quality improvement. Therefore the raw material require for production of corn starch has obtained as:

Flowchart of starch production

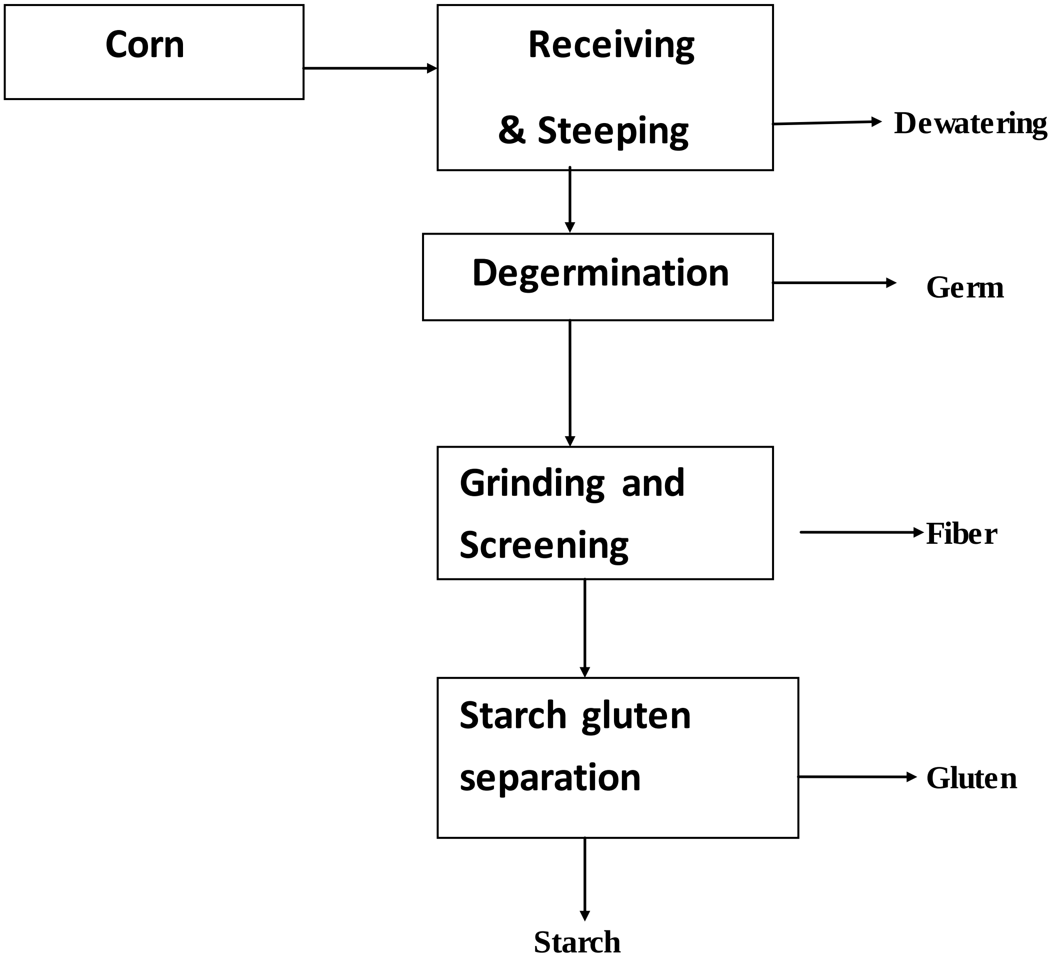


Fig 5.1 Flowchart of wet milling of corn starch production.

From resulted 1Kg of corn we get 55% starch (0.55Kg), 10% germ (0.100Kg), 8% fiber (0.08Kg) and 27% gluten (0.270Kg)

1Kg = 0.55Kg starch

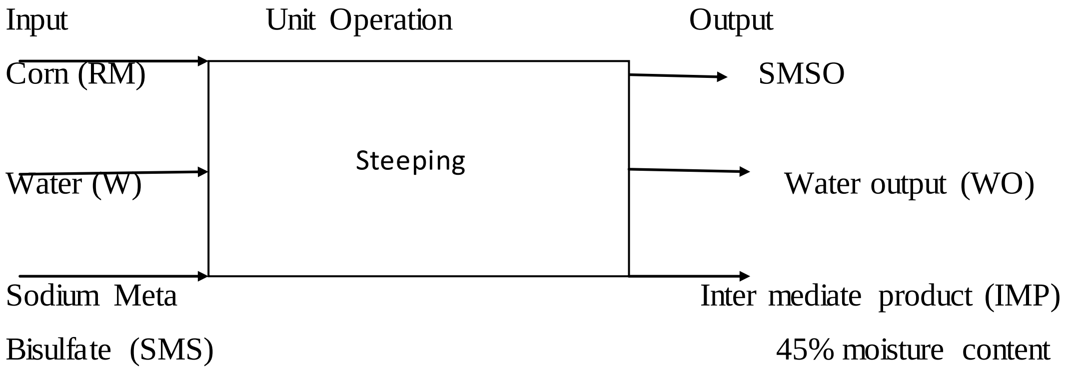
= 0.10 Kg germ

= 0.08 Kg fiber

= 0.27 Kg gluten……………………………………………….5.1

The amount of raw material required to obtained 500 tons of pure starch is

- 1Kg corn = 0.55Kg starch
  X corn = 560 tons of starch
  X = (560 tons of starch x 1Kg corn)/ (0.55 Kg starch)
  = 1,018.2 tons of corn required
- 1Kg of corn need 1L of water
  1,018.2tons of water per annual only starch production
- 0.5% Sodium meta bi sulfite (SMS)
  0.5gm per 100ml=5gm per 1L
  0.005 Kg SMS = 1L
  X = 1,018.2tons
  X = (0.005 Kg SMS x 1,018.2tons) /1L
  =5.091 tons SMS per annual



RM + W + SMS = SMSO + WO + IMP ……………………………………..5.2

WO = W (1-0.45)………………………………………………………5.3

= 1,018.2 (0.55) = 560.01 tons of water out put

W = 1,018.2 – 560.01

= 458.19 tons of water reaming in to intermediate product

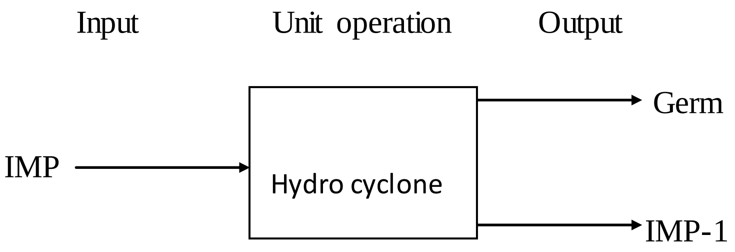
But, SMS = SMSO = 5.091 tons

45% moisture content of intermediate product from Eq. 5.1

RM + W + SMS = SMSO + WO + IMP

1,018.2 + 1,018.2+ 5.091 = 5.091 + 560.01 + IMP

IMP = 1,476.39 tons of IMP (i.e. IMP = water + germ + starch + fiber + gluten)



IMP-1 = Germ + IMP……………………………………………………5.4

From Eq. 5.1 1Kg corn contain 0.1 Kg germ

1,081.2tons corn contain X amount of germ

X = (1,018.2 tons x 0.1 Kg) / 1Kg

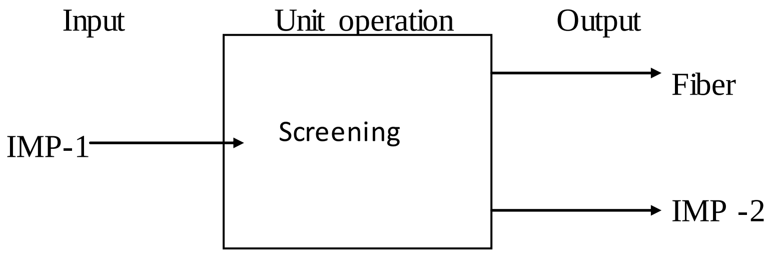
X= 101.82 tons of germs

From Eq. 5.4 IMP = Germ + IMP-1

1,476.39 = 101.82 + IMP-1

IMP = 1,476.39-101.82

= 1,374.57tons of IMP-1 (i.e. IMP-1 = starch + water + gluten + fiber)



IMP-1 = Fiber + IMP-2 ……………………………………………5.5

From Eq. 5.1 1Kg of corn contain 0.08Kg fiber

1,018.2 tons of corn contain X amount of gluten

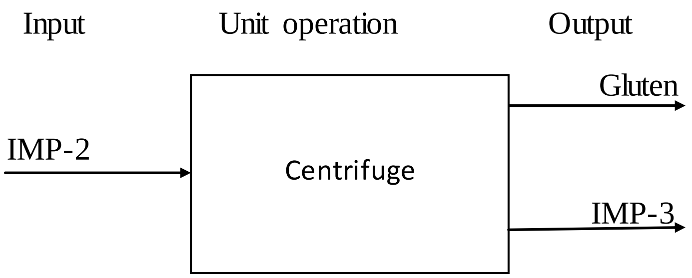
X = (0.08 Kg fiber x 1,018.2 tons) / 1Kg

X = 81.46tons of fiber

IMP-2 = IMP-1 – fiber

=1,374.57tons of – 81.46

= 1,293.11tons of IMP-2 (i.e. IMP-2 = starch + water + gluten)



IMP-2 = Gluten + IMP-3…………………………………………………..5.6

From Eq. 5.1 1Kg corn contain 0.27Kg gluten

1,018.2tons corn contain X amount of gluten

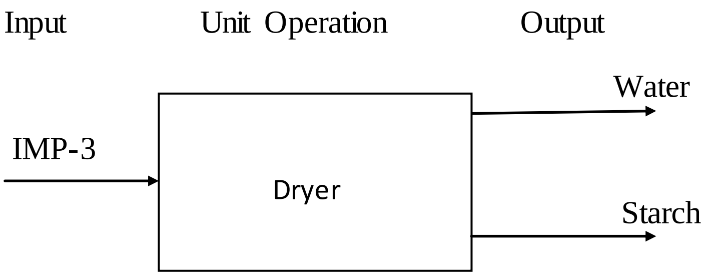
X = (1,018.2 x 0.27) / 1 Kg

= 274.91 tons of gluten

From Eq. 5.6 IMP-2 = Gluten + IMP-3

1,293.11 = 274.91 + IMP-3

IMP-3 = 1,018.196 tons of IMP-3 (i.e. IMP-3 = starch + water= starch slurry)



IMP-3 = water + starch ……………………………………………………….5.7

The moisture contain of commercially available corn starch is up to 15%

Water = 1,018.2(0.45-0.15)

= 305.46 tons water output by drying

From Eq. 5.7 IMP-3 = water + starch

1,018.196 tons = 305.46+ starch

Starch = 1,018.196 – 305.46

=712.74 tons per annual of starch

Annually 803 tons of pure starch produced with 15% of moisture content.

5.5 Annual output product

Table 5.3 Annual output product

Item	Output product (tons)
Corn starch	**712.74**
Fiber	**81.46**
Gluten	**274.91**
Germ	**108.12**

Training requirement

The technical personnel of the plant should be trained by qualified engineers of the machinery supplier. The cost of training shall be Birr 20,000

5.6 Plant Machinery

5.6.1 Production Process

The production process of starch varies depending upon the type of raw material used. However, the method of starch production starts with crushing or grinding of the raw material to destroy its tissues. Thus, in this way the starch is obtained from within the tissues. Hence, the simplified production process is outlined as follows.

- **Steeping:** The purpose of steeping is to soften the kernels so that subsequent milling operations and separations can be carried out efficiently. This unit operation is carried out for about 40 hours.

- **Degermination and Separation:** In this operation, oil-rich germ is separated from starch, gluten, hulls and fiber. First, the corn is ground in attrition mills. This material, leaving the mills, is a mixture of water, starch, gluten, germs and hulls. It is further fed to mills which grind the material to a very small particle size. After removing the hulls and fiber particles by passing it on a set of hydrocyclones, the starch and gluten are allowed to separate using centrifugal separators.

- **Drying:** The purified starch milk is dewatered by means of a thickening filter in order to reach a dry solid content suitable for the following drying. Drying is operated by dosing the starch into the dryer at controlled temperature maintained by an air conditioning unit. The dry starch is then separated by the air stream and stored and packed in 100 kg polypropylene bags

Table 5.4 List of Machinery and Equipment

No.	Item	Qty	Price (birr)
1	**Steeping tanks**	**4**	**90,000**
2	**Mills**	**2**	**80,000**
3	**Screen bends**	**2**	**70,000**
4	**Hydro cyclone**	**2**	**125,000**
5	**Centrifugal Machine**	**2**	**101,000**
6	**Dryers**	**2**	**105,000**
	FOB		**88,200**
Total			**659,200**

Source: Jinan Zhuoda Machinery Equipment co., Ltd. and Frigmaires International Mumbai

5.7 Cost Estimation

Plant capacity	800 tons of corn starch
Number of shift	Two
Working days per year	300

5.8 Man power requirement

The manpower requirement of the plant and the monthly and annual salary expenditure are shown in Table 5.5

Table 5.5 Man power

No.	Manpower	Quantity	Monthly Salary	Annual Cost
1.	General Manager	1	7,000	84,000
2.	Technical Manager	1	5,000	60,000
3.	Human Resource	1	3,000	36,000
	Manager	1	4,000	48,000
4	Production Head	2	2,000	48,000
5.	Supervisor	3	2,500	60,000
6.	Chemist	5	1,500	90,000
7.	Skilled Operator	5	1,000	60,000
8.	Semi-skilled operator	2	2,500	60,000
9.	Maintenance	1	2,000	24,000
10.	Accountant	1	2,000	24,000
11.	Sales man	5	1,000	60,000
12.	Unskilled labor			
	Total	28	35,000	654,000

5.9 Fixed investment cost

Table 5.6 Initial investment cost

No.	Cost Items	Total cost
1	Plant Machinery	659,200
2	Land lease value	79,000
3	Building	6,000,000
4	Office furniture, Fixture, Equipment	500,000
5	Total	7,238,2000
6	Working capital= 0.15 x 7,150,000	1,085,700
	Total capital investment	8,323,930

5.11 Cost of utilities

Table 5.7 Cost of utility

No.	Utility	Unit	Annual Consumption	Cost (birr)
1	Furnace	m^3	10	25,500
2	Water	m^3	819.7	14,877.2
3	Electricity	KWH	25.000	50,000
Total Costs				90,377.2

i.e. water used for steam generation and other purpose

5.12 Cost of raw materials

Table 5.8 Cost of raw materials

Item	Quantity	Cost per Kg	Cost (birr)
Corn	1,018.2tons	5 birr	5,091,500
Sodium Meta bisulfate	5.091 tons	15 birr	76,365
Packing Materials (polypropylene bags)	803,000 pcs	0.5 birr	401,500
Total cost			5,569,365

5.13 Estimation of total production cost

Table 5.9 Total production cost estimation

Item		**Total cost, birr per annual**
I Manufacturing Costs	**A. Material Cost**	**5,569,365**
	B. Labor cost	
	a. Direct cost	366,000
	b. Indirect cost	288,000
	Total of B	**654,000**
	C. Utility	
	a. Electricity	50,000
	b. Furnace oil	25,000
	c. Water	14,000
	Total of C	**89,000**
	D. Production over heads	
	a. Employees benefit	400,000
	b. Maintenance & repairing	100,000
	c. Insurance	75,000
	d. Depreciation	400,000
	e. Production breakages and others	100,000
	Total of D	**715,000**
	Cost of Goods produced (A+B+C+D)	**7,027,765**
II General Expenses	**Sales and distribution expense**	**60,000**
	a. Administrative expense	**50,000**
	Total of General expense	**110,000**
Total product Cost (I + II)		**7,137,765**

5.14 Sales of product

Table 5.10 Sales of annual product

Product	Qty (tons)	Sales per Kg	Total price (birr)
Starch	712.74	18 birr	12,829,320
Germ	108.12	2birr	261,240
Gluten	274.91	2birr	549,820
Fiber	81.46	2birr	162,920
Total cost			13,803,300

5.15 Gross earning

It's expected the entire output product will be sold

Net sales =13,803,300

Cost of goods sold = 7,027,765

Gross profit = Net sales - Cost of goods sold = 6,775,535

General Expense = 110,000

Net profit before tax = Gross profit – General expense = 6,665,535

Profit tax (30%) = 1,999,660.5

Net profit after tax = Net profit before tax – profit tax (35%) = 4,665,874.5

Rate of return

ROI = (net profit) / Total investment cost x 100 = (4,665,874.5) / (8,222,500) x100

=56.74%

Payback period = (FIC) / (NP + depreciation) = (7,238,200) / (4,665,874.5 + 400,000)

= (12,555,000) /(5,065,874.5)

= 2.48years

The investment cost and income statement projection are used to project the pay-back period. The project's initial investment will be fully recovered within 2 years. The project can create employment for 28 persons.

Chapter 6

6. Conclusion and Recommendation

6.1 Conclusion

The goal of this research was to prepare a pharmaceutical grade corn starch from locally available corn. The corn variety was selected from Melkasa Research Center and the corn distributed in Ethiopia. By using the method that was described in experiment part it was prepared different degree of substituted acetylated starch that was low and high. A maximum degree of substitution 2.83 was obtained at a temperature 75 ˚C and at a time of 3 hr and a minimum degree of substitution 0.26 was obtained at a temperature 50 ˚C and at a time of 1 hr. According to the result obtained the maximum degree of substitutions from 0.60-2.83 are used for pharmaceutical purpose. Because pharmaceutical grade corn starch must be a high degree of substitution

6.2 Recommendation

The results of this study suggest further investigation on the following directions:

- Further study should be conducted on Effect of pH on acetylated corn starch with temperature and time
- Study on Micro biological test of high degree of substitute acetylated modified corn starch
- And also energy balance in feasibility study.

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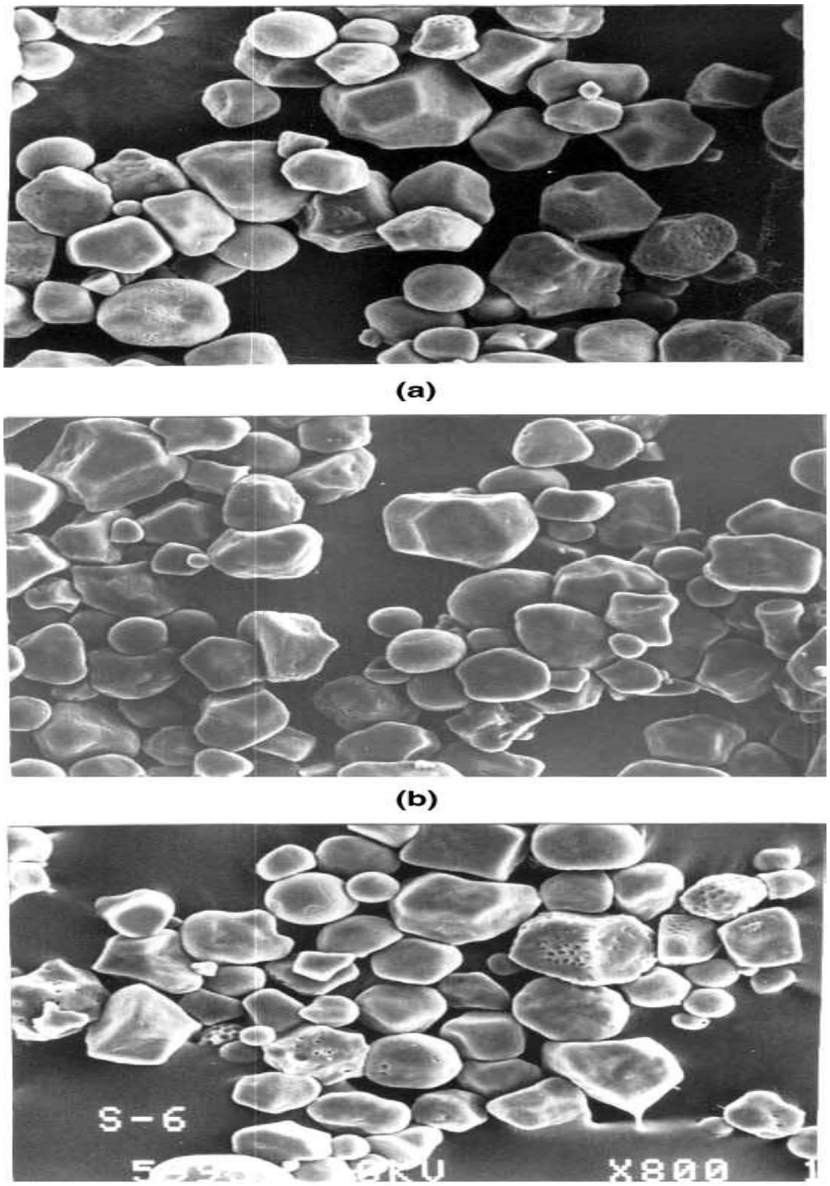
Annexes

Annex 1 Scanning electron micrographs (SEM) of native and acetylated corn starches structure



(a)

(b)

(c)

Fig 4.6 Scanning electron micrographs (SEM) of native and acetylated corn starches: (a) Native, (b) 10 g acetic anhydride, (c) 12 g aceticanhydride (800x, Bar=10 µm).
Source Department of Food Science and Technology, Guru Nanak Dev University, Amritsar 143 005, India