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SCHOOL OF CHEMICAL AND BIO ENGINEERING

OPTIMIZATION OF HYDROLYSIS FOR PRODUCTION OF BIOETHANOL FROM WASTE POTATOES AND POTATO PEELS

By: Fasil Alemayehu

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A Thesis Submitted to Addis Ababa Institute of Technology, School of Chemical and Bio Engineering in Partials Fulfillment of the Requirements for the Degree of Masters of Science in Process Engineering.

Advisor: Dr. S. Anuradha Jabasingh

Declaration

I, Mr. Fasil Alemayehu, declare that this thesis is my original work and has not been presented for the award of a degree in any university.

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List of acronyms

ANOVA	Analysis of variance
CAHP	Concentrated acid hydrolysis process
CCD	Central composite design
CRGE	Climate resilient green economy
DAHP	Dilute acid hydrolysis process
DDGS	Distillers dried grain and soluble
EU	European Union
FT-IR	Fourier transfer infrared
GHG	Greenhouse gasses
GTP	Growth and transformation plan
HMF	Hydroxymethyl furfural
LCB	Lignocellulosic biomass
LCW	Lignocellulosic waste
NIR	Near infrared
NPV	Net present value
pH	Power of hydrogen
RSM	Response surface method
SCB	Sugarcane bagasse
US	United State
UV	Ultraviolet
VIS	Visible
XD	Xylitol dehydrogenase
XK	Xylulokinase
XR	Xylose reductase

Abstract

Green plants and factory byproducts containing carbohydrate can be a source of a product like bioethanol. The major problem of these raw materials is the content of the type of carbohydrate. In this study, waste potatoes and potato peels was chosen as a carbon source; however, a pretreatment process is needed to convert starch of potato to fermentable sugar through hydrolysis. In order to obtain maximum fermentable sugar, optimal value of factors for hydrolysis was determined by Response Surface Methodology (RSM) using Central Composite Design (CCD). The optimum combination of temperature, liquid-solid ratio, and time was determined.

Waste potatoes and potato peels were washed, cut, dried at 105 °C for 24h and ground to the particle size of 2 mm. After soaking in diluted acid for 24h, hydrolysis was carried out. Then after water was added. And then separation of fermentable and non-fermentable sugar with centrifuge was accomplished. Then fermentable part of the hydrolyzate goes to fermentation. After fermentation distillation was carried out for bioethanol separation.

Analysis of starch content of waste potatoes and potato peels and sugar content of hydrolyzate was carried. The starch content was analyzed with iodine solution and the sugar content was analyzed with phenol sulfuric acid. The analysis was done by using UV/Vis spectroscopy. The amount of starch content of waste potatoes and potato peels is 0.095 g/ml and 0.084 g/mL respectively. The optimal value of sugar content of waste potatoes and potato peels is 0.080 g/mL and 0.074 g/mL respectively. The amount of alcohol for waste potatoes and potato peels is 32.0% and 27.5% v/v respectively. The statistical analysis was accomplished by using Design Expert version 7.

***Key words**: sugar, optimum, starch, waste, hydrolysis, and analysis*

1. INTRODUCTION

1.1. Background

Bioethanol, which is one of the energy sources, is known to be a potential alternative to petroleum derived fuels and has the potential to meet the increasing demand of energy for industrial processes, heating and transportation. Bioethanol can be used as an alternative fuel to gasoline. Bioethanol can be produced by fermentation from several renewable sources, such as from potatoes, sugarcane and corn. Globally, there is a growing interest for the production of ecologically sustainable bio-fuels (Kilpimma *et al.*, 2009). In 2013, 24 billion gallons of bioethanol were produced worldwide (Yacob, 2013).

Bioethanol could be produced from rich sources like corn, sugar beet, sweet sorghum, sweet potato or from the abundant cheap cellulosic feed stocks like wheat straw, switch grass, wood etc. known as cellulosic ethanol. In order to prevent the use of staple crops, agricultural wastes like corn cobs, stover, sorghum stalks and byproducts from sugar industries like sugarcane molasses etc. are considered as cheaper sources of bioethanol (Meenakshi *et al.*, 2009). Potato is high value crop as a food source. During processing of potato, potatoes are wasted, therefore, the waste from restaurant, hotels, chips shops and potato processors can be utilized as growth media for the fermentation processes.

In order to ensure the country's continued development program, the national fuel security and sustainability, it is important to increase fuel utilization and satisfy the demand partly by locally produced fuels such as biofuel. The increasing demand of energy, the associated price hikes and environmental concerns have hit Ethiopian economy very hard. Therefore, it is not surprising that the government places high priority on alternative energy sources that partially or totally substitutes imported fuel (Adem, 2011).

Petrol and diesel are the most widely used fuels for automobiles in Ethiopia. Bioethanol can be blended with gasoline known as gasohol for use in vehicles. Ethiopia imports most of the oil from Middle East by spending valuable foreign currencies. If Federal Government regulations are adopted based on the Kyoto agreement, the mandatory blending of bioethanol with traditional gasoline in amounts up to 10% will result in a demand for large quantities of bioethanol. Many

countries have implemented, or are in the process of implementing, programs providing for the addition of bioethanol to gasoline. Fuel ethanol production has increased remarkably due to the global demand to reduce oil importation, thereby contributing towards boosting rural economies and improving air quality (Arapoglou *et al.*, 2010).

Changing life style and craving for processed foods are factors responsible for the significant growth of global food processing industries. Among several varieties of processed foods, the products from potatoes are in great demand. Potato is a starchy tuber with carbohydrates constituting as much as of 13-30% with a little protein (0.7-4.6%). The conventional peeling method presently followed in the processing plants for removing the potato peel, also result in the loss of some portion of the mash which is rich in starch (Chintagunta *et al*., 2015).

This research gives an insight about the production of bioethanol by using acid hydrolysis for the conversion of waste potatoes. And it was tried to cover the production of bioethanol from damaged and wasted potatoes and potato peels are attempted in this study. The fermentation was carried out by using *saccharomyces cerevisie.* The analysis of raw material and glucose content after hydrolysis was carried out. In addition to this the yield of bioethanol from waste potatoes and potato peels was investigated at specific conditions. The processing loss during production must be reduced by employing efficient peeling methods to obtain maximum economic returns and at the same time the disposal problem needs to be addressed by converting the waste into value added products.

1.2. Statement of the problem

The question of sustainable and cleaner energy resources has become prominent in the past few decades. Most countries depend mainly, in some cases almost completely, on fossil fuels. Thus, security of petroleum supply or other sources of energy which can replace petroleum is critical for Ethiopia to diversify the energy mix. However, the future of petroleum products reserve is uncertain with increase in price that makes the foreign currency expenditure intolerably high and affect transport tariff and price of other commodities negatively.

Moreover, due to environmental concerns about air pollution caused by the combustion of fossil fuels, the search for alternative fuels has gained importance. The use of staple crops (like corn,

maize) for biofuel production may cause inflation of cost of these crops leading to food insecurity. To solve such problems, wastes coming from food processors, hotels, restaurants, cafeterias, chips shops and juice house should be used to produce alternative energy. Converting wastes to energy helps to protect the environment from pollution.

Therefore, to produce alternative energy sources require investigation how to produce, with what mechanism and technology, and from what raw materials. In order to obtain maximum energy value from such biomass selection of pretreatment and process design is necessary (Moshi *et al*., 2015). Since bioethanol is a bioenergy source for the future, there is a need to investigate economical bioethanol production from available raw materials. Bioethanol could decrease the dependence on foreign oil and give an opportunity to use wastes coming from potatoes as a carbon source. However, ethanol fermentation from waste potatoes and potato peels still needs to be studied in order to get high yield from the fermentation process. The conversion process of potato waste to glucose should be studied to get maximum amount of bioethanol.

1.3. Goals and objectives

1.3.1. Goal

The main goal of this research was to optimize hydrolysis variables for production of bioethanol from waste potatoes and potato peels.

1.3.2. Specific objectives

- To determine starch content of waste potatoes and potato peels.
- To determine the optimal value of temperature, liquid-solid ratio and time for hydrolysis.
- To produce bioethanol from waste potatoes and potato peels.

1.4. Significance

The production of bioethanol, a type of bioenergy, is explored from various sources all over the world and has immense benefit. Bioethanol produced from waste potatoes and potato peels could serve as a fuel volume extender, an oxygenator, and an octane enhancer. When blended with gasoline, it could increase the octane rating and the oxygen content of the fuel, which results in

more complete combustion and reduction in exhaust emissions such as carbon monoxide and unburned hydrocarbons which have a problem (Izmirlioglu and Demirci, 2012).

This research studied bioethanol production from waste potatoes and potato peels and contributes to develop understanding on long-term bioethanol production and transport fuel substitution shifts in Ethiopia and thus provides a significant value to academic researchers, business societies, industrialists, policy makers, and all interested groups.

This research serves as a platform to give basic information on the development of bioethanol and to create further research on areas of data deficiency. The indication of areas lacking sufficient data and potential areas for additional study would direct academic researchers to select priority areas and undertake further study.

This work could offer important understanding on bioethanol in general and the status in Ethiopia. Recognizing the status in Ethiopia could be important to businesses to have a fundamental data and the opportunities thereon. This thesis attempts to indicate the suggestions connected with the expanded bioethanol development and use, as it is not well understood in the country. Knowing these issues would make possible for supply chain actors to consider them in their deed and when they set up new facilities, thereby available data could be acquired, analyzed, and improvement achieved.

In conclusion, this study urges the government to arrange stakeholders and different other concerning bodies to participate in bioethanol production. Furthermore, the result could strength the policies and strategies promoting green economy using biofuels, as alternative energy source.

2. LITERATURE REVIEW

This chapter consist of two sections, the first section presents background information on bioethanol, feed-stocks, pretreatments, hydrolysis, methods of bioethanol fermentation from waste of potatoes, producer microorganisms of bioethanol, and potato as a raw material for bioethanol production and the second part discusses global and local trends on bioethanol production.

2.1. Bioethanol

The ethanol obtained from biomass based waste materials or renewable sources is called as bioethanol. It can be used as a fuel, chemical feedstock, and solvent in various industries. It has certain advantages as petroleum substitutes, viz., alcohol can be produced from a number of renewable resources, alcohol as fuel burns cleaner than petroleum this aspect is environmentally more acceptable. It is biodegradable and thus, keeps a check on pollution and it is far less toxic than fossil fuels (Domínguez-Bocanegra *et al*., 2014).

Ethanol is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, - OH, bonded to a carbon atom. The word *alcohol* derives from Arabic *al-kuhul*, which denotes a fine powder of antimony used as an eye makeup. *Alcohol* originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage (Wondal, 2012).

Ethanol melts at –114.1 ℃, boils at 78.5 ℃, and has a density of 0.789 g/mL at 20 ℃. Its low freezing point has made it useful as the fluid in thermometers for temperatures below – 40 ℃, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators.

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction, symbolized by the simple equation is actually very complex,

and impure cultures of yeast produce varying amounts of other substances, including glycerin and various organic acids.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

2.2. Feedstocks for bioethanol production

Feed-stocks are typically grouped under the heading biomass and include agricultural residues, wood, municipal solid waste and dedicated energy crops (Kim and Dale, 2004). Biomass is seen as an interesting energy source for several reasons, the main reason being the contribution provided by bioenergy to sustainable development (Sanchez and Cardona, 2008). Resources are often available locally, and conversion into secondary energy carriers is feasible without high capital investments (Lin and Tanaka, 2006).

Different feedstock types are available for the production of bioethanol as it can be derived from any biological raw materials that contain sugar, such as sugarcane, sugar beets and potatoes, or materials that can be converted into sugar from starch or cellulose, such as corn, wheat and other cereals (Yacob, 2013). Ethanol may be obtained from the agricultural products, products from food and biomass processing industries that are divided into three groups: raw sugar e.g. sugar beet and sugar cane, raw starch e.g. corn, rye, and triticale and lignocellulose materials such as grass, wood (Gumienna *et al*., 2015).

2.2.1. Starch based feedstock

Starch containing crops form an important constituent of the human diet and a large proportion of the food consumed by the world's population originates from them. In spite of the large number of plants able to produce starch, only a few plants are important for industrial starch processing. The major industrial sources are maize, tapioca, potato, and wheat (van der Maarel *et al*., 2002). Amongst various starchy materials available throughout the world; corn, wheat, potato, and purified starch have been successfully utilized for the commercial production of bioethanol (Pervez *et al*., 2014). Root and tuber crops contain on average 70-80% water, 16-24% starch, and trace quantities of proteins and lipids (Peroni *et al*., 2006).

Grains. The most common grains used in alcohol fuel production are corn, barley, wheat and sorghum. With the exception of sorghum, all of these grains have been used extensively for years as feedstock for the production of beverage grade alcohols. Now, with the new demand for non-fossil liquid fuels, these same grains are being called upon as basic feedstock for production of fuel grade alcohols as well. At least in the early stages of the small scale ethanol production movement, grains have been the most popular and widely used feedstock of all varieties mentioned (U.S. Department of Energy, 1982).

Tubers. The most common tubers used in alcohol fuels production are potatoes and sweet potatoes. Both are common farm products containing high concentrations of starch and are excellent feedstock for conversion to alcohol fuels.

Nevertheless, despite of using staple crops for ethanol production, wastes coming from staple crops could be used for ethanol production. During starch and bioethanol processing from cassava in Asian countries like Thailand and Indonesia, enormous amounts of waste consisting of peels and pulp are generated. In Nigeria, the world leader in cassava production, cassava is mainly processed into gari (popular West African food made from cassava tubers) by micro, small and medium scale enterprises and for every unit of raw cassava processed, there is generation of 30%, 20% and 16% of solid (mainly peels and pulp), liquid and gaseous waste (moisture and cyanide), respectively (Moshi *et al*., 2015).

2.2.2. Sugar based feedstock

In sugar crops, the majority of the six-carbon sugar units occur individually or in bonded pairs. Once a sugar crop has been crushed to remove the sugar, no additional processing is needed prior to fermentation since the six-carbon sugar units are already in a form that the yeast can use. This fact is both an advantage and a disadvantage. Preparation of the feedstock for fermentation involves comparatively low cost equipment, labor, and energy costs, since the only major steps involved are milling and extraction of the sugar. However, sugar crops tend to spoil easily. Numerous types of microorganisms, including the type of yeast that produces ethanol thrive on these crops during storage because of their high moisture and sugar content.

Sugarcane: Sugarcane is considered a favorable feedstock because of its high yield of sugar per acre, as high as 50 tons per acre per year and a correspondingly high yield of crop residue, known as bagasse that can be used as a fuel for production of process heat. The major drawback with this feedstock is the limited availability of land suitable for economical cultivation.

Brazil is the largest producer of sugarcane with about 27% of global production and a yield of 18 million gram of dry sugarcane per hectare, and highest yield occurs in Peru, which produces more than 32 million gram of dry sugarcane per hectare (Kim and Dale, 2004). In Asia countries like India, Thailand, and Philippines, sugarcane is produced on small fields owned by small farmers. For example, India has around seven million small farmers with an average of around ¼ hectare sugar cane fields (Ayele, 2011).

Ethiopia is endowed with large areas of suitable low lands, rivers and conducive climate for sugar cane growth. The climate and soil types in the country have both proven to be highly conducive for sugar cane growth and productivity. However, the total area developed for the production of sugar cane in the country is only about 8% of the total identified suitable areas. Experiences of existing sugar factories show that because of the suitable soil, adequate water and conducive climate, an average sugar cane production per hectare per month of the land under irrigation is very high as compared to other countries (Ethiopian Investment Agency, 2012).

Sugar beets: Sugar beets are a much more versatile crop than sugarcane. An important advantage of sugar beets is the comparatively high yield of crop coproducts: beet pulp and beet tops. Beet pulp, the portion of the root that remains after the sugar has been removed, is bulky and palatable and may be fed in either wet or dry form. Beet tops have alternative uses that include leaving them on the field for organic material, fertilizer and as cover to lessen soil erosion.

Sweet sorghum: Sweet sorghum is a name given to varieties of a species of sorghum, *Sorghum bicolor*. This crop has been cultivated on a small scale in the past for production of table syrup, but other varieties can be grown for production of sugar. The most common types of sorghum species are those used for production of grain. Sweet sorghum is one of the most drought resistant agricultural crops as it has the capability to remain dormant during the driest periods. Of the many crops being investigated for energy and industry, sweet sorghum is one of the most promising

candidates, particularly for bioethanol production principally in developing countries (Kumar *et al*., 2006).

2.2.3. Lignocellulosic based feedstock

Bioethanol can be produced from lignocellulosic biomass (LCB) such as crop residues for instance wheat straw, rice straw, corn stover, sugarcane bagasse (SCB), rice hulls, barley straw, sweet sorghum bagasse, and olive stones, hardwood, softwood, cellulose wastes, herbaceous biomass and municipal solid wastes (Ramadoss and Muthukumar, 2015). A large amount of lignocellulosic waste is generated through agro-industrial activities each year. These materials most often are underutilized and are sometimes disposed of in the environment without any treatment, leading to serious environmental pollution problems (Moshi *et al.,* 2015).

Lignocellulosic wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin. They may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, nonfood seeds, bagasse, domestic wastes (lignocellulose garbage and sewage), food industry residues, municipal solid wastes and the like. The lignocellulosic biomass, which represent the largest renewable reservoir of potentially fermentable carbohydrates on earth, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Due to their abundance and renewability, there has been a great deal of interest in utilizing LCW for the production and recovery of many value added products (Mtui, 2009).

Cellulose is a chain of six-carbon sugars called glucose joined by β-1, 4-glycosidic linkages. Hydrogen bonds between the chains lead to the formation of sheets lying on top of one another in a staggered position. This makes cellulose chemically stable, insoluble, and difficult to break down.

Hemicellulose is a highly branched heteropolymer containing sugar residues such as hexoses (D-galactose, L-galactose, D-mannose), pentose (D-xylose, L-arabinose), and uronic acids (Zalvidar *et al*., 2001). It strengthens the plant wall by forming a short-chain polymer that interacts with cellulose and lignin (Hauser, 2008).

Lignin is the most abundant aromatic polymer in nature, and is what makes up the woody part of the lignocellulose biomass. It is composed primarily of carbon ring structures interconnected by polysaccharides, which are very valuable chemical intermediates. Separation and recovery of these lignin structures is also very difficult to accomplish.

2.3. Pretreatments

Pretreatment of biomass is technically challenging and forms a large part of the process cost and therefore will need to be optimized prior to commercialization. Due to the different types of carbohydrates contained in biomass, a package of enzymes or microbes will be required for hydrolysis and fermentation; this package is a significant process cost and requires optimization. Pretreatment must meet the following requirements: Improve the formation of sugars or the ability to subsequently form sugars by acidic or enzymatic hydrolysis; avoid the degradation or loss of carbohydrate; avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes. Physical, chemical, physico-chemical, and biological processes have been used for pretreatment (Wondale, 2012).

The pretreatment techniques that can be used include acid, alkaline, biological pretreatment, wet oxidation, organosolv, ozonolysis, ultrasound pretreatment and hydrogen peroxide with metal salts pretreatment. These methods are significantly different from one another in terms of reaction conditions, process efficiency and complexity (Ramadoss and Muthukumar, 2015). In addition to these pretreatments the raw material should be washed and reduced in size.

2.4. Hydrolysis

Acids have been used to catalyze the hydrolysis of starch in starch cookers operating at temperatures of 50 to 150 ℃, a process referred to as acid hydrolysis (Wondale, 2012). Acid hydrolysis aims the mass production of fermentable sugar from starch. Two main developing or commercialized acid hydrolysis processes of starch; Dilute Acid Hydrolysis Process (DAHP) and Concentrated Acid Hydrolysis Process (CAHP) have been introduced.

CAHP is operated at the condition of high acid concentration (above 1M HCl) and at low temperature (below 70 ℃), which takes a long reaction time. The neutralization and the separation of acid are the main problems in CAHP.

DAHP is carried in a few second or minute at high temperature (between 150 and 200 ℃) and dilute acid (below 3 wt%). DAHP involves the undesired byproducts from degradation, reversion and retrogradation while it has some merits on the hydrolysis rate and alleviation of the neutralization. Starch hydrolysis has been also performed at moderate temperature between 90 and 120 ^{o}C for analytical application. This takes about 3h to complete the reaction in the case of 0.7M HCl (Hong and Bae, 1994).

The dilute-acid and concentrated-acid hydrolysis are relatively mature techniques for which no major improvements are likely to happen. Nevertheless, the enzyme cost is still high for the enzymatic process. The cost of enzyme in the processes has been calculated to be 10-20% of the total ethanol production cost (Wondale, 2012). Acid hydrolysis is used for alcohol production from potato industry starchy waste. However, byproduct of acid hydrolysis has inhibitory effects on yeast growth. Neutralization of hydrolyzers and expensive constructional equipment are also indicated as disadvantages of acid hydrolysis in industry.

2.5. Fermentation of waste of potatoes

Fermentation is a metabolic process of microorganisms to obtain energy by breaking down organic compounds. While microorganisms derive their energy, some byproducts are: lactic acid, butane, carbon dioxide, ethanol, cellulose, nisin. In ethanol fermentation, derivation of energy from sugars by either yeast or bacteria, produce carbon dioxide and ethanol are produced. Because yeasts produce their energy without the need for oxygen, ethanol fermentation is a facultative anaerobic process (Izmirlioglu and Demirci, 2012).

Utilization of waste potatoes and potato peels for high yield ethanol production is the main purpose of the fermentation of potato medium. Because potato is a starchy material the fermentation process of waste potatoes and potato peels starts with hydrolysis. To convert starch into the fermentable sugars, either acid hydrolysis or enzymatic hydrolysis needs to be performed.

The dilute-acid and concentrated-acid hydrolysis are relatively mature techniques for which no major improvements are likely to happen. Nevertheless, the enzyme cost is still high for the enzymatic process. The cost of enzyme in the processes has been calculated to be 10-20% of the total ethanol production cost (Wondale, 2012). Acid hydrolysis is used for alcohol production from

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Fermentation methods are important aspects of ethanol fermentation. Batch, semi-continuous, and continuous processes have been applied in the ethanol industry. There are also some other fermentation types, such as immobilized cultures.

2.6. Producer microorganisms of bioethanol

Microorganisms, termed ethanologens, presently convert an inadequate portion of the sugars from biomass to bioethanol. There are a number of microorganisms that produce significant quantities of bioethanol (Ayele, 2011). Yeast is the most commonly used microorganism in fermentation processes. Yeasts are minute, often unicellular, fungi. The yeasts used are typically bakery yeasts. Yeasts capable of fermenting the decaying biomass include, but are not limited to, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. Non-*Saccharomyces* yeasts, also known as non-conventional yeasts, are also used to make a number of commercial products. Some examples of non-conventional yeasts include *Kuyberomyces lactis, Yarrowiali polytica, Hansenula polymorpha and Pichia pastoris* (Wondale, 2012).

Microorganisms other than yeast can also be useful in making fermentation products. There are bacteria which are used in ethanol production called *Clostridium ijungdahlii*. Mid to long term technology under development are expected to improve the fermentation efficiency of the organism, producing higher yields in less time, and an organism requiring less detoxification of the hydrolysate (Wondale, 2012). Although *Zymomonas mobilis, Kluyveromyces spp., Schizo saccharomyces pombe,* and some recombinant bacteria and yeast can ferment sugars to ethanol, *Saccharomyces cerevisiae* is still the standard microorganism in the industry (Kunz, 2008).

2.7. Potato

Potatoes are starchy crops which do not require complex pre-treatments. Potato is a tuberous crop vegetable from the perennial *Solanum tuberosum* of the *Solanaceae* family. Potato is composed of 80% moisture, 18% starch and the remaining are protein, fat and minerals and is a high value crop

as a food source. Although potato is a high value product, the cost of bioethanol production can be decreased by using wastes from the potato industry (Abouzied and Reddy, 1986).

Potato is an important food and cash crop especially on the highland and mid altitude areas of Ethiopia. Potato provides food and income as a cash crop for over 2.3 million households in different part of Ethiopia. In recent years, potato production has dramatically increased by about 64%, from 349,000 tons in 1993 to 572,332 tons in 2010. Potatoes are mostly consumed after boiling and are incorporated as an ingredient. Nevertheless, there is an increasing demand for potato as an ingredient in other fast foods that entail salad and processed products such as French fries and crisps, as a result of dietary diversification among urban dwellers, emerging fast food restaurants and roadside small scale fryers (Mitiku *et al.,* 2015).

The direction of waste potatoes and potato peels for animal feed may be a risky option. Firstly, the material as it comes out as a waste has high moisture content, 70-80% which limits its value as an animal feed. Drying might prove very costly and increase significantly haulage costs. Second, the amount of protein, 8%, of dry matter is considered low for animal feed even for ruminants. However, this level may be enhanced by appropriate semisolid fermentation, (Han and Callihan, 1974) especially with the use of edible basidiomycete strains (*Lentinula edodes*, *Pleurotusostreatus*) which contain powerful lignocellulolytic enzymes (Arapoglou *et al*., 2010).

2.8. Bioethanol production in global

The production and use of bioethanol is increasing worldwide due to various driving factors explained in the preceding sections. The production in 1990 was only 4 billion gallons and it took 15 years for the quantity to double to around 8 billion gallons in 2005. But in recent years it only took three years to double again to around 17 billion gallons in 2008 and in 2011 around 23 billion gallons of ethanol is produced annually worldwide. Moreover, predictions indicate that the production would reach 34 and 48 billion gallons by the year 2020 and 2030 respectively as shown in Figure 2:1. With this the global future demand of bioethanol is expected to surpass the supply which signifies that there will be market opportunities for low cost producer in developing countries, especially for tropical countries with low labor and land costs (Yacob, 2013).

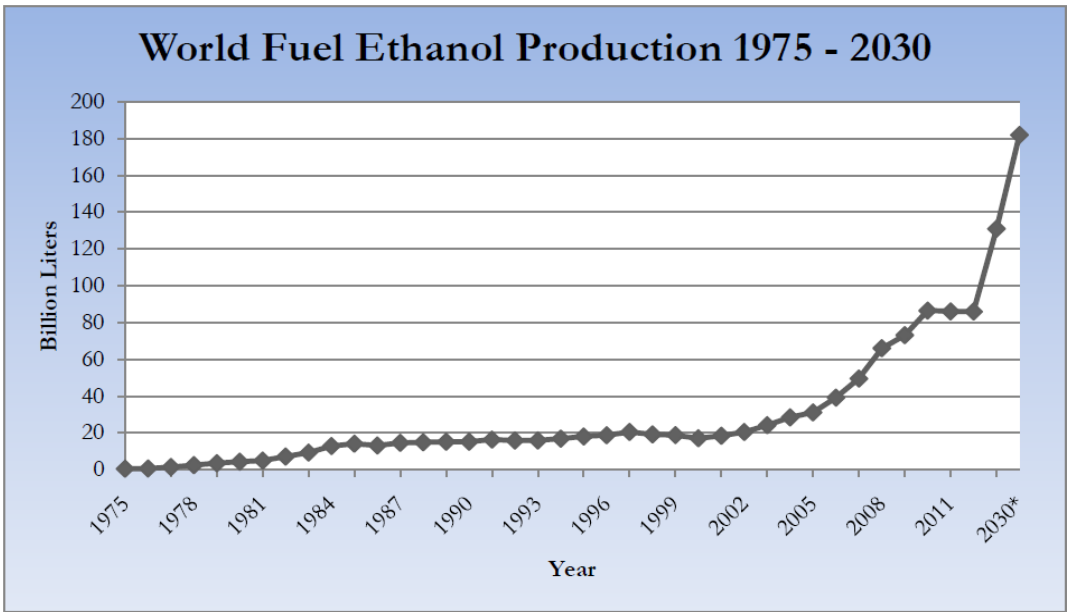

Figure 2:1 World fuel ethanol production trend

Source: (Yacob, 2013)

2.9. Bioethanol production in Ethiopia

Ethanol production in Ethiopia is linked with sugar factories and aimed for import substitute of petroleum products, enhance agricultural development and agro processing, job creation, and export earnings. However, only a small fraction of the potentials is utilized yet, 5% blend has accessed only in the capital city of the country. Moreover, at present only two of the sugar factories, Finchaa and Metehara, are producing bioethanol (Gebreegziabher *et al*., 2014).

Previously, there was only one biofuel factory in Ethiopia, a power alcohol plant that has been producing bioethanol as a byproduct at Finchaa Sugar Factory. Finchaa has a distillery, an ethanol plant annexed to its sugar mill with a capacity of 12 million liters per year. The plant was commissioned in 1998 and produces ethanol from sugarcane molasses, it had a stock of about four million liters of bioethanol at the end of December 2001. However, although the government had issued a directive allowing Finchaa to produce and sell fuel alcohol to oil companies, who would in turn blend it with gasoline and distribute it to motorists, it could not sell its fuel alcohol on the market at that time. The major reasons for the refusal of the oil companies appeared to be the need for rehabilitating the existing old fuel stations and lack of interest in investing in a fuel sales

operation that gives them little profit. This was also viewed as a lack of understanding and absence of commitment to alleviate one of the major problems of the country (Gebreegziabher *et al*., 2014).

Meanwhile, the corporation will also boost the current 11.1 million liters of annual ethanol production capacity of the country to 181.6 million liters and the current 100 MWh annual electrical energy generated through cogeneration to 607 GWh. And will enable the country to export 623,000 tons of raw sugar and 623,000 tons of white sugar which in total makes the amount 1,246,000 tons as a source of export earnings by the end of the GTP period. It also allows to use fuel ethanol to substitute imported gasoline through blending and to replace imported kerosene from abroad by the extra ethanol produced in order to minimize the foreign currency the country spent on oil import (Yacob, 2013).

Table 2.1 Summary of sugar factories production plan in Ethiopia

Year	Description	Unit	Sugar Factories					
			Wonji/Shoa	Metehara	Fincha	Tendaho	New	Total
2012	Annual Sugar Production	Million tons	0.075	0.137	0.11	-	-	0.322
	Annual Ethanol Production	m^3	-	12500	8000	-	-	18000
	Grid Power Generation	MW	-	-	-	-	-	0
2015	Annual Sugar Production	Million tons	0.174	0.137	0.27	0.62	1.049	2.25
	Annual Ethanol Production	m^3	10299	25500	20000	55400	70405	181604
	Grid Power Generation	MW	17	11	-	73	-	101
2020	Annual Sugar Production	Million tons	0.174	0.137	0.27	0.62	3.067	4.268
	Annual Ethanol Production	m^3	10299	25500	20000	55400	278942	390141
	Grid Power Generation	MW	17	11	-	91	-	119

Source: *Wondal, 2012.*

2.10. Uses of bioethanol

The main use of ethanol is as a motor fuel and fuel additive. Ethanol and other alcohols can be used to power motor vehicles instead of gasoline. In almost all cases the ethanol is mixed with gasoline (Robati and Vazirzadeh1, 2013). Efficient method for conversion of biomass into fuel is by ethanol production because ethanol is an economical as well as environmentally friendly fuel. Ethanol has the advantages of being renewable, cleaner burning and produces no GHG (Altintas *et al*., 2002).

A number of market segments are available in the ethanol industry serving a wide range of uses in the medical sectors, pharmaceuticals, beverages, industrial, household, and transport uses. The market potential for bioethanol is therefore not just limited to transport fuel or energy production but has potential to supply the existing chemicals industry and house hold uses. However, the most prevalent use of bioethanol in Ethiopia is as a transport fuel in spark ignition engine vehicles and the current amount of ethanol fuel blended with gasoline is 10% and the government is working to increase the share. The government is also working to start export in two years of time and to substitute household cooking fuel in the future (Yacob, 2013).

The other uses of ethanol are in alcoholic beverages. Alcoholic beverages vary considerably in their ethanol content and in the foodstuffs from which they are produced. Most alcoholic beverages can be broadly classified as fermented beverages, beverages made by the action of yeast on sugary foodstuffs, or as distilled beverages, beverages whose preparation involves concentrating the ethanol in fermented beverages by distillation. Fermented beverages can be broadly classified by the foodstuff from which they are fermented. Beers are made from cereal grains or other starchy materials, wines and ciders from fruit juices, and meads from honey. Fermented beverages may contain up to 15–20% ethanol by volume, the upper limit being set by the yeast's tolerance for ethanol, or by the amount of sugar in the starting material.

Absolute ethanol and 95% ethanol are themselves good solvents, somewhat less polar than water and used in perfumes, paints and tinctures. Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% (Ayele, 2011).

3. MATERIALS AND METHODS

The purpose of this section is to describe how to determine starch content of waste potatoes and potato peels, the hydrolysis of waste potatoes and potato peels in the laboratory, experimental designs to get the optimal value of glucose concentration, and the procedure of analysis of glucose concertation after hydrolysis for ethanol production from waste potatoes and potato peels by *Saccharomyces cerevisiae.* The experiments of production of ethanol from waste of potatoes were carried out in the laboratories of School of Chemical and Bio-Engineering at Addis Ababa University Institute of Technology.

3.1. Determination of starch content of waste potatoes and potato peels

To perform this analysis, the materials required are listed as follows with their functions. Graduated measuring cylinder were used to measure the solutions and distilled water. Test tubes were required to hold samples. Beaker to hold the solution of iodine. Magnetic stirrer to stir the iodine solution. Balance to measure the sample and ingredient. And the other equipment was spectroscopy to measure the absorbance based on the given concentration. Dropper to drop the iodine solution.

To work this analysis chemicals that are used for analysis were prepared. Standard starch solution was prepared by dissolving reagent grade starch in distilled water. The amount of starch solution is 1%. This was carried out by mass, therefore 1g starch/100g water. Water has a density of 1 g/mL, therefore 1g starch/100mL water. The other chemical required was iodine solution. Iodine solution was prepared as follows. For 100 mL of 0.1M solution, measure out 3.0 g of potassium iodide (KI) into an appropriate beaker. Moisten the potassium iodide with a few drops of water. Measure out 2.54 g of iodine and add to the moistened potassium iodide. Add a small volume of water and stir. Pour the solution into a graduated cylinder and dilute with distilled water to the final volume of 100 mL. If there are any bits of iodine remaining, return the solution to the beaker and leave it on a magnetic stirrer for several minutes.

Prepare the solution by adding starch and distilled water according to the Table 3.1. The standard solutions were numbered 1-5. The unknown solutions were labeled A, B, and C. For three replicate the samples were prepared.

Table 3.1 Criteria for preparation of starch solution

Tube	1	2	3	4	5	A	B	C
Starch solution (mL)	0	2.5	5	7.5	10	1.25	1.25	1.25
Distilled water	10	7.5	5	2.5	0	8.75	8.75	8.75

To do the analysis the procedure is, at first label 5 test tubes 1, 2, 3, 4, and 5. Place 10 mL of distilled water in test tube 1 and measure 2.5 mL, 5.0 mL, 7.5 mL, and 10 mL of the 1% starch solution into tubes 2 through 5. Add distilled water to each tube to reach a final volume of 10 mL. And then add one drop of iodine solution to each tube (1, 2, 3, 4, and 5, A, B, and C) and mix thoroughly. Vortex immediately, if possible.

Each solution was transferred to a cuvette, filling the cuvette approximately 2/3 full. If necessary, wipe each cuvette with a Kimwipe. Spectrophotometer was used to record the absorbance of each solution at 610 nm. The absorbance data from the tubes 1 through 5 were used to plot a standard curve, concentration versus absorbance. The plotted data was used to calculate concentration for tubes A, B, and C from their absorbance.

Safety: While using iodine crystal and potassium iodide for analysis try to use skin and eye protecting safety keeping devices. Solid iodine is a skin irritant. Iodine solution is damaging to the eyes. In case of contact with the eyes wash for 15 minutes and seek medical attention.

3.2. Determination of optimal value of temperature, liquid-solid ratio and time for hydrolysis

Hydrolysis

Hydrolysis was carried out at high temperature (90-110 °C); however, at low temperatures, it was possible and can contribute to energy savings (Duhan *et al*., 2013, Izmirlioglu and Demirci, 2012). Based on these literature this thesis work selected the amount of temperature required for hydrolysis of waste potato starch.

Waste potatoes were collected from Atikilt Tera, Addis Ababa City market and potato peel from chips shop and prepared for pretreatment, hydrolysis, fermentation and distillation. Sample preparation process includes, washing with running tap water, manual size reduction with knife,

drying, grinding and sieving after the samples were collected. The drying process was takes place in digital oven at 105 ℃ for 24h to prevent decomposition of the waste potatoes and potato peels during storage (Hauser, 2008, Duhan *et al.*, 2013). The dried waste potatoes and potato peels were ground using rotary mill.

Samples based on the waste potatoes or potato peels to acid solution ratio were put in Erlenmeyer flask. 1M of H_2SO_4 was used for this purpose. The ratio between waste potatoes or potato peels and acid solution was 1:2 (Tasic *et al*., 2008). The mixture of waste potatoes or potato peels and acid solution made 200 mL in Erlenmeyer flask. Then the solution was kept in autoclave for hydrolysis at a given temperature and time according to the experimental design. After that the solution was made dilute using distilled water to satisfy liquid to solid ratio of the experimental design.

And then the solid particles which were non-fermentable were removed from the liquid in the hydrolyzate by centrifugation. Then the separated solid part was washed with distilled water two times. The washing was performed in order to extract all soluble sugars. The non-fermentable part could be used as animal feed after drying or for soil fertilizer. These are the options for handling the byproduct of bioethanol production from waste potatoes and potato peels. The hydrolyzate was putted in refrigerator until used.

Experimental design

Response Surface Method (RSM) was employed to get optimal values of experimental factors for hydrolysis of waste potatoes and potato peels. RSM with a three-factor, three-level CCD was used. The actual factor levels corresponding to coded factor levels are shown in Table 3.2. The optimal values were estimated by statistical analysis using Design Expert.

Table 3.2 Levels of factors chosen for the experimental design of acid hydrolysis

	Name	Units	-1 Level	0 Level	+Level	-alpha	+alpha
A:	Temperature	^oC	70	90	110	56.3641	123.636
B:	Liquid to solid ratio	g/100mL	10	15	20	6.59104	23.409
C:	Time	h	1	2	3	0.318207	3.68179

Determination of sugar content

The sugar content was determined through phenol-sulfuric acid method by taking anhydrous D–glucose as standard as described below. For this analysis, balance, UV-Vis spectrometer, test tubes, measuring cylinder, and beaker, distilled water, sulphuric acid, D-glucose, and phenol were the major materials and chemicals that were used respectively.

The total sugar concentration was determined by using UV-visible spectrophotometer at 490 nm wavelength of glucose absorbance and the quantification was made from calibration curve using glucose as standard and calculation was performed by equation of the linear regression obtained from calibration curve.

Stock glucose solution was made by dissolving 0.5 g of glucose in 100 mL of distilled water. Various dilutions of the stock glucose solutions were made separately by pipetting a known volume of the stock solution (1, 2, 3, 4 and 5 mL) into a 100 mL volumetric flask and filling the volume with distilled water up to the mark. The concentrations made for this study were: 0.05, 0.1, 0.15, 0.2 and 0.25 mg/mL.

The basic principle of this method was that carbohydrates when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color. The standard procedure of this method was as follows. A 2 mL aliquot of a carbohydrate solution was mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm was recorded on a spectrophotometer. Reference solutions were prepared in identical manner as above, except that the 2 mL aliquot of carbohydrate was replaced by distilled water (Albalasmeh *et al*., 2013).

Safety: Phenol and sulfuric acid are skin irritating and dangerous for eyes and take safety measures. The reaction between sulfuric acid and phenol is exothermic and try to add sulfuric acid solution slowly.

3.3. Production of bioethanol from waste potatoes and potato peels

The objective of this experiment was to produce bioethanol from waste potatoes and potato peels. The fermentation starts from media preparation to fermentation in shaker incubator. Before conducting fermentation, the media for the preparation of yeast was prepared. For preparing one liter of media, the following ingredients were added: dextrose 20 g, yeast extract 5 g, Urea 4 g, $MgSO_4.7H_2O$ 1.0 g, peptone water 5 g and add distill water to make up one liter (Izmirlioglu and Demirci, 2012, Arapoglou *et al*., 2010). After checking for the pH and the adjustment of the pH in the range of 4.5-5.5 (Wondal, 2012) 5 g of yeast, *Saccharomyces cerevisiae* was added to the prepared media. The media was placed in a shaking incubator for 24 hours, at a temperature of 30 ℃ and 150 rpm. Temperature, revolution per minute and culturing duration of shaker incubator was adjusted. And the culture was placed for 24h. The sterilization was carried out at a temperature of 121 ºC for 15 minutes using autoclave.

The next step was fermentation of fermentable part. Currently, most bioethanol production is prepared by batch fermentation. Because batch operations have the advantages of low capital and operational costs, simple controls, and entire processes that do not require specialized labor, total sterilization and the supply of raw materials are easier to obtain than in other processes (Tian *et al.*, 2015).

To perform the fermentation the sample was conditioned to temperature of 30 ℃ before fermentation step is started. This is the temperature at which all fermentation experiments are carried out. Then the pH was checked before adding the cultured media and it was maintained by 10M of NaOH. The pH 5.0 was optimum for yeast culture. The cultured media and the hydrolyzate sample were mixed with the proportion of 1:10 in Erlenmeyer flask. Then after using aluminum foil the flasks were covered. At the end the mixture was placed in the shaker incubator at a temperature of 30 ℃ and 150 rpm for 72 hours. And after 72 hours of fermentation, the samples will be taken out and distilled.

Distillation was the final step in the production of ethanol from waste potatoes and potato peels. Distillation is a method used to separate two liquid based on the difference of their boiling points.

In this experiment simple distillation set up was used at a temperature of 85 ºC for 3 hours on oil bath. Oil bath was used in order to control the temperature at set point.

The ethanol concentrations of the samples collected every 3 hours of intervals by distillation of fermented solution was measured by alcoholmeter that is found in alcohol factory. The samples were checked at National Alcohol and Liquor Factory using Mettler Toledo alcoholmeter. This instrument enables to determine the percentage of alcohol in a solution.

To describe the functional group of ethanol FT-IR analysis were done in Natural Science College of Addis Ababa University, Department of Chemistry. The samples were examined and the result was checked by comparing with standard ethanol graph.

4. RESULTS AND DISCUSSION

In this section raw material and product analysis results are presented. The amount of starch concentration in the raw material, glucose concentration after hydrolysis and the amount of ethanol found in the samples was measured and the results are shown as follows.

4.1. Determination of starch content of waste potatoes and potato peels

The major contents of potato are moisture and starch. There are also other components in addition to these constituent, fat, protein and minerals are also existing. The starch content of waste potatoes and peels was investigated using iodine solution and with aid of Perkin Elmer Lambda 950 UV/VIS/NIR Spectrometer. From the calibration curve drawn using standard starch reagent at different concentration the amount of starch content of waste potatoes was 0.095 g/mL and potato peels was 0.084 g/mL. The amount of starch content of waste potatoes was higher than potato peels. This might happen due to potato peels contained lignin part, the outer coverage. The same amount of waste potatoes and potato peels might do not have the same amount of starch do to the presence high amount of lignin part on potato peels.

4.2. Determination of optimal value of temperature, liquid-solid ratio and time

Starch is polysaccharide and is not used by microorganisms for production of ethanol. Hydrolysis is necessary to convert starch to its monomeric building unit, glucose. The amount of glucose produced was found using phenol-sulfuric method and analyzed using Perkin Elmer Lambda 950 UV/VIS/NIR Spectrometer. The standard, calibration curve was drawn at different concentration of D-glucose with respect to absorbance and from that the amount of ethanol concentration was determined as shown in the Table 4.1 and Table 4.2.

Table 4.1 Concentration of glucose for waste potatoes

Glucose concentration (g/mL)						
Std	Run	Block	Factor 1 A: Temperature (℃)	Factor 2 B: Liquid to solid (g/100mL)	Factor 3 C: Time (h)	Response Glucose (g/mL)
17	1	Block 1	90.00	15.00	2.00	0.075
15	2	Block 1	90.00	15.00	2.00	0.084
18	3	Block 1	90.00	15.00	2.00	0.079
6	4	Block 1	110.00	10.00	3.00	0.063
16	5	Block 1	90.00	15.00	2.00	0.078
11	6	Block 1	90.00	6.59	2.00	0.061
9	7	Block 1	56.36	15.00	2.00	0.044
4	8	Block 1	110.00	20.00	1.00	0.060
13	9	Block 1	90.00	15.00	0.32	0.065
12	10	Block 1	90.00	23.41	2.00	0.073
2	11	Block 1	110.00	10.00	1.00	0.058
10	12	Block 1	123.64	15.00	2.00	0.064
5	13	Block 1	70.00	10.00	3.00	0.052
19	14	Block 1	90.00	15.00	2.00	0.083
1	15	Block 1	70.00	10.00	1.00	0.048
7	16	Block 1	70.00	20.00	3.00	0.050
8	17	Block 1	110.00	20.00	3.00	0.070
3	18	Block 1	70.00	20.00	1.00	0.055
14	19	Block 1	90.00	15.00	3.68	0.068
20	20	Block 1	90.00	15.00	2.00	0.073

By using Design Expert Software Table 4.1 was generated at a given combination of the factors. The laboratory experiment was carried out and the result in Table 4.1 was found for the response variable, glucose concentration with the change in temperature, liquid to solid ratio and time. The maximum amount of glucose yield is high at run number 2.

Table 4.2 Concentration of glucose for potato peels

Glucose concentration (g/mL)							
Std	Run	Block	Factor 1 A: Temperature (^{o}c)	Factor 2 B: Liquid to solid (g/100mL)	Factor 3 C: Time (h)	Response Glucose (g/mL)	Yield (%)
17	1	Block 1	90.00	15.00	2.00	0.0692	82.42
15	2	Block 1	90.00	15.00	2.00	0.0677	80.58
18	3	Block 1	90.00	15.00	2.00	0.0615	73.23
6	4	Block 1	110.00	10.00	3.00	0.0708	84.26
16	5	Block 1	90.00	15.00	2.00	0.0681	81.04
11	6	Block 1	90.00	6.59	2.00	0.0544	64.72
9	7	Block 1	56.36	15.00	2.00	0.0416	49.55
4	8	Block 1	110.00	20.00	1.00	0.0666	79.25
13	9	Block 1	90.00	15.00	0.32	0.0638	75.99
12	10	Block 1	90.00	23.41	2.00	0.0669	79.66
2	11	Block 1	110.00	10.00	1.00	0.0654	77.82
10	12	Block 1	123.64	15.00	2.00	0.0551	65.64
5	13	Block 1	70.00	10.00	3.00	0.0553	65.82
19	14	Block 1	90.00	15.00	2.00	0.0704	83.80
1	15	Block 1	70.00	10.00	1.00	0.0419	49.83
7	16	Block 1	70.00	20.00	3.00	0.0476	56.68
8	17	Block 1	110.00	20.00	3.00	0.0760	90.47
3	18	Block 1	70.00	20.00	1.00	0.0515	61.28
14	19	Block 1	90.00	15.00	3.68	0.0743	88.40
20	20	Block 1	90.00	15.00	2.00	0.0687	81.73

Here again using Design Expert Software Table 4.2 was generated at a given combination of the factors. Table 4.2 was found for the response variable, glucose concentration. The maximum yield of glucose is at run number 17. From Table 4.1. and Table 4.2, the amount of glucose concentration is high in waste potato compared to potato peels.

4.2.1. Optimization of experimental variables on hydrolysis of waste potatoes

Starch hydrolysis can be affected by many factors and for this study temperature, time and liquid to solid ratio was chosen. In order to study the combined effect of these factors experiments were performed for different combinations of these physical parameters using statistically designed experiments. The range and level of these factors is shown in Table 3.2.

To accomplish this task statistical analysis by using Design Expert version 7 was carried out. The main tasks done here were selecting the model based on literature and then making ANOVA analysis. After that the effect of the variables were checked and plotted to see the interaction of the factors and effect by using one factor plot, two factor plot, contour plot and 3D surface plot. In addition to this the optimal value of the factors were found from the optimization part of the software. The optimum amount of glucose concentration was found for waste potatoes.

4.2.1.1. ANOVA analysis for waste potatoes

The model chosen for this analysis was quadratic model. In order to check whether the quadratic model is the right model or not, it was crucial to perform analysis of variance (ANOVA). The probability, P-values were used to check the significance of each coefficient of regression model equation. The P-values of corresponding coefficient should be less than or equals to 0.05. This is the bench mark for checking the significance of the proposed model.

Appendix A shows that the model F-value of 13.34 implies the model is significant. There is only a 0.02% chance that the model F-value this large could occur due to noise. Values of Prob > F less than 0.05 indicate model terms are significant. In this case, A, A^2, B^2, and C^2 are significant model terms. Values greater than 0.10 indicate the model terms are not significant. If there are many insignificant model terms, not counting those required to support hierarchy, model reduction may improve the model. The lack of fit F-value of 1.05 implies the lack of fit is not significant relative to the pure error. There is a 47.78% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good; the model is needed to fit.

Development of regression model equation

The application of RSM gives an empirical relationship between the response function and the independent variables. The mathematical relationships between the response, glucose concentration and the independent variables temperature (A), liquid to solid ratio (B) and time (C) in terms of coded and actual factors can be determined by Design Expert software. The model equation that correlates the response (Y) to the hydrolysis process variables in terms of coded factor and actual factors for waste potatoes is shown by equation (4.2) and (4.3).

Final equation in terms of coded factors for waste potatoes

$$\begin{aligned}\text{Glucose} = {} & 0.079 + 5.86\times10^{-3}\,A + 2.57\times10^{-3}\,B + 1.47\times10^{-3}\,C + 4.34\times10^{-4}\,A\times B \\ & + 2.31\times10^{-3}\,A\times C - 5.31\times10^{-4}\,B\times C - 9.60\times10^{-3}\,A^2 - 5.23\times10^{-3}\,B^2 - 5.26\times10^{-3}\,C^2\end{aligned} \quad (4.1)$$

Where, A = Hydrolysis temperature

B = Liquid to solid ratio

C = Hydrolysis time

Final equation in terms of actual factors for waste potatoes

$$\begin{aligned}\text{Glucose} = {} & -0.20 + 4.32\times10^{-3}\,\text{Temperature} + 6.61\times10^{-3}\,\text{Liquid to solid ratio} + 0.02\,\text{Time} \\ & + 4.34\times10^{-6}\,\text{Temperature}\times\text{Liquid to solid ratio} + 1.16\times10^{-4}\,\text{Temperature} \\ & \times\text{Time} - 1.06\times10^{-4}\,\text{Liquid to solid ratio}\times\text{Time} - 2.40\times10^{-5}\,\text{Temperature}^2 \\ & - 2.09\times10^{-4}\,\text{Liquid to solid ratio}^2 - 5.26\times10^{-3}\,\text{Time}^2\end{aligned} \quad (4.2)$$

The results showed that the concentration of glucose produced from waste potato was dependent on the linear terms, on the quadratic terms and also on the interactions of variables. On the basis of the coefficients in equations (4.2) and (4.3) it was evident that the produced glucose concentration increases with the increase in temperature (A), liquid to solid ratio (B) hydrolysis time (C). Compared to hydrolysis temperature and liquid to solid ratio hydrolysis time has weighty effect on the produced glucose concentration.

Table 4.3 Model statistics for waste potatoes

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.011	0.2215	0.0755	-0.1065	2.94E-03	
2FI	0.012	0.2391	-0.1122	-1.3674	6.30E-03	
Quadratic	**4.52E-03**	**0.9231**	**0.854**	**0.6335**	**9.75E-04**	**Suggested**
Cubic	5.29E-03	0.937	0.8004	-4.696	0.015	Aliased

The predicted R-squared of 0.6335 is not as close to the adjusted R-squared of 0.8540 as one might normally expect. This may indicate a large block effect or a possible problem with the model and/or data. Things to consider are model reduction, response transformation, outliers, etc. Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.573 indicated on ANOVA analysis result is an adequate signal. This model can be used to navigate the design space.

In order to conclude from the analysis of variance adopted, the adequacy of the underlying model should be checked. And the normality assumption can be checked by constructing a normal probability plot of the residuals. An extremely useful procedure is to construct a normal probability plot of residuals. A normal probability plot of the raw data is used to check the assumption of normality. In the analysis of variance, it is usually more effective and straight forward to do this with the residuals. If the underlying error distribution is normal, this plot will resemble a straight line. In visualizing the straight line, place more emphasis on the central values of the plot than on the extremes.

Figures 4:1 to Figures 4:15 shows how exactly the produced glucose concentration is modeled. In Figure 4:1 the points line up well and the deviation of points for glucose concentration from normality is insignificant. In addition, the normal probability plot indicates the residuals following a normal distribution. In the case of this experiment the points in the plots shows fit to a straight line, this shows that the quadratic polynomial model satisfies the assumptions of analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

Ideally the normal plot is a straight line, indicating no abnormalities. There is no severe indication of abnormality, nor is there any evidence pointing to possible outliers. The points are coded by color to the level of response and they represent going from cool blue for lowest values to hot red for the highest. Only the studentized form of residuals will produce valid diagnostic graphs.

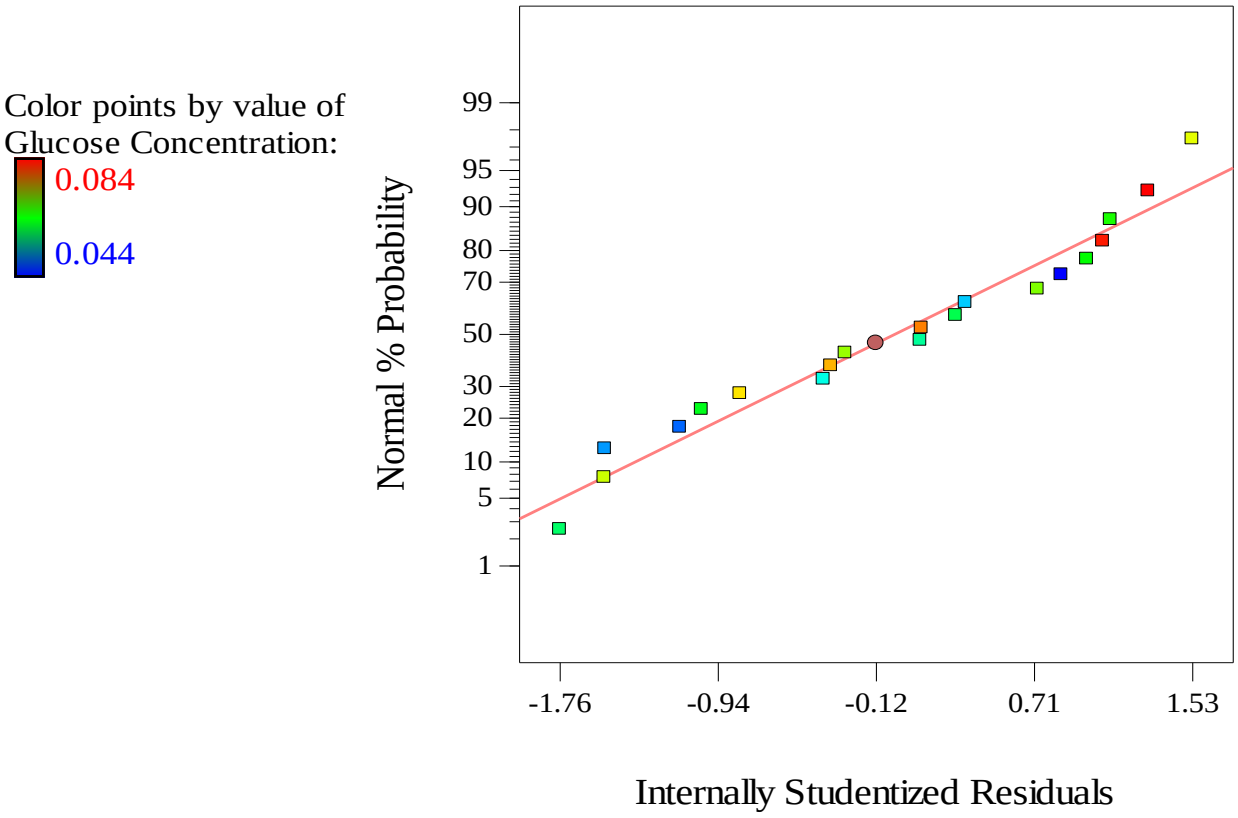

Figure 4:1 Normal plots of residuals for glucose concentration of waste potatoes

And also the assumptions of the analysis of variance (ANOVA) should be checked using the plots of residuals versus predicted. This could be useful to know how much the model is acceptable. In the analysis of variance, it is usually more effective to do this with the residuals. When the model is correct and the assumptions are satisfied, the residuals is structure less; in particular, they are unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted, predicted values.

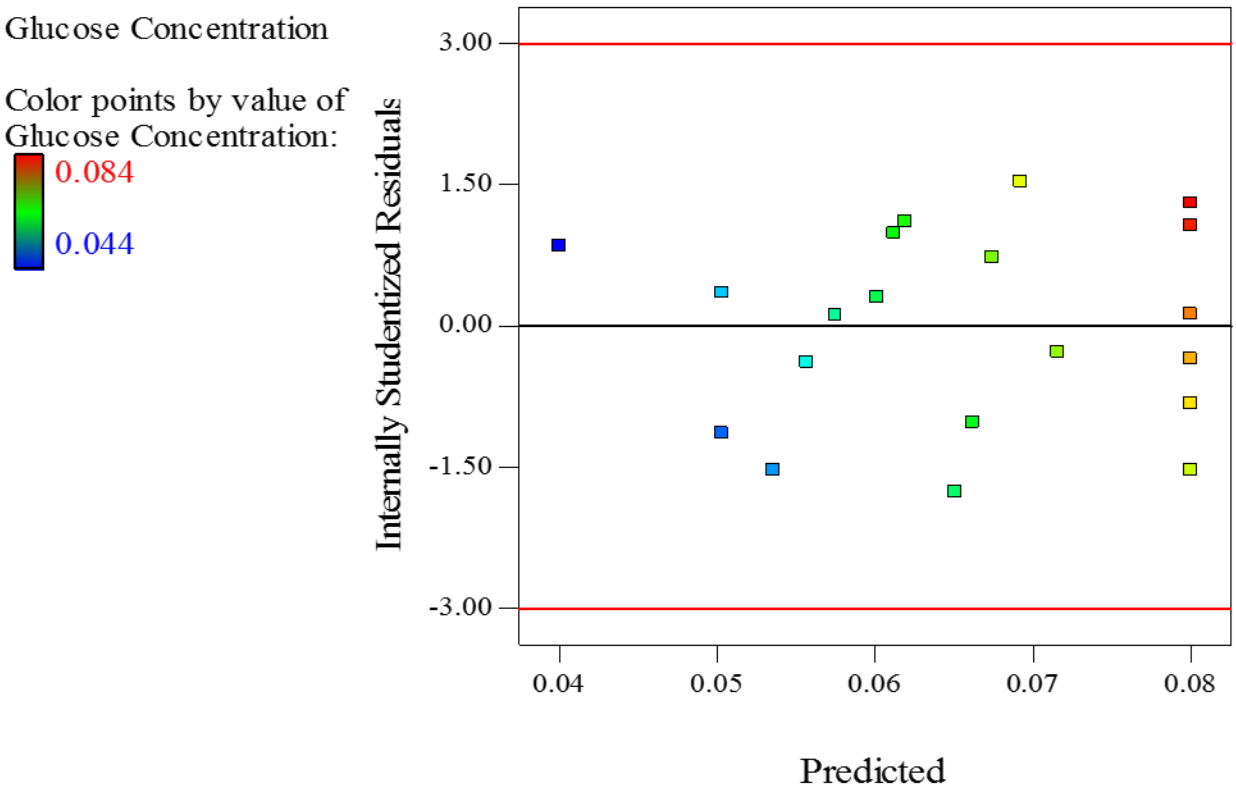

Figure 4:2 Residual of predicted values

From the plot of the residuals versus the predicted response values tests the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error. Figure 4:2 plots the residuals versus the fitted values. There should be no relationship between the size of the residuals and the fitted values. This plot reveals nothing of unusual interest. These plots are potentially very informative. If there is more scatter in the residuals for a particular treatment, that could indicate that this treatment produces more unpredictable response reading than the others. More scatter in the residuals for a particular block could indicate the block is not homogenous.

In addition to Figure 4:2 predicted versus actual value of glucose concentration was plotted in the Figure 4:3. The plot in Figure 4:3 shows how precisely the model is modeled. The points show how the predicted value and actual values of each run approach the straight line. The straight line

shows how the predicted and actual values are closer to each other. When the point is above the straight line predicted value is greater than the actual value and the reverse is also true.

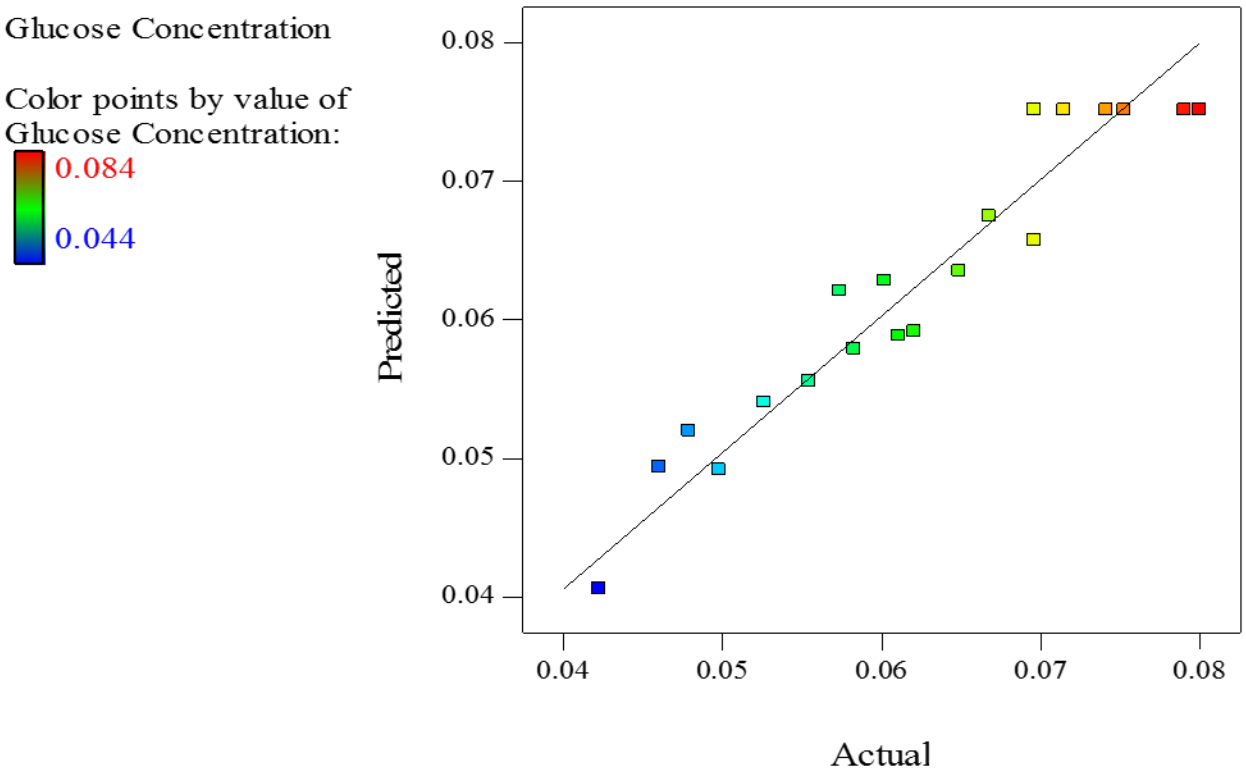

Figure 4:3 Predicted versus actual values of glucose concentration of waste potatoes

That means when the actual value is less than the predicted value the point lies below the straight line. The other condition is when the point lies on the straight line that run has the same actual and predicted value. To sum up, in Figure 4:3 the points laid on the straight line, so the actual and predicted values have closer value.

4.2.1.2. The effect of temperature and liquid to solid ratio on glucose concentration

The effect of temperature at fixed liquid to solid ratio

The effects of temperature at fixed liquid to solid ratio on glucose concentration when time was at the center point is shown in Figure 4:4. The interaction plots have black and red line which indicates low and high level of fixed parameter liquid to solid ratio respectively. At higher level

of liquid to solid ratio the amount of glucose concentration is high compared to lower level of liquid to solid ratio. There are two cases, the first case is when liquid to solid ratio is at higher level, the amount of glucose concentration is low at low temperature level and this might be due to the temperature is insufficient for hydrolysis of starch and the amount of glucose concentration is low at higher temperature and this might be due to the reason that the starch is converted to other byproduct.

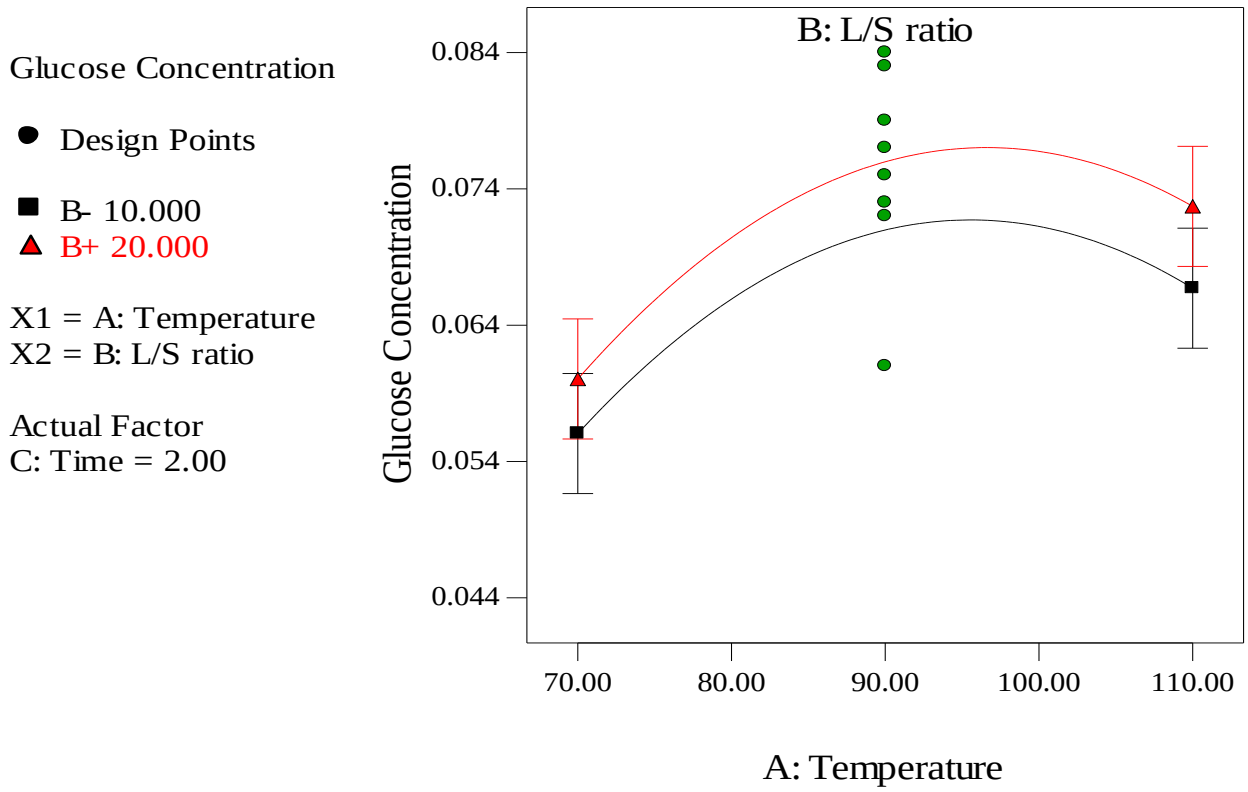

Figure 4:4 Effects of temperature at fixed liquid to solid ratio on glucose concentration when time was at the center point

The second case is when liquid to solid ratio is at lower level, the amount of glucose concentration is low at low temperature level and this might be due to the reason that the temperature is insufficient for hydrolysis of starch and the amount of glucose concentration is low at higher temperature and this might be due to the reason that the starch is converted to other byproduct.

The effects of liquid to solid ratio at fixed temperature

The effects of liquid to solid ratio at fixed temperature on glucose concentration when time was at the center point is shown in Figure 4:5. From figure at higher level of liquid to solid ratio the amount of glucose concentration is high compared to lower level of liquid to solid ratio. There are two cases, the first case is when temperature is at higher level, the amount of glucose concentration is low at low liquid to solid ratio level and this might be due to the amount of liquid to solid ratio is low and the amount of glucose concentration is low at higher liquid to solid ratio and this might be due to the reason that the amount of starch is high to hydrolysis.

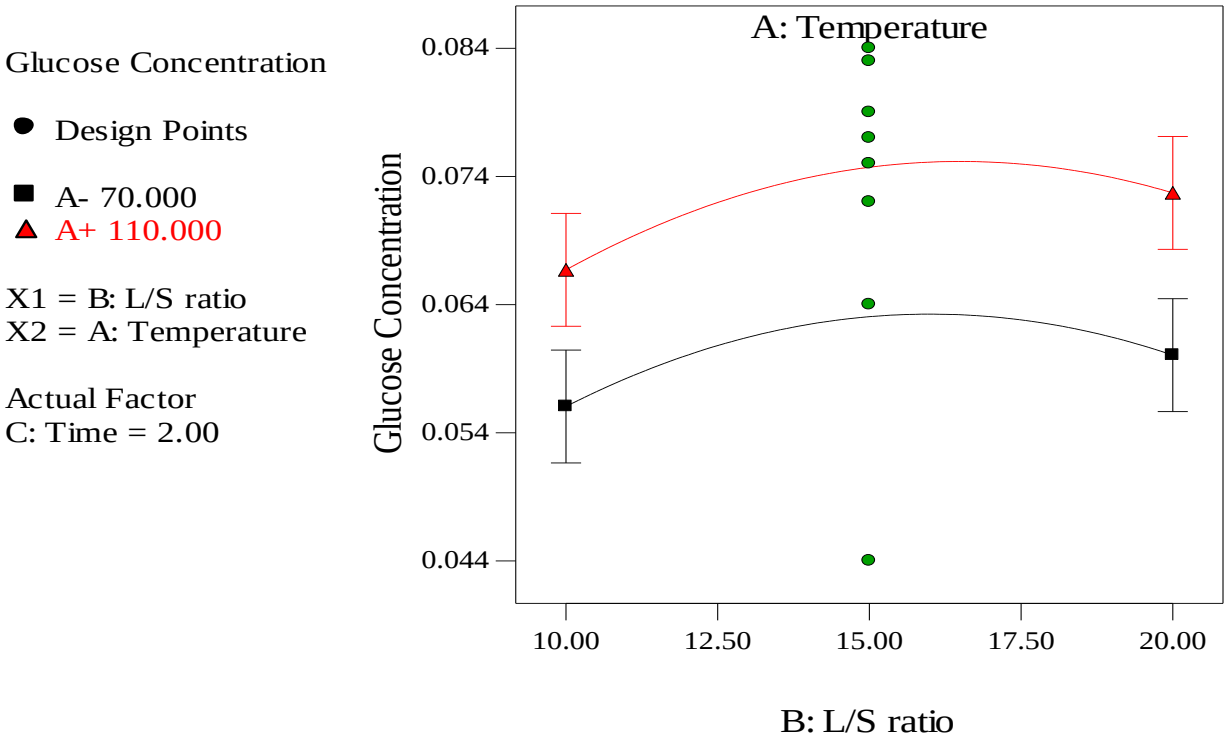

Figure 4:5 Effects of liquid to solid ratio at fixed temperature on glucose concentration when time was at the center point

The second case is when temperature is at lower level, the amount of glucose concentration is low at low liquid to solid ratio level and this might be due to the reason that the liquid to solid ratio is insufficient to produce the required amount of glucose and the amount of glucose concentration is

low at higher liquid to solid ratio this might be due to the reason that the starch is too high to hydrolysis.

Contour plot for the effect of temperature and liquid to solid ratio

Figure 4:6 presents the contour plot of glucose concentration with the effect of temperature and liquid to solid ratio. There is color change on the graph and the response variable is increasing from green to red color. The graph suggests operating at the center point where the response variable shows maximum amount. Operating in the red region is good to have high amount of glucose concentration. The line of the contour plot is not straight line due to there is high interaction between temperature and liquid to solid ratio.

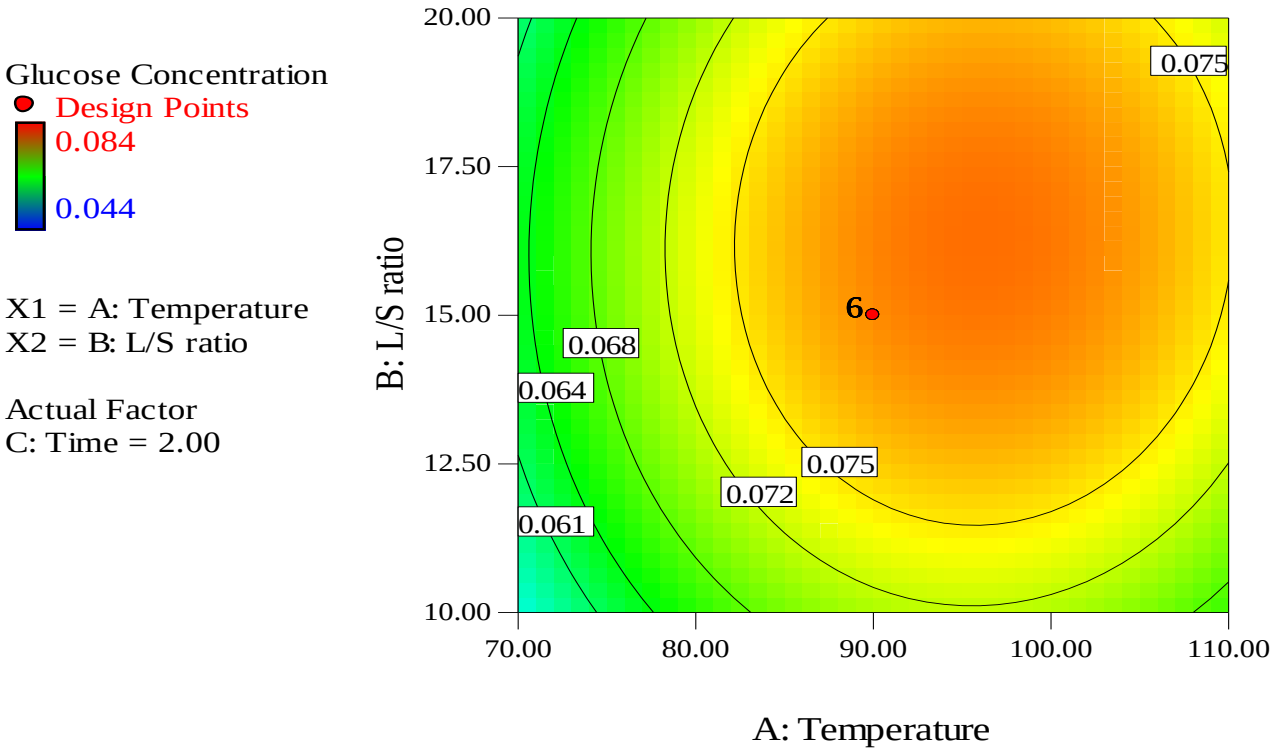

Figure 4:6 Contour plot of the effects of temperature and liquid to solid ratio on glucose concentration when time was at the center point

This slice includes six center points as indicated by the dot at the middle of the contour plot. By replicating center points, a very good power of prediction could be get at the middle of experimental region.

Response surface plot for the effects of temperature and liquid to solid ratio

Figure 4:7 shows response surface plot of glucose concentration with respect to temperature and liquid to solid ratio. All of the coefficients in the assumed model are significant. The quadratic effect is easily observable in the response surface plot. At the base of the graph contour plot is shown. The response surface plot obtained from hydrolysis temperature and liquid to solid ratio has curvature. This is an indication that there is interaction between the two factors temperature and liquid to solid ratio on glucose concentration. A hole in the middle of the surface plot is shown.

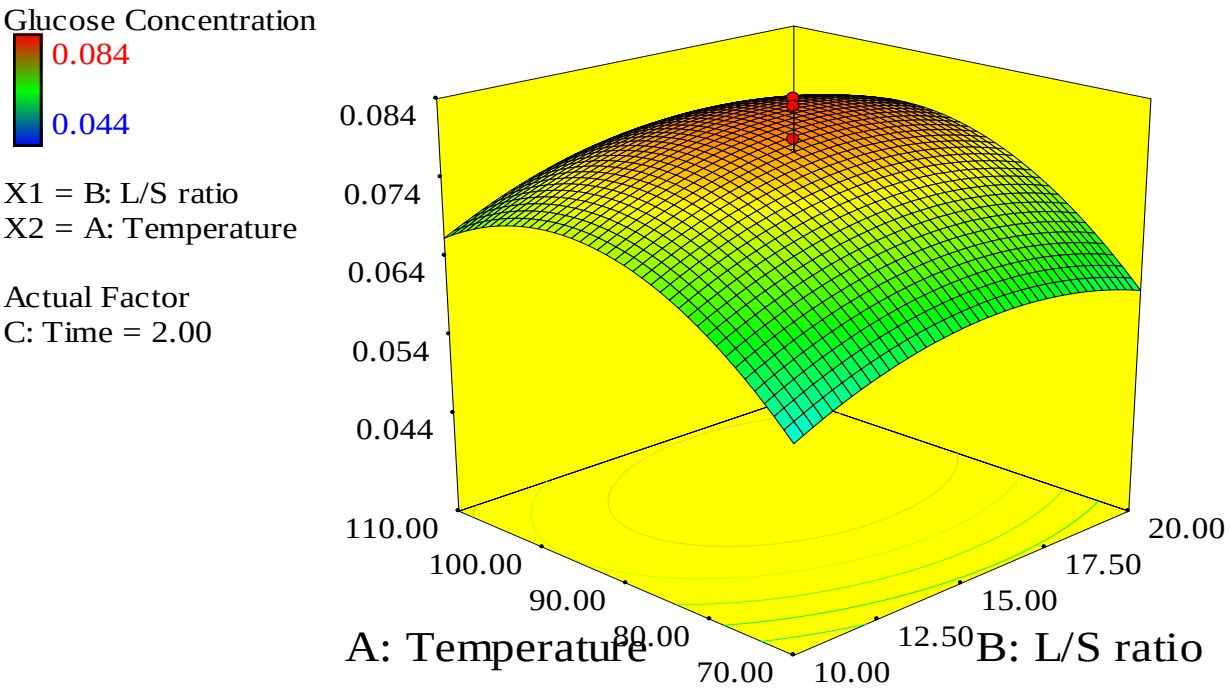

Figure 4:7 Response surface plots the effects of temperature and liquid to solid ratio on glucose concentration when time was at the center point

That is a clue that more points, lightly shaded for easier identification, fall below the surface. The maximum amount of glucose concentration could be found in the middle of the plotted surface. At the edge of the surface plotted where the factors are at high and low level the amount of glucose concentration is low.

4.2.1.3. The effect of temperature and time on the glucose concentration

The effect of temperature at fixed time

Figure 4:8 demonstrates the effect of temperature at fixed time on the glucose concentration, when liquid to solid ratio was at the center point. At high and low hydrolysis time the glucose production increases as temperature increases. In the figure it is shown that the amount of glucose concentration is not high always at the time is high. Before reaching 0.0645 g/mL glucose concentration the amount of glucose concentration is low at high level of time. The reverse is true after 0.0645 g/mL; the amount of glucose concentration is high at high time of hydrolysis. The amount of glucose concentration is low at higher level of time and at low temperature.

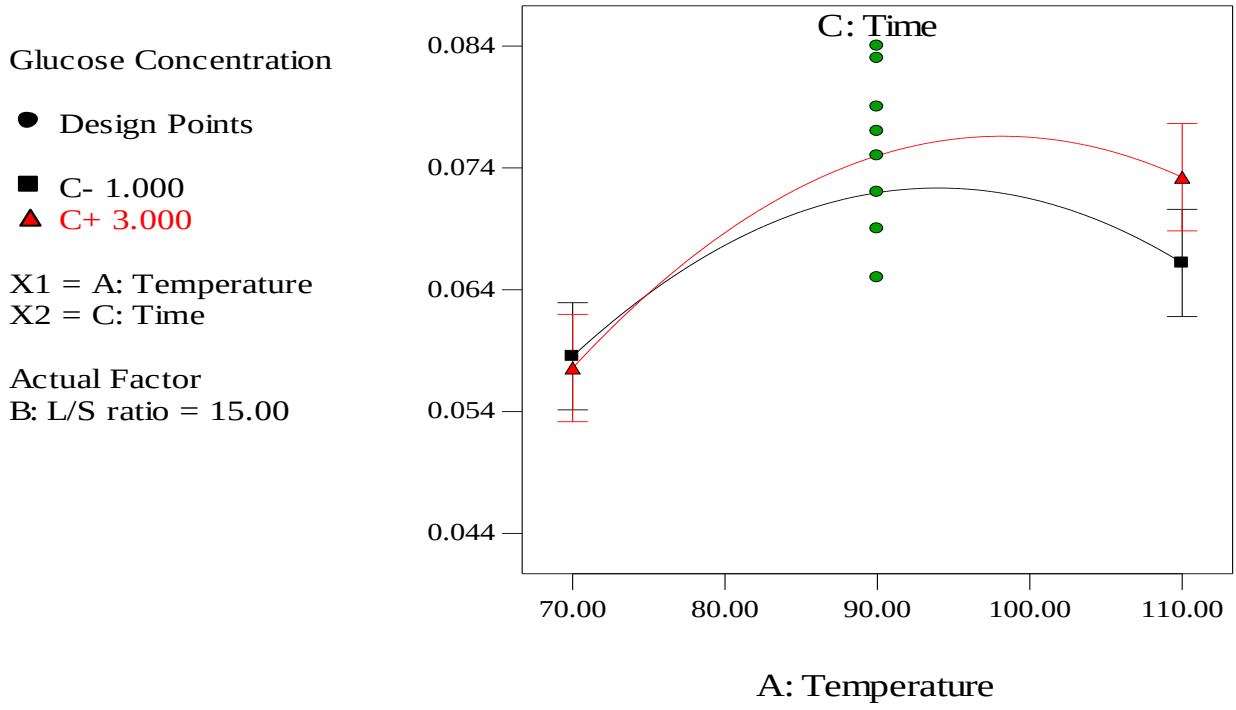

Figure 4:8 Effects of temperature at fixed time on the glucose concentration when liquid to solid ratio was at the center point

This might happen due to the reason that the amount of temperature is not enough to hydrolysis the starch. And also the amount of glucose concentration is low at higher level of time and high

temperature. This also true for high hydrolysis time. However, the maximum amount of glucose concentration is found around the middle point.

The effect of time at fixed temperature

Figure 4:9 displays the effect of hydrolysis time at fixed temperature on the glucose concentration, when liquid to solid ratio was at the center point. At high and low hydrolysis temperature the glucose production increases as hydrolysis time increases. The amount of glucose concentration is high at higher level compared to lower level of temperature. From the graph the amount of glucose increases as time increases and reaching maximum around the middle it starts to decreases. At lower time the time is not sufficient to hydrolysis starch and at higher time the starch is converted to other byproduct. At lower level of temperature and lower level of time the amount of glucose produce is low. These two combination of time and temperature resulted lower amount of glucose concentration.

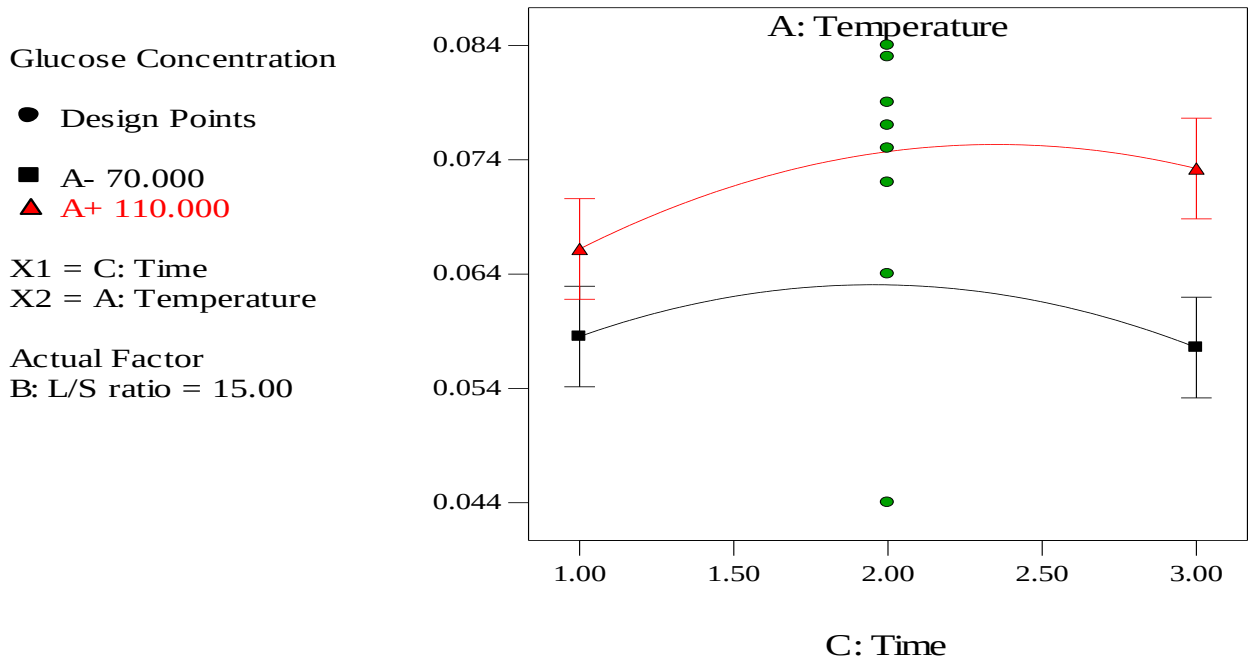

Figure 4:9 Effects of time at fixed temperature on the glucose concentration when liquid to solid ratio was at the center point

The reason for this the amount of time and temperature level is not enough to hydrolysis starch to glucose. The other instance is the amount of glucose is high at higher temperature level and higher time level compared to the lower level of both factors. Around the middle level of the two factor the amount of glucose is high at higher level of temperature.

Response surface contour plot of the effect of temperature and time

The contour plot in terms of factors temperature and time with liquid to solid ratio at the center point is shown in Figure 4:10. From the contour plot graph showing predicted response of glucose concentration as a function of temperature and time there is color change on the graph and the response variable is increasing from green to red color. The graph suggests operating at the center point where the response variable is at maximum. Operating in the red color region is good to have high amount of glucose concentration. There is high interaction between temperature and time.

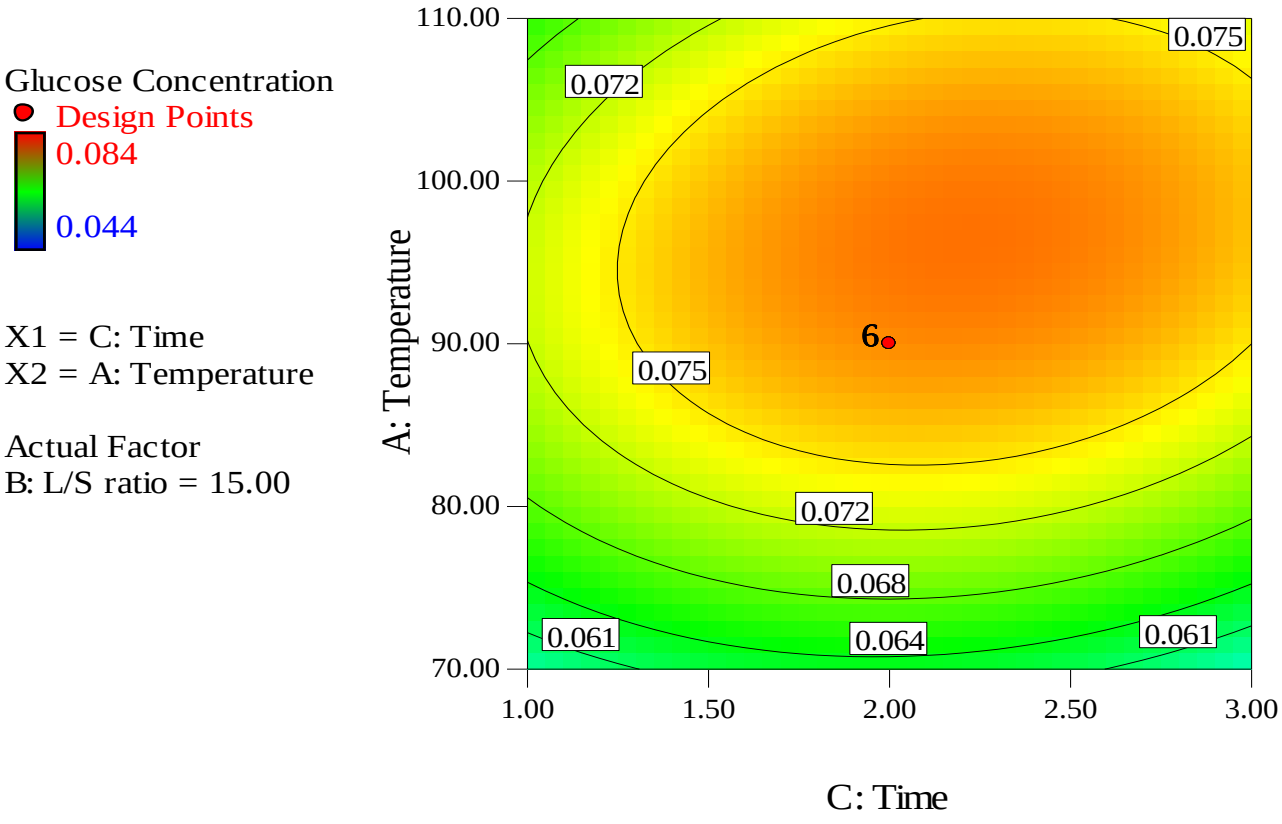

Figure 4:10 Contour plots of the effect of temperature and time on the glucose concentration, when liquid to solid ratio was at the center point

Response surfaces plot of the effect of temperature and time

Figure 4:11 shows response surface plot of glucose concentration with respect to temperature and time. All of the coefficients in the assumed model are significant. The quadratic effect is easily observable in the response surface plot. The response surface plot is obtained from hydrolysis temperature and liquid to solid ratio has curvature. This is an indication that there is interaction between the two factors temperature and liquid to solid ratio on glucose concentration. The plot proposes that there were well defined optimum operating conditions. The maximum amount of glucose is found in the middle part of the surface plot. At the edge of the surface plot the amount of glucose concentration is low.

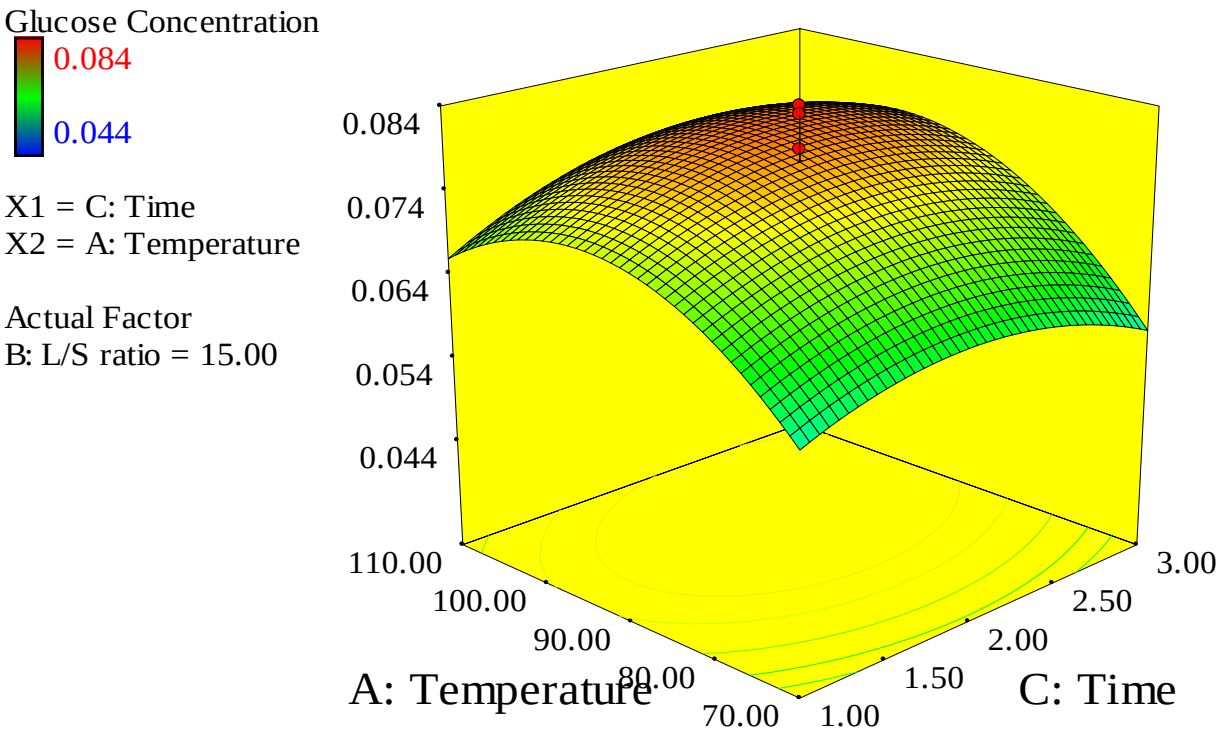

Figure 4:11 Response surfaces plots of the effect of temperature and time on the glucose concentration when liquid to solid ratio was at the center point

4.2.1.4. The effects of liquid to solid ratio and time on the glucose concentration

The effects of liquid to solid ratio at fixed time

Figure 4:12 indicates the response of glucose concentration when liquid to solid ratio changes at a fixed time holding temperature at center point. The trend of the graph shows increasing and decreasing. From the figure the amount of glucose concentration increases as liquid to solid ratio increases and reaching maximum around the middle it starts to decrease. At lower level of liquid to solid ratio the amount of glucose concentration is low and the reason for this is the amount of liquid to solid ratio is not enough to provide high amount of glucose concentration.

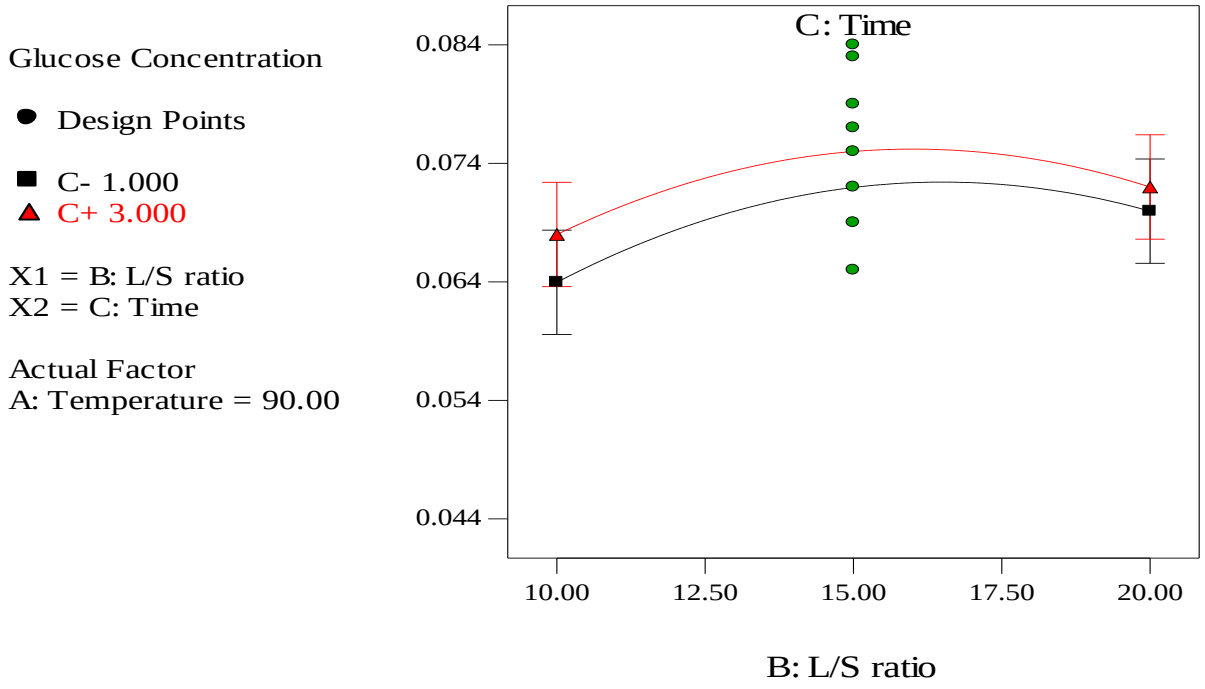

Figure 4:12 Effects of liquid to solid ratio at fixed time on the glucose concentration when temperature was at the center

And also the amount of glucose concentration is low at higher level of liquid to solid ratio. The reason for this is the amount of raw material is to be hydrolyzed is high. On the graph at low level of liquid to solid ratio and at low level of time the amount of glucose concentration is low compared to at high level of both factors.

The effects of time at fixed liquid to solid ratio

Figure 4:13 has the same character like the previous. The plotted graph explains the effect of time at fixed liquid to solid ratio. At high level of liquid to solid ratio hydrolysis time effect has high glucose concentration compared to low level liquid to solid ratio. The amount of glucose produced is dependent on time and liquid to solid ratio. As time increases the amount of glucose concentration increases and reaching maximum around the middle it starts to decrease. The amount of glucose is low at low time and this result came due to the time is not enough for hydrolysis.

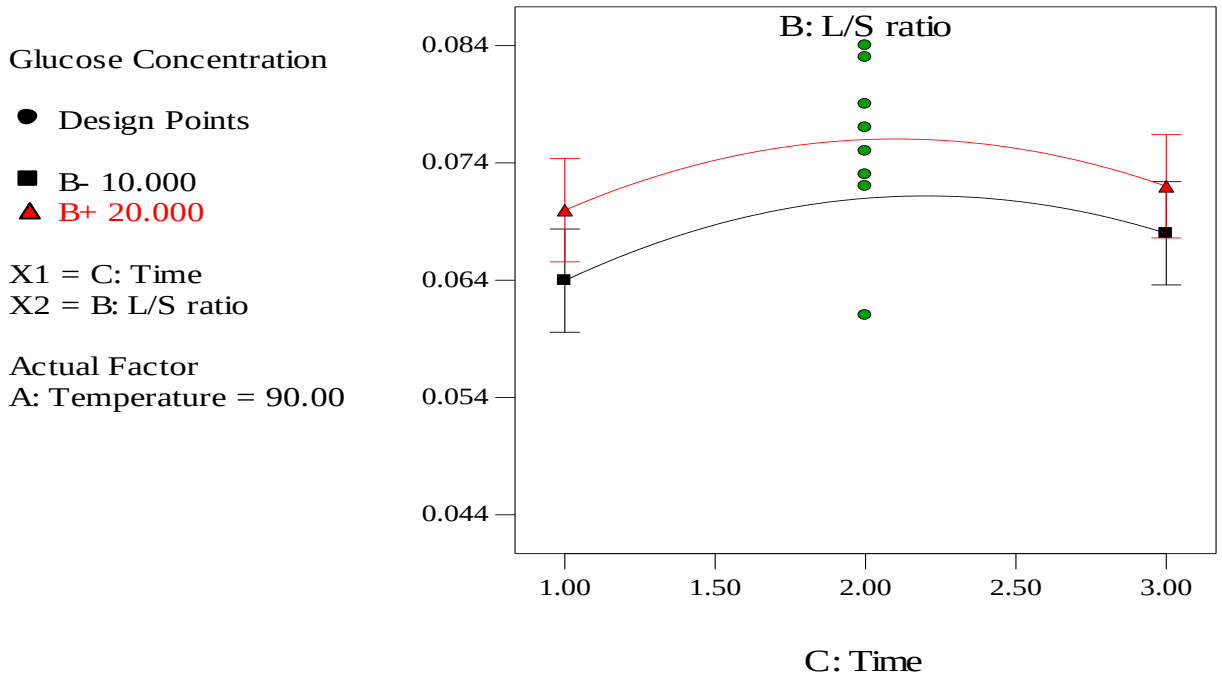

Figure 4:13 Effects of time at fixed liquid to solid ratio on the glucose concentration when temperature was at the center

And the amount of glucose concentration is low at higher temperature, this might happen due to starch was converted to other product. On the graph the amount of glucose is low at lower level of liquid to solid ratio and at lower temperature. The amount of glucose is high at high level of liquid to solid ratio and time compared to the low level of both factor.

Response surface contour plots of the effects of liquid to solid ratio and time

The contour plot of glucose concentration with respect to the change in liquid to solid ratio and time at temperature center point is shown in Figure 4:14. From the contour plot graph showing predicted response of glucose concentration as a function of liquid to solid ratio and time there is color change on the graph and the response variable is increasing from green to red color. The graph suggests operating at the center point where the response variable is at maximum glucose concentration is good. Operating in the red color region is good to have high amount of glucose concentration. There is high interaction between time and liquid to solid ratio.

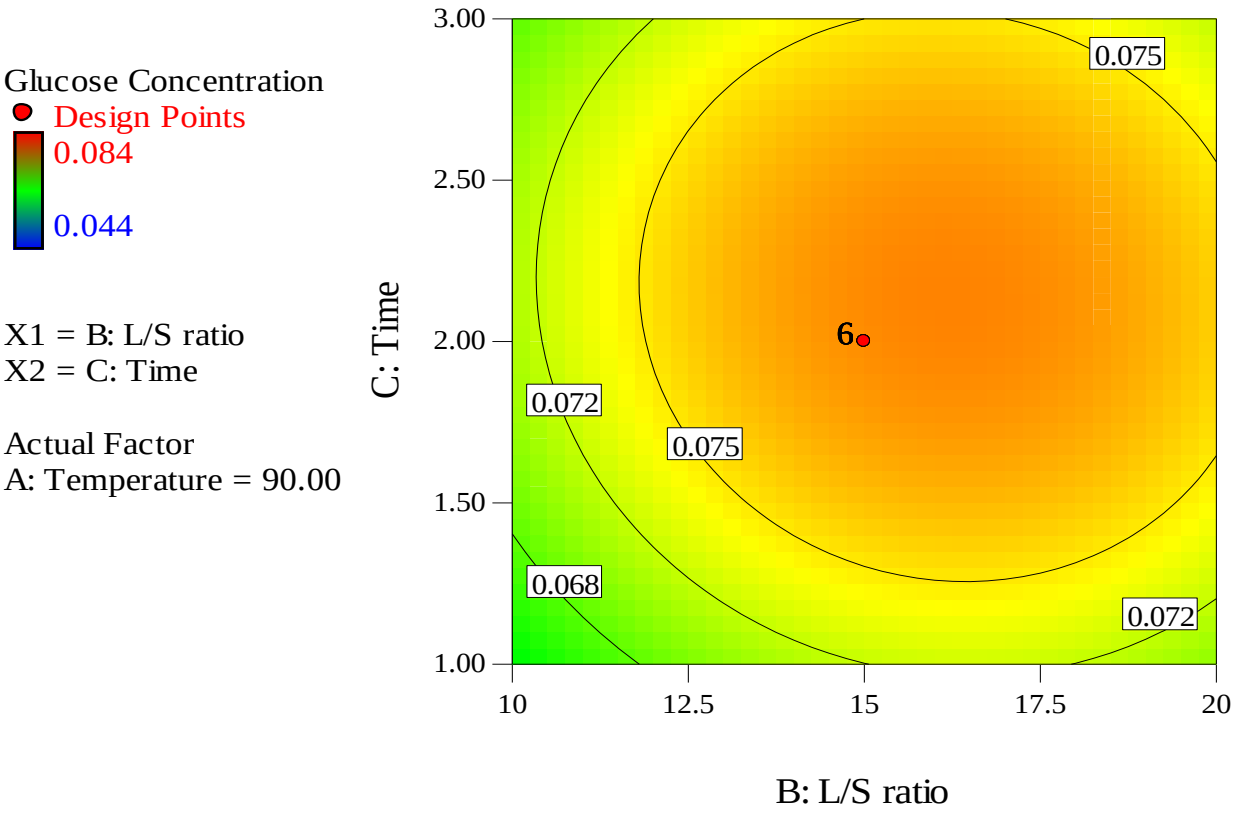

Figure 4:14 Contour plots of the effects of liquid to solid ratio and time on the glucose concentration when temperature was at the center

Response surface plots of the effects of liquid to solid ratio and time

All of the coefficients in the assumed model are significant. The response surface plot and the contour plot in terms of factors liquid to solid ratio and time with temperature at the center point is shown in Figure 4:15. There is interaction between the two factors liquid to solid ratio and hydrolysis time on glucose concentration. The plot proposes that there were well defined optimum operating conditions.

In addition, at the maximum point of the plotted surface the maximum amount of glucose could be found at the best combination of liquid to solid ratio and time. The sharp age of the surface indicates where the amount of glucose is low at low liquid to solid ratio and time. The sharp age of the surface indicates also the lower amount of glucose at higher liquid to solid ration and time.

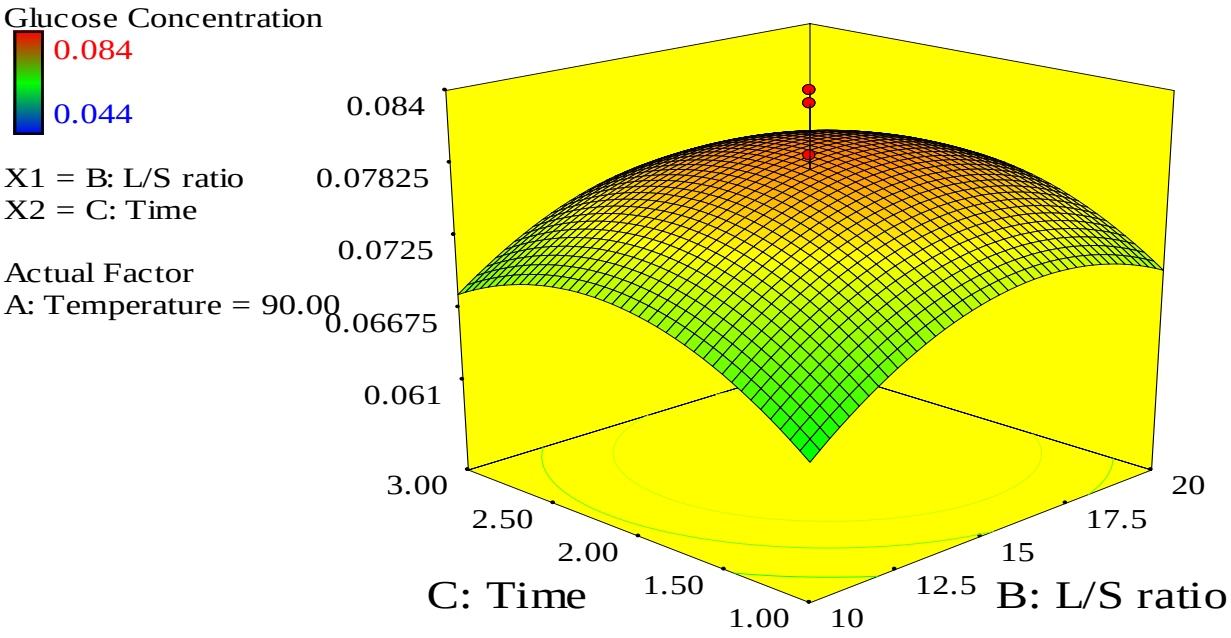

Figure 4:15 Response surface plots of the effects of liquid to solid ratio and time on the glucose concentration when temperature was at the center

Generally, the one factor, two factor, contour and response surface plot suggest the operating point for the factors temperature, liquid to solid ratio and time. The graph for contour plot suggests the

operating area to get maximum amount of glucose concentration. The optimum values of the factors and the response variable should be determined.

4.2.1.5. Finding optimal value of glucose concentration

The optimization of hydrolysis criteria for glucose production from waste potatoes using dilute acid treatment are summarized in table 4.4.

Table 4.4 Criteria for finding optimal value of glucose concentration

Optimization criteria for optimum glucose concentration			
Parameters	Purpose	Minimum value	Maximum value
Temperature (°C)	In the range	70	110
Liquid to solid ratio (g/100mL)	In the range	10	20
Time (hour)	In the range	1	3
Glucose concentration(g/mL)	Maximize	0.044	0.084

By using the criteria in Table 4.4, the Design Expert solution was obtained. The possible solution for this model with the given hydrolysis factors, temperature, liquid to solid ratio and time is 96.71 ^{o}C, 16.25g/100mL and 2.2 h respectively that resulted the amount of the produced glucose concentration 0.080 g/mL.

Validation of the model

To validate this solution two run of hydrolysis was carried out with the given optimal values of the three factors, hydrolysis temperature, liquid to solid ratio and hydrolysis time were conducted. The average result of the two runs are 0.0815 g/mL of glucose concentration and the yield of ethanol is 32.0%. This implies that he experiments was carried out at the optimized conditions. As a result, the model was considered to be accurate and reliable for predicting the concentration of glucose from waste potatoes using dilute acid hydrolysis. Compared to the data obtained from literature, the optimum hydrolysis conditions of glucose concentration lie in the accepted limits of starch hydrolysis conditions as suggested by (Tasic *et al*., 2009 and Arapoglou *et al*., 2010). Therefore, this study showed that waste potato is a potential source for the glucose production for bioethanol production. This waste can be the source for production of bioethanol.

4.2.2. Optimization of experimental variables on hydrolysis of potato peels

After getting the optimum value of the factors and the response variable for waste potatoes the next task is evaluating the experiments done on potato peels. The experiment done was hydrolysis of potato peels starch to glucose with dilute acid and effect of the factors was evaluated.

4.2.2.1. ANOVA analysis for potato peels

To confirm whether quadratic model is the right model or not, it was necessary to perform analysis of variance (ANOVA). The probability, P-values were used to check the significance of each coefficient of the regression model equation. The P-values of corresponding coefficient should be less than or equals to 0.05. This is the bench mark for checking the significance of the proposed quadratic model.

From Appendix B the model F-value of 7.42 implies the model is significant. There is only a 0.22% chance that a model F-Value this large could occur due to noise. The terms A and A^2 are significant model terms. The lack of fit F-value of 4.35 implies there is a 6.62% chance that a lack of fit F-value this large could occur due to noise.

Development of regression model equation

The model equation that correlates the response (Y) to the hydrolysis process variables in terms of coded factors and actual factor was given in equation (4.3) and (4.4) respectively.

Final equation in terms of coded factors for potato peels

$$\begin{aligned} \text{Glucose} = {} & 0.07 + 8.44\times10^{-3}A + 2.88\times10^{-3}B + 8.76\times10^{-4}C + 1.81\times10^{-3}A\times B \\ & + 1.91\times10^{-3}A\times C - 4.13\times10^{-4}B\times C - 6.43\times10^{-3}A^2 - 2.08\times10^{-3}B^2 \\ & + 8.90\times10^{-4}C^2 - 1.09\times10^{-3}A\times B\times C \end{aligned} \quad (4.3)$$

Where, A = Hydrolysis temperature

B = Liquid to solid ratio

C = Hydrolysis time

Final equation in terms of actual factors for potato peels

$$\begin{aligned}\text{Glucose} = & -0.06 + 2.53\times10^{-3}\,\text{Temperature} - 3.52\times10^{-4}\,\text{Liquid to solid ratio} - 0.03\text{Time} \\ & + 3.99\times10^{-5}\,\text{Temperature}\times\text{Liquid to solid ratio} + 2.59\times10^{-4}\,\text{Temperature} \\ & \times\text{Time} - 8.96\times10^{-4}\,\text{Liquid to solid ratio}\times\text{Time} - 1.61\times10^{-5}\,\text{Temperature}^2 \\ & - 8.31\times10^{-5}\,\text{Liquid to solid ratio}^2 - 8.90\times10^{-4}\,\text{Time}^2 \\ & - 1.09\times10^{-5}\,\text{Temperature}\times\text{Liquid to solid ratio}\times\text{Time}\end{aligned} \quad (4.4)$$

Depending on the coefficients in equations (4.3) and (4.4) it was clear that the amount of glucose concentration increases with the temperature (A), while decrease with liquid to solid ratio (B) and hydrolysis time (C). Hydrolysis time has a weighty effect on glucose concentration as compared to temperature and liquid to solid ratio. Temperature has positive effect on glucose concentration since it has positive coefficient. However, the other two variables liquid to solid ratio and time has negative effect on glucose concentration because of negative coefficient the two variables possessed.

Table 4.5 Model summary statistics for potato peels

Model Summary Statistics						
Source	Std.Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	7.93×10^{-03}	0.4994	0.4056	0.1738	1.66×10^{-03}	
2FI	8.67×10^{-03}	0.5134	0.2888	-0.3107	2.63×10^{-03}	
Quadratic	**5.12×10^{-03}**	**0.8697**	**0.7524**	**0.1155**	**1.78×10^{-03}**	**Suggested**
Cubic	2.88×10^{-03}	0.9752	0.9216	0.8728	2.55×10^{-04}	Aliased

The predicted R-squared of 0.1155 is not as close to the adjusted R-squared of 0.7524 as one might normally expect. Adequacy precision measures the signal to noise ratio. The ratio of 10.721 indicated on ANOVA analysis is an adequate signal. This model can be used to navigate the design space.

Before concluding from the analysis of variance adopted, the adequacy of the underlying model should be checked. Figure 4:16 to Figure 4:30 shows how exactly the produced glucose concentration is modeled.

Figure 4:16 shows the points line up well and the deviation of points for glucose concentration from normality is insignificant. In addition, the normal probability plot indicates the residuals following a normal distribution. In the case of this experiment the points in the plots shows fit to a straight line, this shows that the quadratic polynomial model satisfies the assumptions of analysis of variance (ANOVA) i.e. the error distribution is approximately normal. Ideally the normal plot is a straight line, indicating no abnormalities. There is no severe indication of abnormality, nor is there any evidence pointing to possible outliers in the figure.

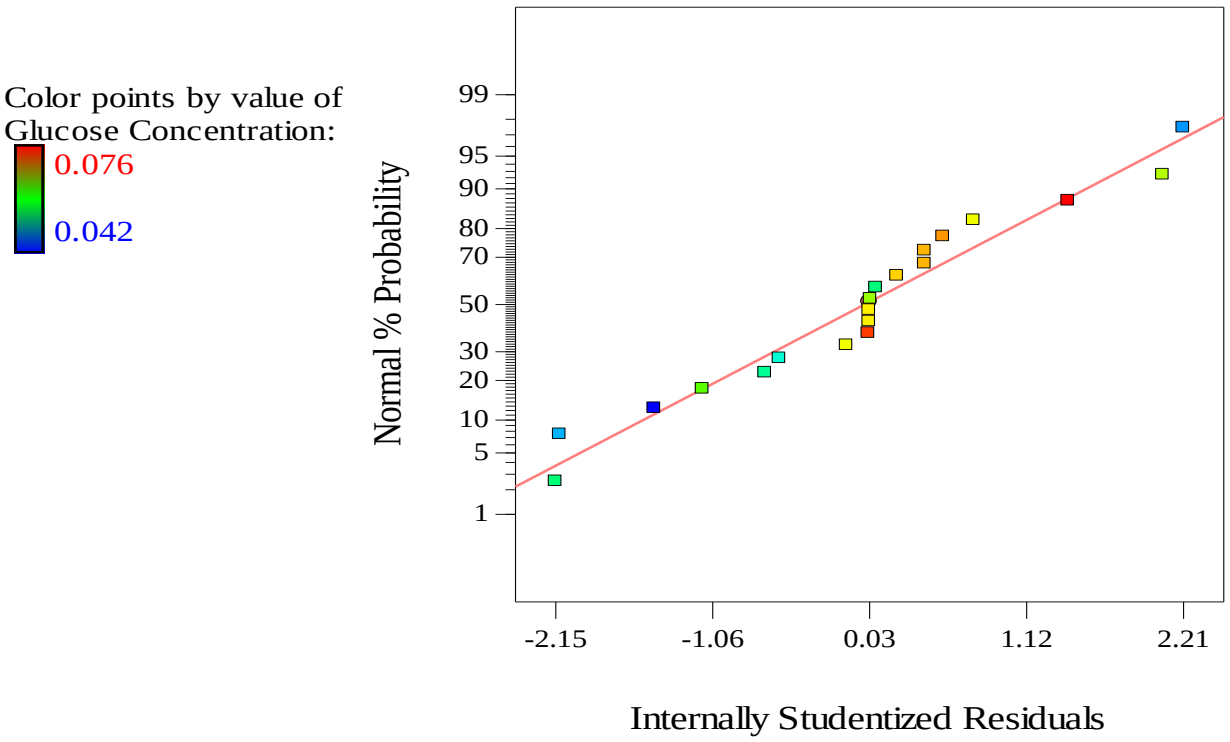

Figure 4:16 Normal plots of residuals glucose concentration of potato peels

The plot in Figure 4:17 shows the residuals versus the rising predicted response values tests the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error. A plot of the residuals versus the predicted response values tests the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error.

There should be no relationship between the size of the residuals and the fitted values. This plot reveals nothing of unusual interest. This plot is potentially very informative. If there is more scatter in the residuals for a particular treatment, that could indicate that this treatment produces more unpredictable response reading than the others. More scatter in the residuals for a particular block could indicate the block is not homogenous.

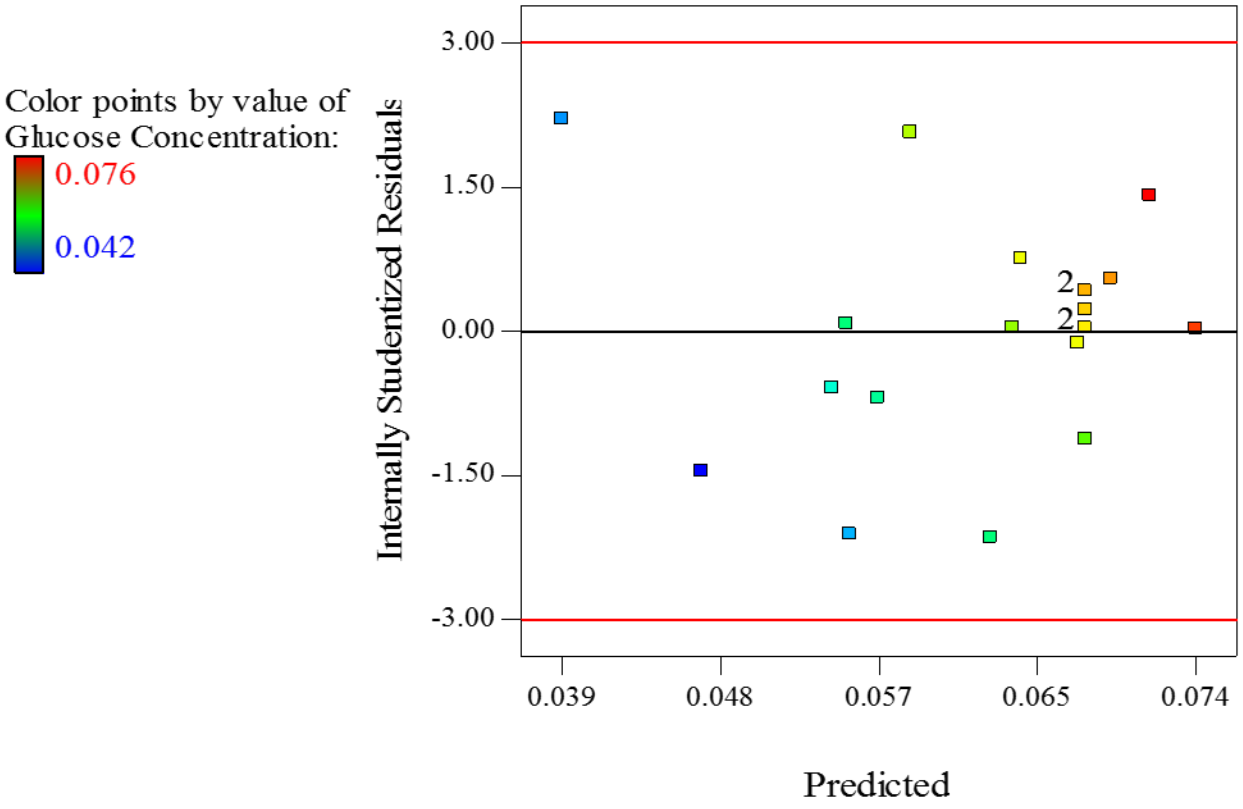

Figure 4:17 Residual of predicted values

Figure 4:18 shows how precisely the model is modeled. The graph of the predicted values obtained using the developed correlation versus actual values. The points show how the predicted value and actual values of each run related. The straight line shows how the predicted and actual values are closer to each other. When the point is above the straight line predicted value is greater than the actual value and the reverse is also true. That means when the actual value is less than the predicted value the point lies below the straight line. The other condition is when the point lies on the straight line that run has the same actual and predicted value. To sum up, in Figure 4:18 the points laid

slight away from the straight line at some points so the actual and predicted values have different values at those points.

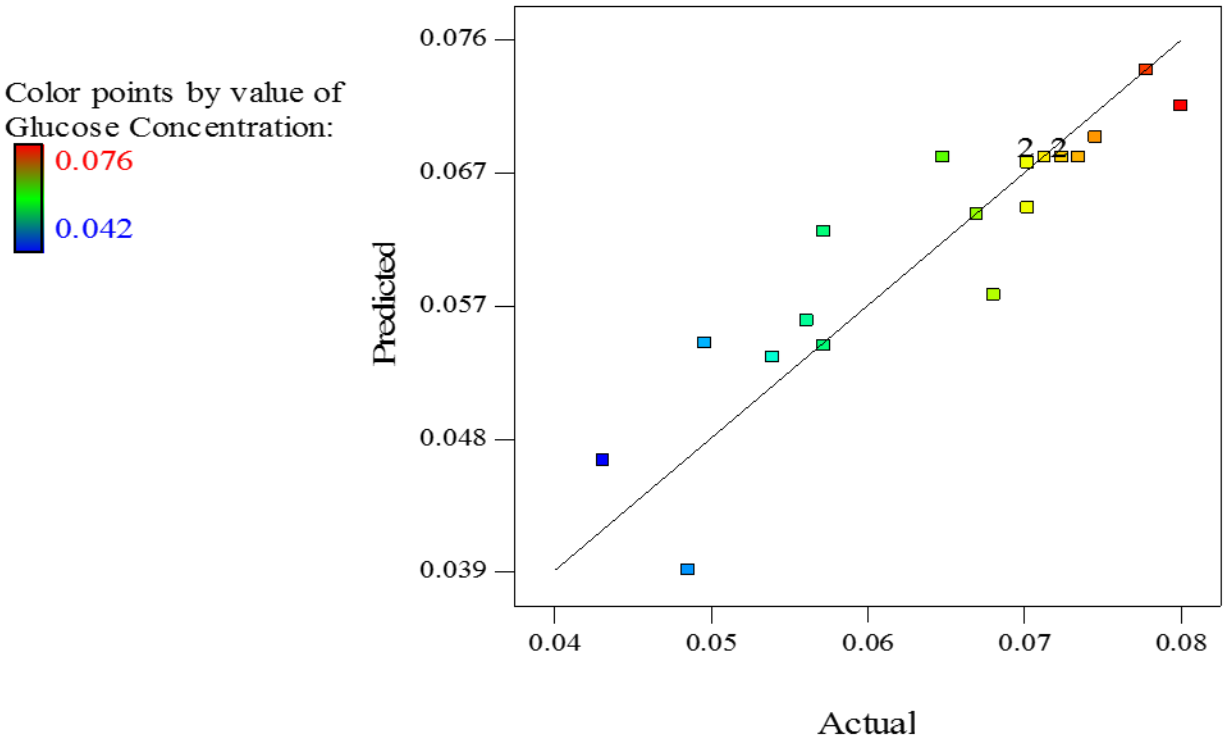

Figure 4:18 Predicted values versus actual values of glucose concentration of potato peels

4.2.2.2. The effect of temperature and liquid to solid ratio on glucose concentration

Effects of temperature at fixed liquid to solid ratio

The two factor plot of hydrolysis temperature versus glucose concentration at fixed liquid to solid ratio and holding hydrolysis time is shown in Figure 4:19. The graph shows the effect of temperature at high and low level of liquid to solid ratio. The graph shows the increase of glucose concentration when temperature increases and decreases after reaching maximum. At low temperature the amount of glucose concentration is low due to the reason of temperature is low to hydrolysis starch. At high level of temperature, the amount of glucose concentration is low and the reason is starch might be converted to other byproducts. At higher level of liquid to solid ratio the amount of glucose concentration is high compared to low level of liquid to solid ratio.

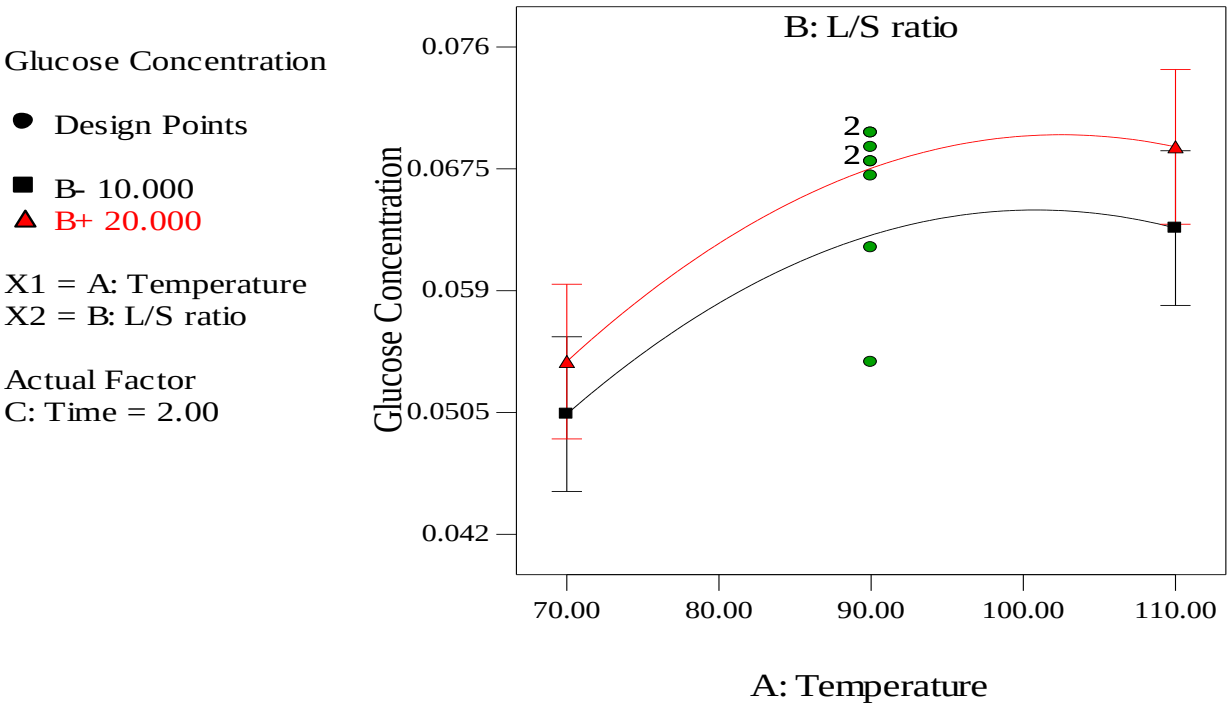

Figure 4:19 Effects of temperature at fixed liquid to solid ratio on glucose concentration when time was at the center point

In Figure 4:19 the amount of glucose concentration is low at low level of temperature and liquid to solid ratio compared to the amount of glucose concentration at high level of both factors. The amount of glucose concentration is high in the middle of the points.

The effects of liquid to solid ratio at fixed temperature

The two factor plot of liquid to solid ratio versus glucose concentration is show in Figure 4:20. The graph presents the effect of liquid to solid ratio on the amount of produced glucose concentration hydrolysis temperature at center point and holding liquid to solid ratio at center point. There is high difference on concentration of glucose produced at fixed temperature. At high level of liquid to solid ratio the amount of glucose concentration is high compared to low level. The reason for this is, at low level the amount of starch is low to give high amount of glucose, while at high liquid to solid ratio the amount of starch could be enough to provide the required amount of glucose.

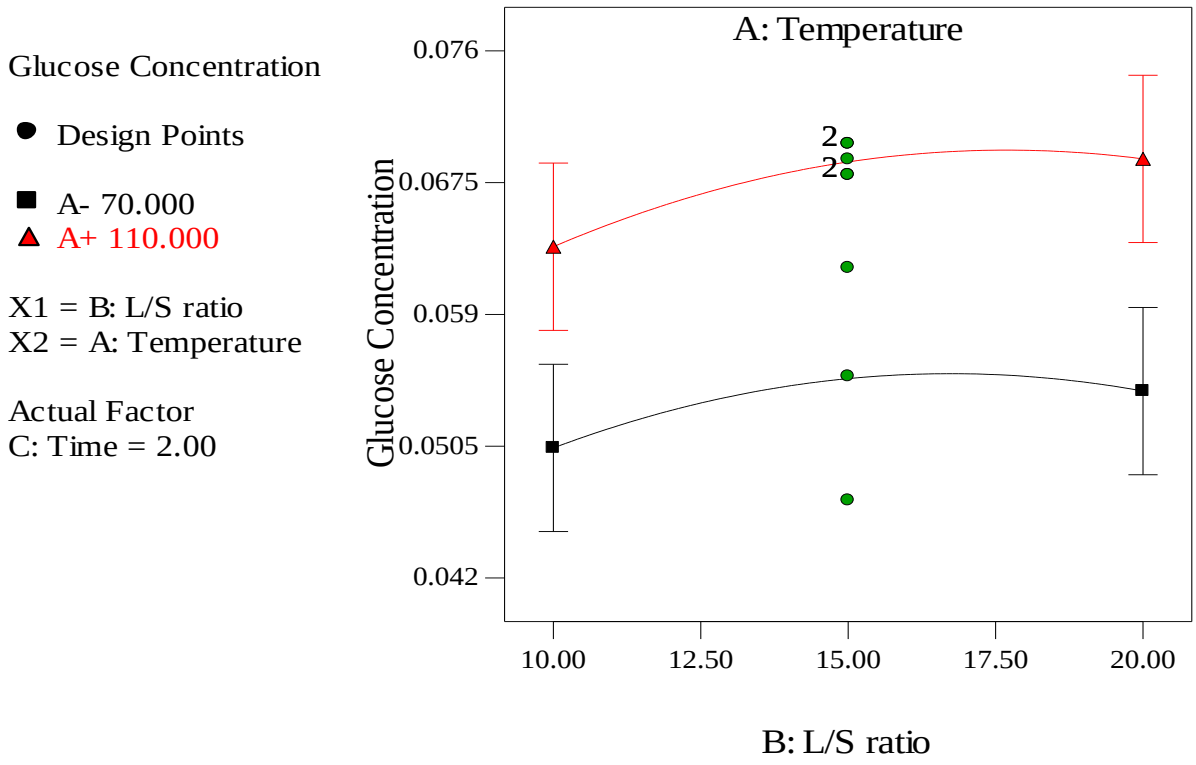

Figure 4:20 Effects of liquid to solid ratio at fixed temperature on glucose concentration when time was at the center point

On the graph the amount of glucose produced is low at low liquid to solid ration and temperature. However, the amount is high at high temperature and liquid to solid ratio. The maximum amount of glucose is produced in the middle of the high level and low level.

Contour plot of the effects of temperature and liquid to solid ratio

The contour plot of the effects of hydrolysis temperature and liquid to solid ratio on glucose concentration when hydrolysis time was at the center point is shown in Figure 4:21. From the contour plot graph showing predicted response of glucose concentration as a function of temperature and liquid to solid ratio there is color change on the graph and the response variable is increasing from green to red color.

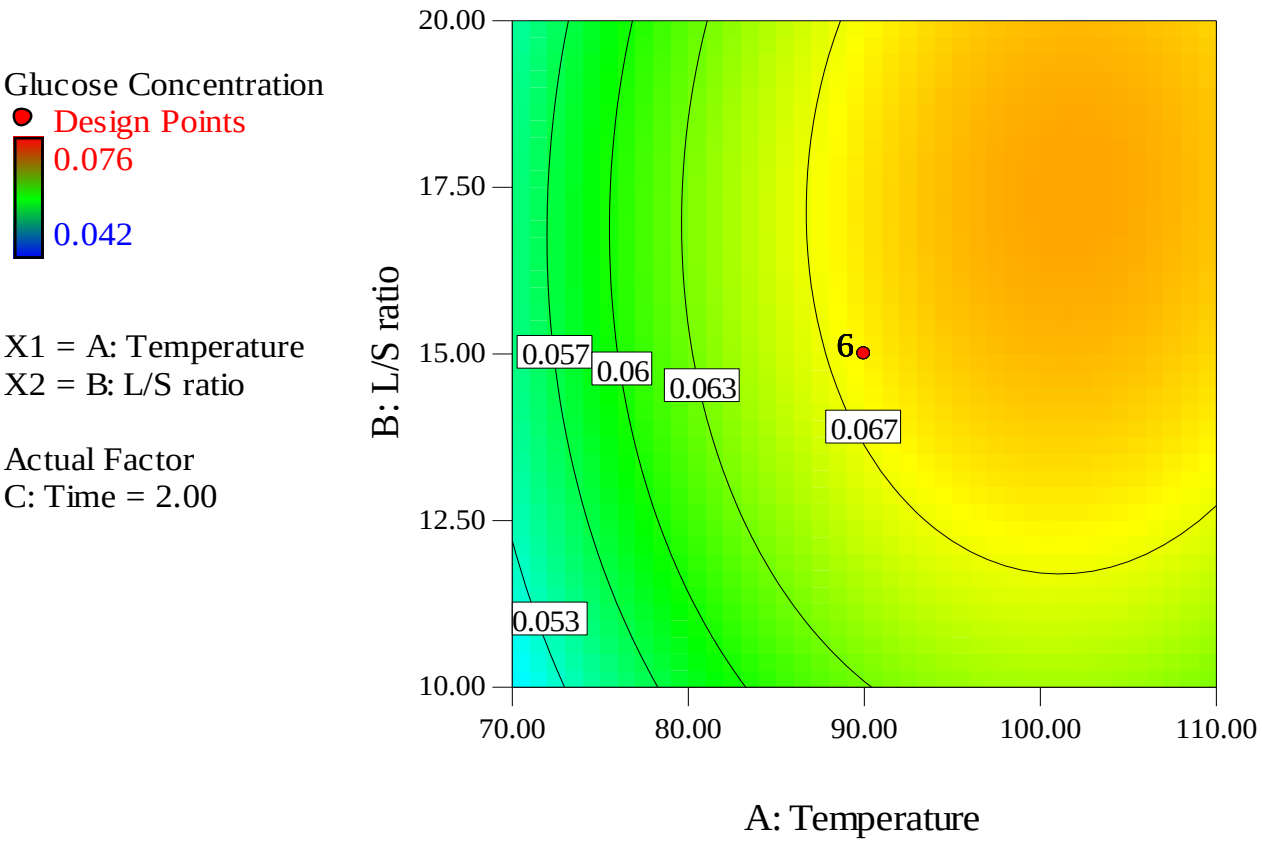

Figure 4:21 Contour plot of the effects of temperature and liquid to solid ratio on glucose concentration when time was at the center point

The graph suggests operating at the center point where the response variable is high. Operating in the red region is good to have high amount of glucose concentration.

Response surface plots the effects of temperature and liquid to solid ratio

The surface plot for the response variable is shown in the Figure 4:22. The plot indicates the effect of temperature and liquid to solid ratio on glucose production. All of the coefficients in the assumed model are significant. The quadratic effect is easily observable in the response surface plot.

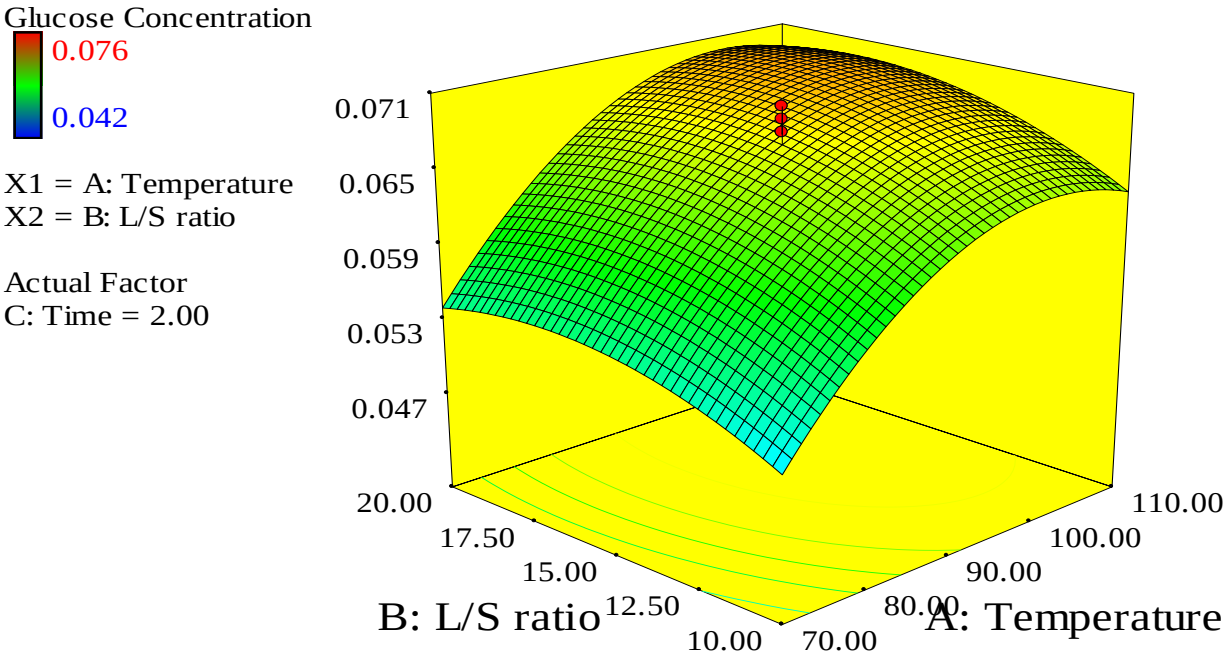

Figure 4:22 Response surface plots the effects of temperature and liquid to solid ratio on glucose concentration when time was at the center point

There is interaction between the two factors liquid to solid ratio and hydrolysis time on glucose concentration. The plot proposes that there were well defined optimum operating conditions. The maximum amount of glucose concentration is shown when the temperature and liquid to solid ratio are at high level. The best combination of temperature and liquid to solid ratio also found at this place. The sharp age of the surface plot indicates the lower amount of glucose concentration at low level of temperature and liquid to solid ratio.

4.2.2.3. The Effects of Temperature and Time on the Glucose Concentration

The effects of temperature at fixed time

The effects of hydrolysis temperature at fixed hydrolysis time is revealed in Figure 4:23. The graph was plotted at high and low level of hydrolysis time. The black and red colored graph shown in figure increases with time and decreases after reaching maximum. However, at the end the lines

do not returned back to the original place. The amount of glucose concentration at high temperature is higher than at lower temperature. The maximum amount is found in between the two points.

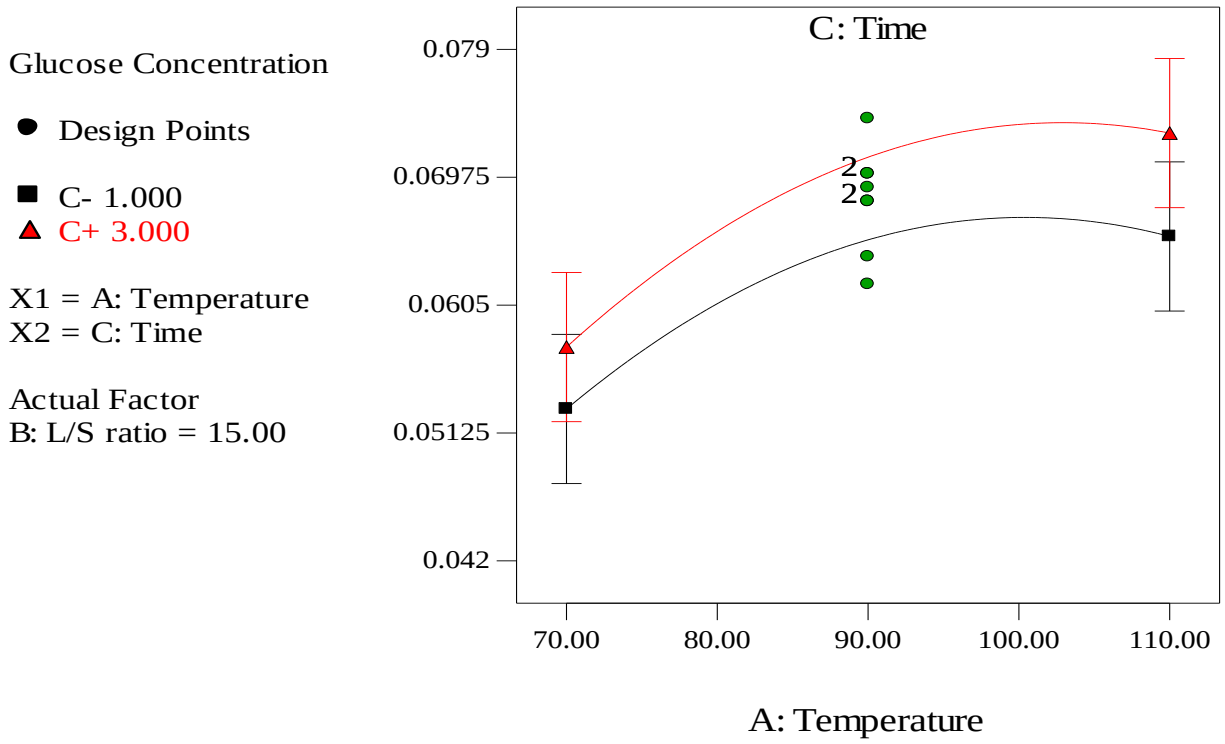

Figure 4:23 Effects of temperature at fixed time on glucose concentration when liquid to solid ratio was at the center point

At fixed hydrolysis time at low level of temperature the amount of glucose concentration is low. This might be due to the reason that the starch was not converted to glucose, therefore the concentration of glucose is low. At high level of hydrolysis temperature, the amount of glucose concentration is low compared to the middle maximum point. This might be due to the reason that starch was converted to other by products.

The effect of time and at fixed temperature

Figure 4:24 shows the effect of hydrolysis time at fixed temperature holding liquid to solid ratio at the center point. At high level of temperature, the amount of glucose concentration produced is higher than at the low level of temperature. And the concentration of glucose increases with time for both high level and low level of time and start to decrease. On the graph the amount of glucose

concentration is low at low level of time and temperature and the reverse happen at high level of the two factors.

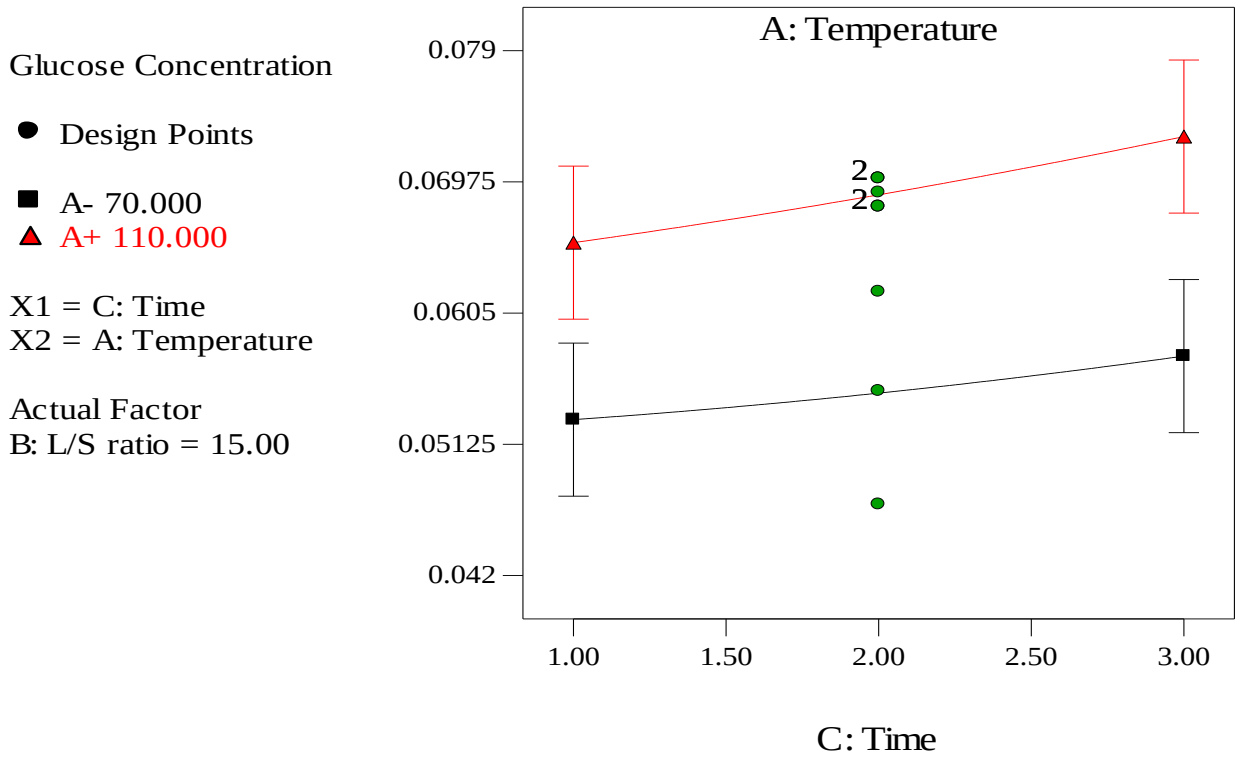

Figure 4:24 Effects of time and at fixed temperature on glucose concentration when liquid to solid ratio was at the center point

Contour Plots of the Effect of Temperature and Time

Contour plot of the response variable glucose is shown in Figure 4:25. The combined effect of hydrolysis temperature and time on the hydrolysis is clearly indicated on the graph. There is color change on the graph and the response variable is increasing from green to red color. The graph proposes operating at the center point where the response variable is high. Operating in the red region is good to have high amount of glucose concentration. The interaction of temperature and time is high.

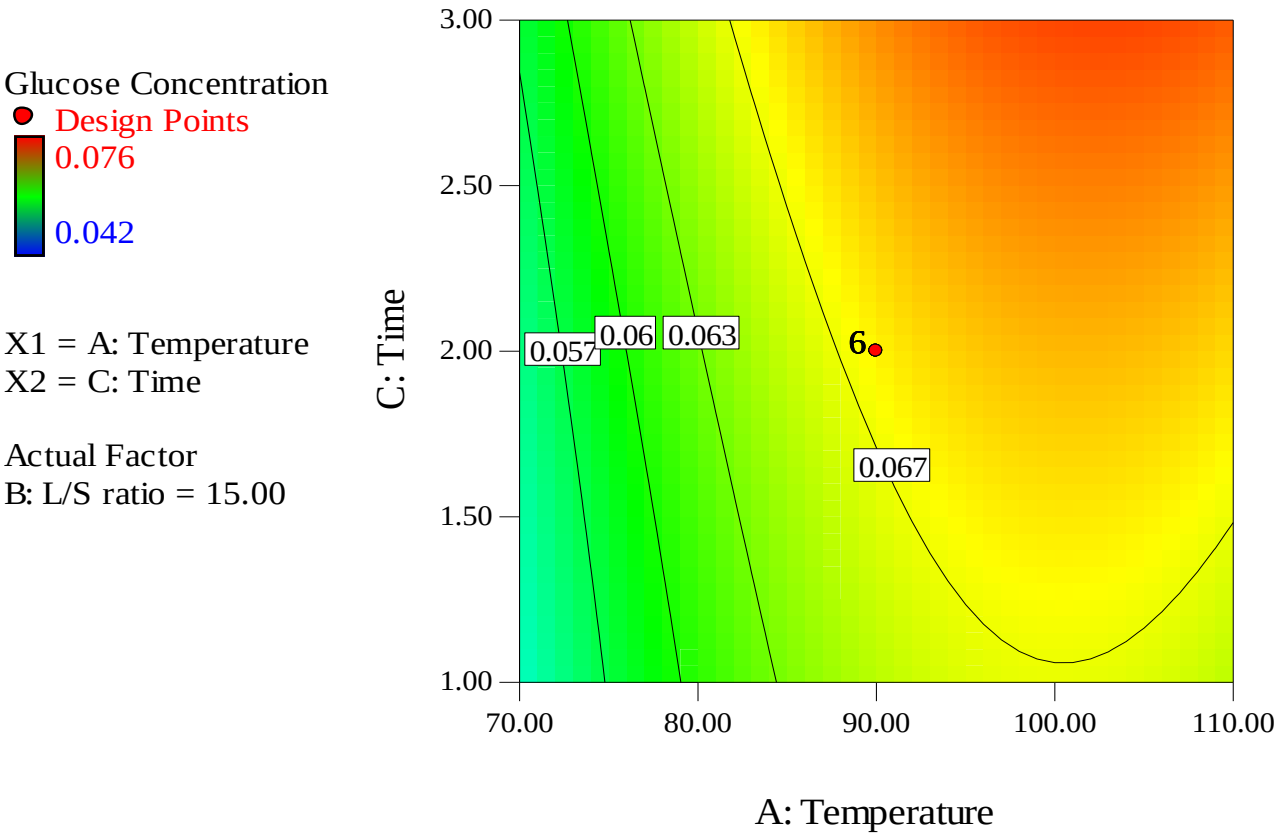

Figure 4:25 Contour plots of the effect of temperature and time on glucose concentration when liquid to solid ratio was at the center point

Response Surfaces Plots of the Effect of Temperature and Time

The effect of time and temperature on response variable, glucose concentration is shown in Figure 4:26. The response surface plot obtained from temperature and time has curvature. This is an indication that there is interaction between the two factors temperature and hydrolysis time on glucose concentration. The plot proposes that there were well defined optimum operating conditions. The amount of maximum glucose concentration is found at the point where temperature and time are at high level. At other edge of the surface the amount of glucose concentration is low. Low amount of glucose is produced at lower level of temperature and time.

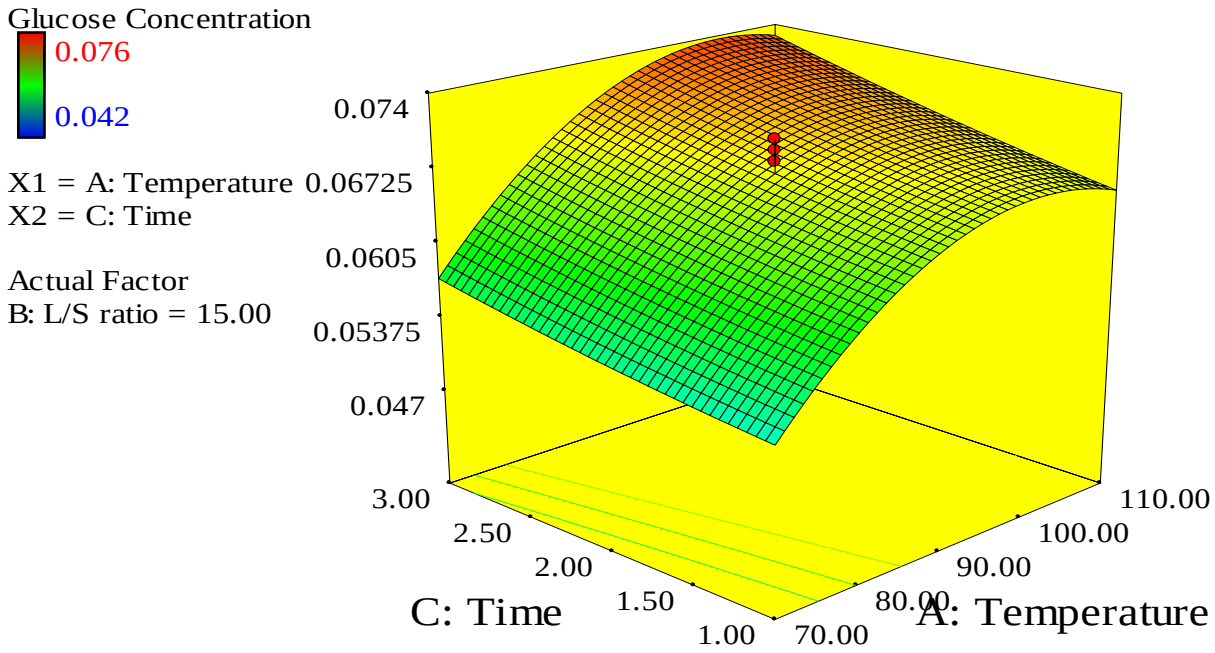

Figure 4:26 Response surfaces plots of the effect of temperature and time on glucose concentration when liquid to solid ratio was at the center point

4.2.2.4. The effect of liquid to solid ratio and time on the glucose concentration

The effect of liquid to solid ratio at fixed time

Figure 4:27 shows the effect of liquid to solid ratio on glucose concentration at fixed time when the temperature held at center point. When the liquid to solid ratio increases for both high level and low level of time the amount of glucose concentration increases and reaching the maximum it starts to decrease. At low level of both liquids to solid ratio and time the amount of glucose concentration is low compared to the high level of both factors. This could be due to the reason that the amount of starch is low at low level of the factors liquid to solid ratio and time. At high level of liquid to solid ratio and time the amount of glucose concentration is low compared to the middle point.

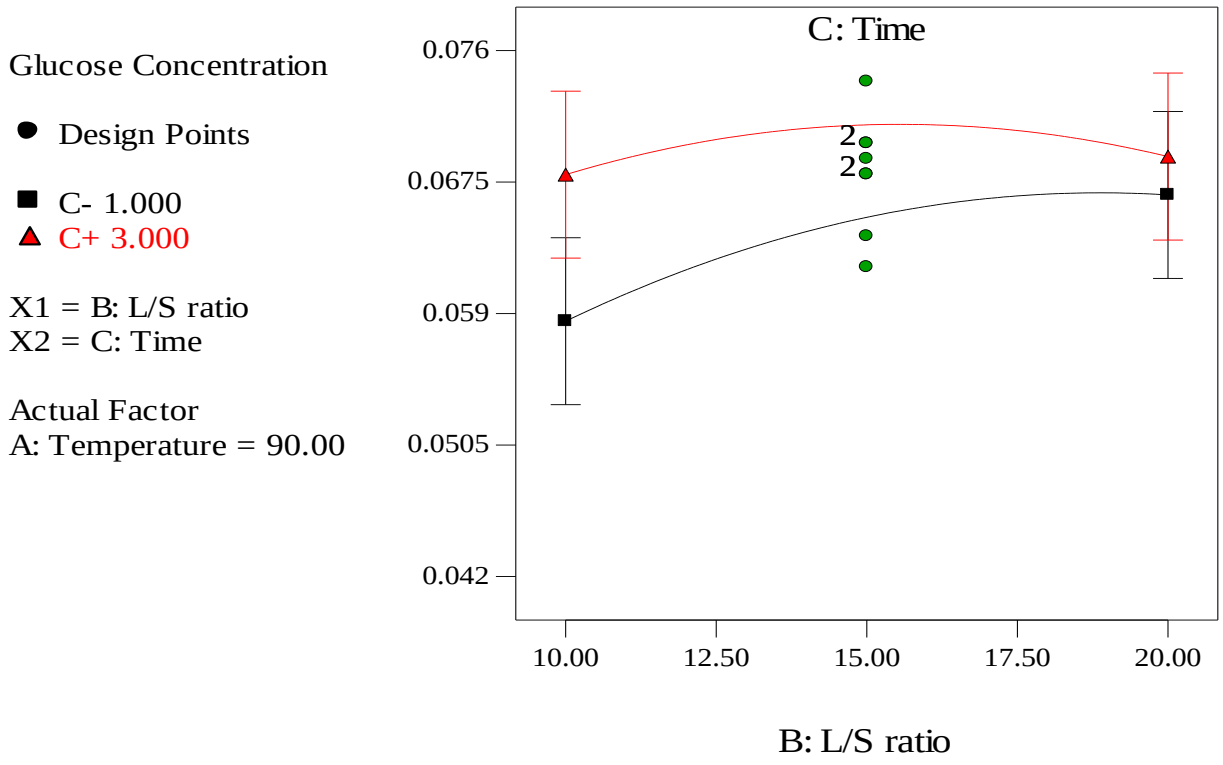

Figure 4:27 Effects of liquid to solid ratio at fixed time on glucose concentration when temperature was at the center

The effects of time at fixed liquid to solid ratio

The two factor plot of hydrolysis time versus glucose concentration at a fixed liquid to solid ratio when hydrolysis temperature held at center point is shown in Figure 4:28. The effect of hydrolysis time and liquid to solid ratio on the amount of glucose concentration is shown in the graph. The high level line shows slight increment of glucose concentration as time goes on. However, the low level line displays that the amount of glucose concentration increases rapidly as time goes on compared to low level of liquid to solid ratio. On the graph the amount of glucose is low at low level of time and liquid to solid ratio. This might happen due to the level of the factors is not good enough to provide the best amount of glucose. At high level of both factors the amount of glucose is high and this is due to the reason that the point is where maximum amount of glucose is found.

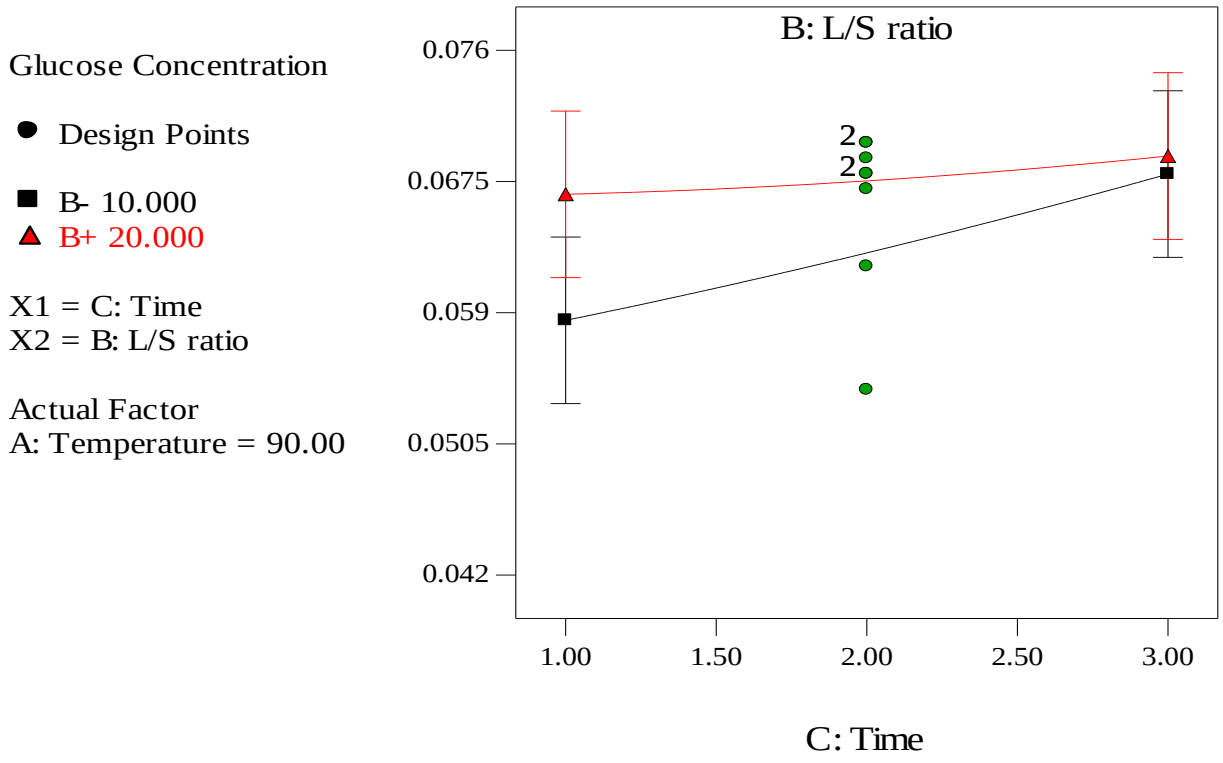

Figure 4:28 Effects of time at fixed liquid to solid ratio on glucose concentration when temperature was at the center

Contour plots of the effects of liquid to solid ratio and time

Figure 4:28 shows the contour plot of glucose concentration with change in both liquids to solid ratio and hydrolysis time. The effect of changing liquid to solid ratio and hydrolysis time and is shown on the graph. There is color change on the graph and the response variable is increasing from green to red color. The graph suggests operating at the center point where the response variable is high. Operating in the red region is good to have high amount of glucose concentration. This is the point where maximum amount of glucose is produced with the combined effect of liquid to solid ratio and time. There is high interaction between liquid to solid ratio and time.

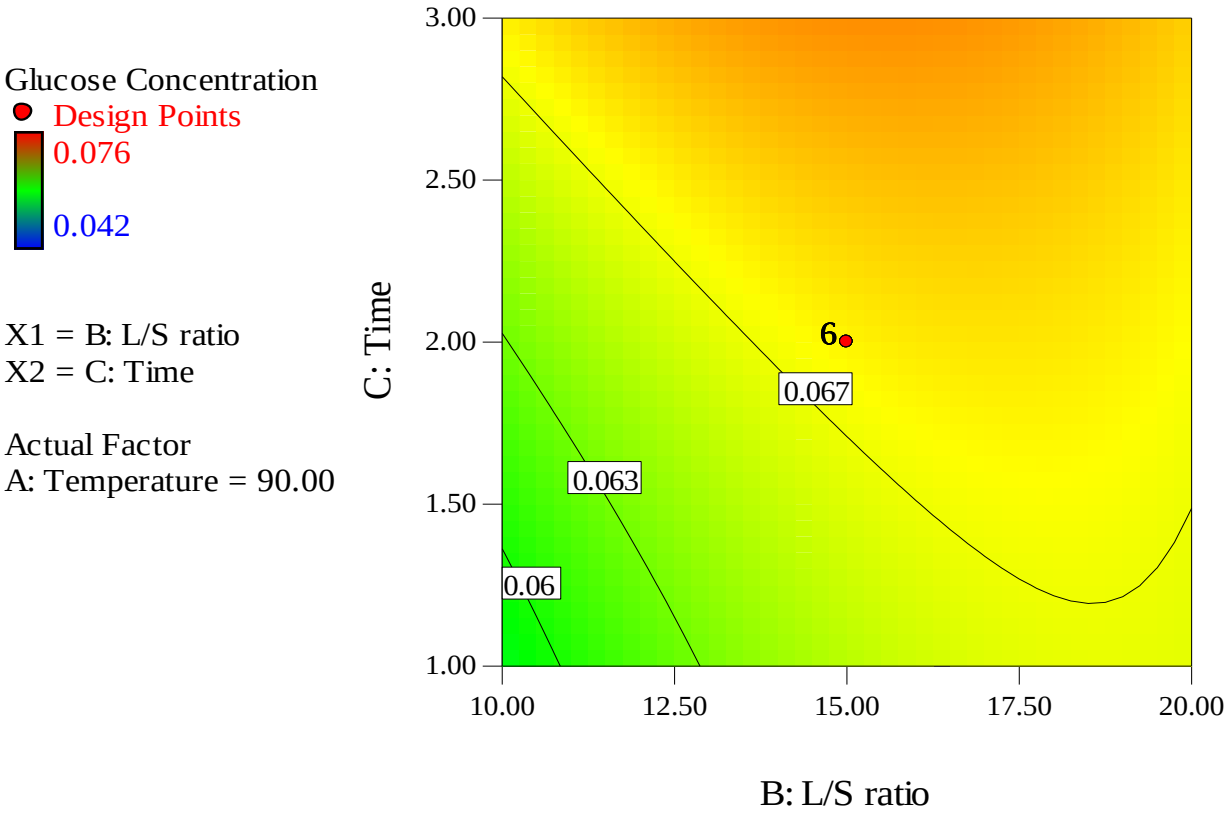

Figure 4:29 Contour plots of the effects of liquid to solid ratio and time on the glucose concentration when and temperature was at the center

Response surface plots for the effects of liquid to solid ratio and time

The graph shown in Figure 4:30 shows the surface plot of the response variable, glucose concentration. The change in glucose concentration with change in time and liquid to solid ratio is indicated on the graph. The response surface plot obtained from temperature and time has curvature. This is an indication that there is interaction between the two factors liquid to solid ratio and hydrolysis time on glucose concentration. The contour plot is also shown on the base of the graph. The plot proposes that there were well defined optimum operating conditions. The amount of maximum glucose concentration is found at the point where liquid solid ratio and time is at the middle point. At other edge of the surface the amount of glucose concentration is low.

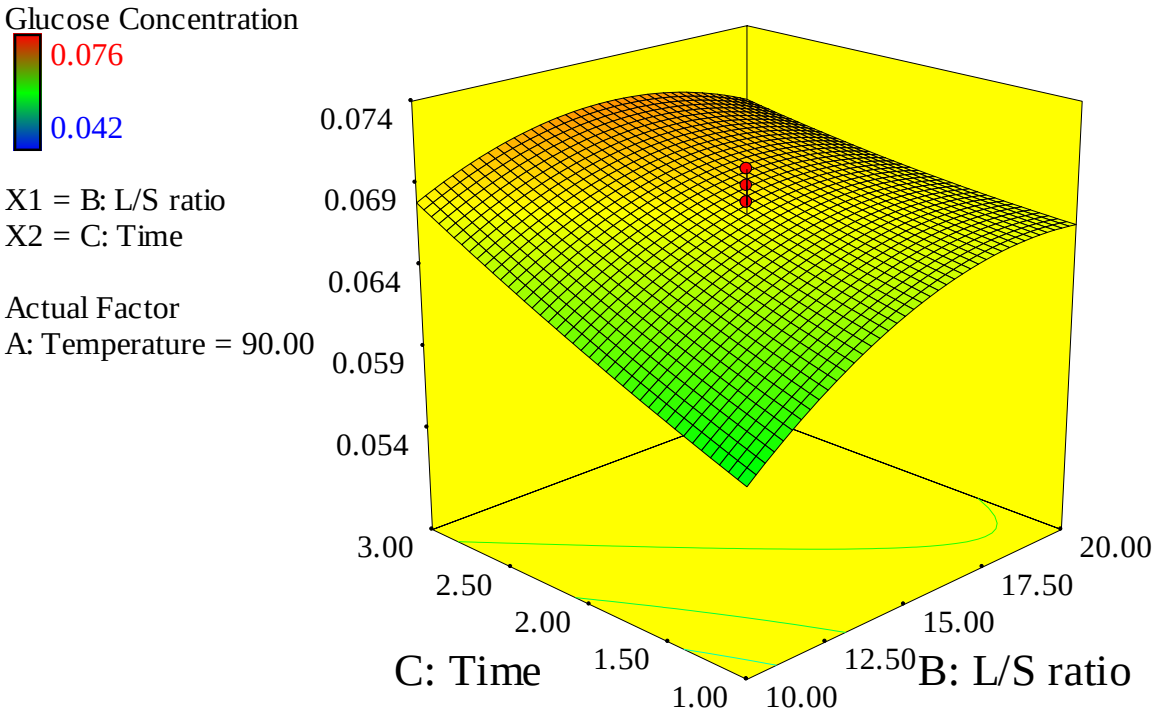

Figure 4:30 Response surface plots for the effects of liquid to solid ratio and time on glucose concentration when and temperature was at the center

4.2.2.5. Finding optimal value glucose concentration

This section describes the optimal value of glucose concentration. The optimal value of the factors was found from Design Expert. The factor considered are temperature, liquid to solid ratio, and time. The criteria for glucose production from potato peels using dilute acid treatment are summarized as follows in Table 4.9.

Table 4.6 Criteria for finding optimal value of glucose concentration

Optimization criteria for optimum glucose concentration			
Parameters	Purpose	Minimum value	Maximum value
Temperature (ºC)	In the range	70	110
Liquid to solid ratio (g/100mL)	In the range	10	20
Time (hr)	In the range	1	3
Glucose concentration(g/mL)	Maximize	0.042	0.076

The criteria in Table 4.9 were considered for getting the numerical optimal possible solutions for hydrolysis of potato peels. The optimal value of temperature, liquid to solid ration and hydrolysis time for glucose production are 102.66 ºC, 15.9g/100mL and 3 h respectively. The amount of glucose concentration is 0.074 g/mL. The results showed that the three process variables namely, temperature, liquid to solid ratio and time, and the interaction among the variables affect the concentration of glucose.

Validation of the model

In order to validate this prediction, laboratory experiments were performed and the average results were similar with the prediction. It was found that the experimental value of 0.0740 g/mL glucose concentration and 27.5 alcohol percentage, which well agreed with the predicted value. Compared to the data obtained from literature, the optimum hydrolysis conditions of glucose concentration lie in the accepted limits of starch hydrolysis conditions as suggested by (Tasic *et al*., 2009 and D. Arapoglou *et al*., 2010). Therefore, this study showed that potato peels waste is a potential source for the glucose production for bioethanol production. This waste can be the source for production of bioethanol.

4.3. Production of bioethanol from waste potatoes and potato peels

The fermentation was carried out on shaker incubator for 72 hours. Yeast, *Saccharomyces cerevisea* has done his job in changing glucose to ethanol. For survival *Saccharomyces cerevisea*, bakery yeast uses glucose as a food. In this biological process glucose is converted into ethanol

and carbon dioxide. Then at controlled temperature on oil bath distillation was executed. The amount of ethanol produced was measured using alcoholmeter.

Table 4.7 Ethanol percentage in solution from waste potatoes

Run No.	**% ethanol by weight**
1	28.6
2	33.2
3	30.5
4	22.4
5	29.9
6	21.0
7	12.5
8	20.5
9	23.2
10	27.3
11	19.4
12	23.0
13	16.3
14	32.8
15	14.5
16	15.4
17	26.0
18	18.3
19	25.0
20	27.3

The result presented in Table 4.11 shows the amount of ethanol produced from waste potatoes. Run 2, 3, 5, and 14 shows maximum amount of ethanol. However, run 7, 13, 15, 16 and 18 shows minimum amount of ethanol.

Table 4.8 Ethanol percentage in solution from potato peels

Run	% ethanol by weight
1	25.5
2	24.7
3	21.5
4	26.3
5	24.9
6	17.8
7	11.2
8	29.3
9	22.7
10	24.3
11	23.5
12	18.2
13	13.1
14	26.1
15	16.5
16	14.3
17	29
18	16.3
19	28.1
20	25.2

The result presented in Table 4.12 shows the amount of ethanol produced from waste potatoes. The measurement was carried out by alcoholmeter. Run 8, 17, and 19 shows maximum amount of ethanol. However, run 7, 13, 15, 16 and 18 shows minimum amount of ethanol.

The standard IR spectrum graph of ethanol is shown in Figure 4:31. The figure shows O-H, C-H, C-C and C-O stretch of ethanol. The peaks which actually pointing down show region of strong

absorption and the peaks which pointing up shows region of weak absorption. The broadest peak in the IR spectrum of ethanol comes from O-H stretches, similar to O-H bonds in water. This absorption at 3400cm^{-1} corresponds to a wavelength of about 2.9µm. Other strong stretching modes are C-H at 3000cm^{-1}, C-C at 1102cm^{-1}and C-O at 1050cm^{-1}, corresponding to 3.3µm and 9.5µm, respectively.

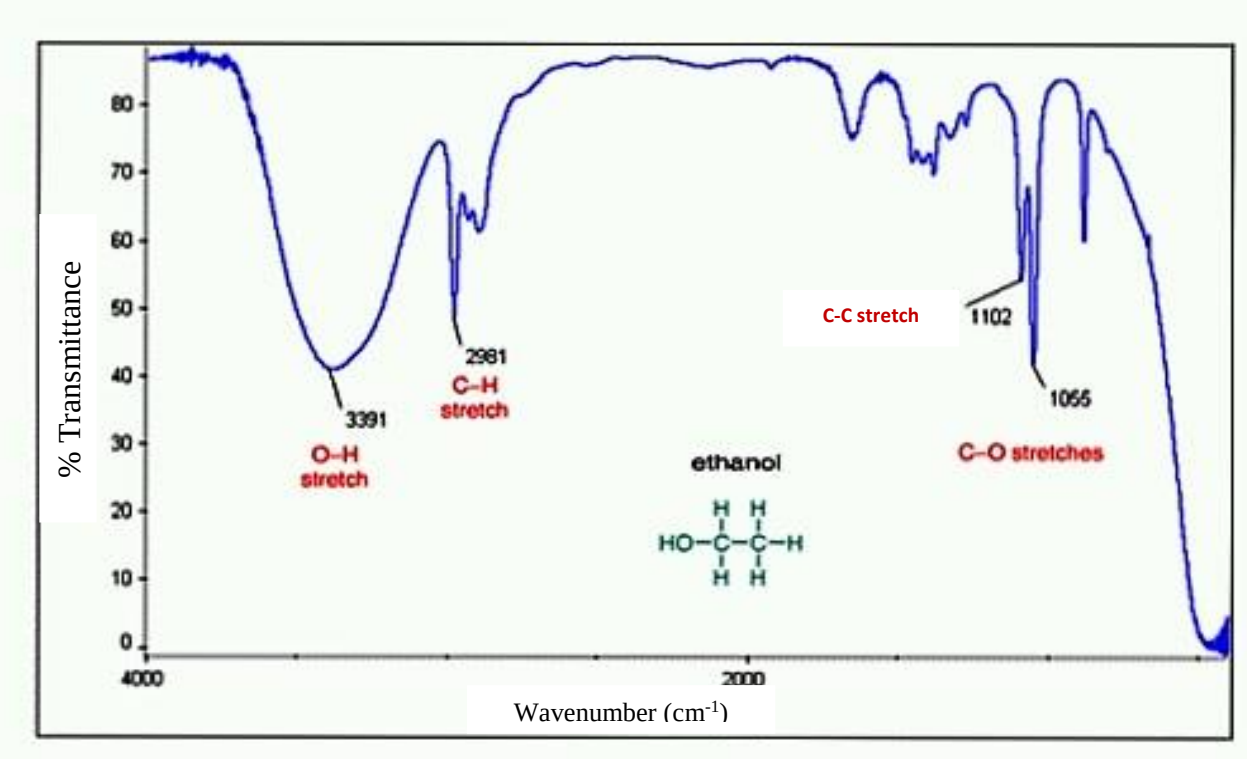

Figure 4:31 Standard ethanol FT-IR spectrum graph

IR spectrum of ethanol produced from waste potatoes is demonstrated in Figure 4:32. And it showed the same trend like the standard IR graph of ethanol. The molecular formula of ethanol is CH_3CH_2OH. The existing bonds are O-H, C-H, C-C and C-O in molecular formula of ethanol.

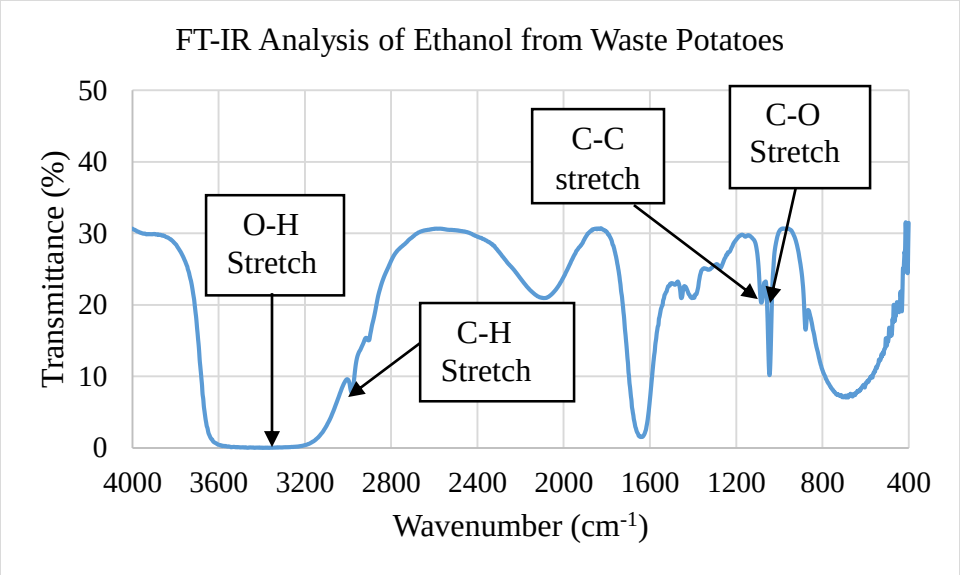

Figure 4:32 FT-IR spectrum analysis of ethanol from waste potatoes

Figure 4:32 shows FT-IR Spectrum analysis of ethanol from waste potatoes. The O-H, C-H, C-C and C-O stretch looks like the same as standard ethanol. This implies that the existence of ethanol in the produced alcohol solution.

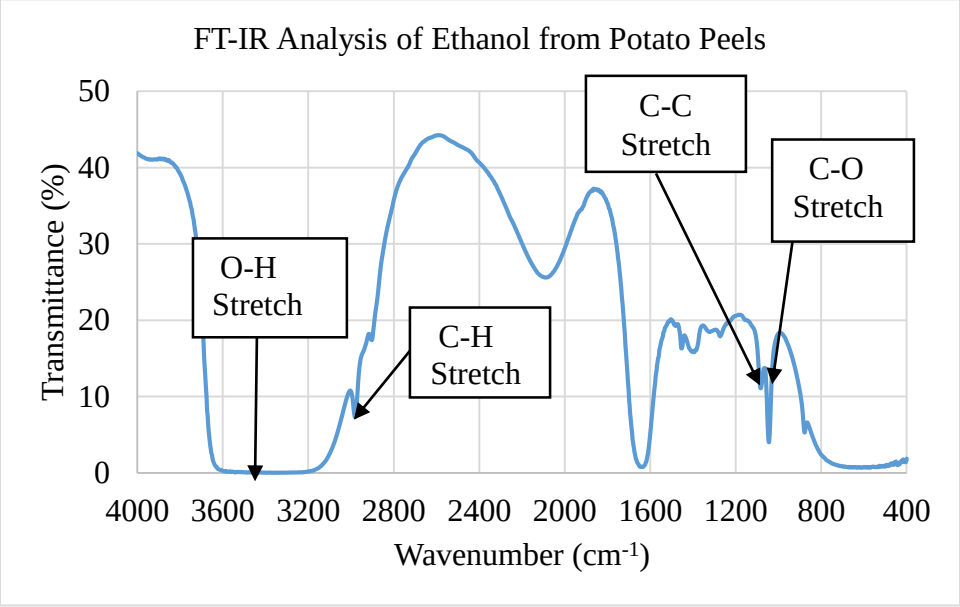

Figure 4:33 FT-IR spectrum analysis of ethanol from potato peels

IR spectrum of ethanol produced from waste potatoes is displayed in Figure 4:33. Like the Figure 4:33 for waste potatoes, Figure 4:33 shows FT-IR Spectrum analysis of ethanol from potato peels.

The O-H, C-H, C-C and C-O stretch looks like the same as standard ethanol. This recommends that the presence of ethanol in the produced alcohol solution.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

In this research ethanol was produced from wastes generated from potatoes. Starch hydrolysis with dilute acid was carried out. The effect of temperature, liquid to solid ratio and time on hydrolysis of waste potatoes and potato peels were investigated. From experimental results, all the process variables have significant interaction effect on glucose concentration. The maximum and minimum value of temperature, liquid to solid ratio and time contribute negative effect on glucose concentration. The optimal value of these three factor is in between low and high level. Samples containing high amount of glucose concentration produced high amount of ethanol as one might expect.

5.2. Recommendation

This research would like to suggest the following recommendation.

- Waste potatoes and potato peels should be utilized for ethanol production.
- Works done with regard to bioethanol production should not remain on paper and they should be implemented.
- The government should involve on bioethanol production from wastes generated from home, restaurant, juice house, hotels, cafeterias and chips shops to encourage business owners.
- Dilute acid was used for hydrolysis of starch, however hydrolysis with enzyme has to be checked.
- Further researches have to be done on hydrolysis of starch so as to get high amount of glucose concentration which gives high amount of ethanol.

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APPENDIX

Appendix A: ANOVA analysis for waste potatoes

Response	1	Glucose Concentration				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.46×10^{-3}	9	2.73×10^{-4}	13.34	0.0002	significant
A-Temperature	4.69×10^{-4}	1	4.69×10^{-4}	22.95	0.0007	
B-L/S ratio	9.04×10^{-5}	1	9.04×10^{-5}	4.42	0.0618	
C-Time	2.97×10^{-5}	1	2.97×10^{-5}	1.45	0.256	
AB	1.51×10^{-6}	1	1.51×10^{-6}	0.074	0.7914	
AC	4.29×10^{-5}	1	4.29×10^{-5}	2.1	0.1779	
BC	2.26×10^{-6}	1	2.26×10^{-6}	0.11	0.7467	
A^2	1.33×10^{-3}	1	1.33×10^{-3}	64.93	< 0.0001	
B^2	3.94×10^{-4}	1	3.94×10^{-4}	19.27	0.0014	
C^2	3.99×10^{-4}	1	3.99×10^{-4}	19.53	0.0013	
Residual	2.05×10^{-4}	10	2.05×10^{-5}			
Lack of Fit	1.05×10^{-4}	5	2.10×10^{-5}	1.05	0.4778	not significant
Pure Error	9.96×10^{-5}	5	1.99×10^{-5}			
Cor Total	2.66×10^{-3}	19				

Appendix B: ANOVA analysis for potato peels

Response	1	Glucose Concentration				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.75×10^{-3}	9	1.94×10^{-4}	7.42	0.0022	significant
A-Temperature	8.11×10^{-4}	1	8.11×10^{-4}	30.98	0.0002	
B-L/S ratio	6.35×10^{-5}	1	6.35×10^{-5}	2.43	0.1503	
C-Time	1.29×10^{-4}	1	1.29×10^{-4}	4.92	0.0508	
AB	2.51×10^{-6}	1	2.51×10^{-6}	0.096	0.7632	
AC	3.45×10^{-6}	1	3.45×10^{-6}	0.13	0.7241	
BC	2.21×10^{-5}	1	2.21×10^{-5}	0.84	0.3802	
A^2	6.44×10^{-4}	1	6.44×10^{-4}	24.61	0.0006	
B^2	7.95×10^{-5}	1	7.95×10^{-5}	3.04	0.1119	
C^2	5.54×10^{-6}	1	5.54×10^{-6}	0.21	0.6552	
Residual	2.62×10^{-4}	10	2.62×10^{-4}			
Lack of Fit	2.13×10^{-4}	5	4.26×10^{-5}	4.35	0.0662	not significant
Pure Error	4.89×10^{-5}	5	9.78×10^{-6}			
Cor Total	2.01×10^{-3}	19				