ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

PROCESSING *PROSOPIS JULIFLORA* AS A CATTLE FEED: CASE STUDY OF AFAR REGION AND AWASH RIVER BASIN

By

Girmaye Haile

JUNE 2011

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDY

DEPARTMENT OF CHEMICAL ENGINEERING

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in Partial fulfillment of the requirements of the Degree of Masters of Science in Chemical Engineering (Process Engineering Stream).

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June, 2011

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Acknowledgement

I wish to express my sincere gratitude and earnest appreciation to my research advisors, Mr.Gizachew Shiferaw and Dr. Getenet Assefa for their guidance, advice, patience, and encouragement in the development of this project and for their assistance in the preparation of this manuscript.

I am highly grateful to Dr-Ing Zebene Kifle for his encouragement, friendly approach and help whenever I need him. My gratitude also goes to Ethiopian Agricultural Research Institute, Holeta Genet for providing the results of my samples early which were crucial for my research work. Special thanks to my friends, Gezachew Assefa, Shimles Bekele, Sintayehu Nibret, Sisay Semere, Temesgen Matteous, members of Chemical Engineering Department staffs at Addis Ababa University. I would also like to acknowledge W/ro Seble wengel for all support she provides me during my research work.

My appreciation goes to Chemical Engineering Laboratory supervisors Alemayehu Mengsta, Biruk yohannes, Biruk Tefera, Henstaselassie, Maheteb Wedu, Nebiyu Getachew and Yosan Teshome for their invaluable support, friendship and making my laboratory work easier.

It is my pleasure to express my sincere appreciation to my mother, Tigist and brothers Ephrem and Tsegaye for their unconditional love and support in my education.

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List of Abbreviation

ADF	Acid Detergent Fiber
AOAC	Association of Official Analytic Chemists
CCD	Central Composite Design
CP	Crude Protein
CF	Crude Fiber
$C_6H_{10}O_5$	Anhydroglucopyranos
$C_6H_{12}O_6$	Glucose
DMI	Dry Matter Intake
DM	Dry Matter
DMD	Dry Matter Digestibility
IVDMD	In-vitro Dry Matter Digestibility
Lingo- cellulose	lignin and cellulose combination
NFE	Nitrogen Free Extract
NDF	Neutral Detergent Fiber
OMD	Organic Matter Digestibility
OM	Organic Matter
pH	Power of Hydrogen
P.Juliflora	Prosopis Juliflora
RSM	Response Surface method
TDN	Total Digestible Nutrient
USFS	the US Department of Agriculture Forestry Service

Abstract

In the Ethiopia, Prosopis juliflora was introduced in the 1970's by Ministry of Agriculture in the Awash River basin in the Afar National Regional State (ANRS) of Northeast Ethiopia. The plant rapidly invaded vast areas of a these region. Variety of material has been done on utilization of this plant for different application and benefit including as feed ration. Despite the high content of Nutrition available for the cattle feed, it is less digestible for ruminant animals. Therefore, Thermochemical Treatment methods of processing P.Juliflora to improve its digestibility have been suggested by different researchers. This paper assesses the effectiveness of treatment of Prosopis Juliflora using Sulfurous acid (SO_2 + H_2O) and was found to have the better potential.

The effect of reaction time, concentration of Sulfurous acid, and heat treatment for improving digestibility of Prosopis was investigated. The treatment was efficient that it almost increase the total digestibility of this plant by two fold making it comparative to other commercial roughage feeds. The treatment efficiency of this thermochemical method was increased with concentration of sulfurous acid and cooking temperature. The In-vitro Dry Matter Digestibility was appreciable when reaction time is also longer.

Laboratory scale reactor were conducted and showed good result on improving the digestibility of P.Juliflora. The In-Vitro DMD shows a maximum of 70% digestibility under condition of 6% SO_2, 120 ^oC and 2.5 hours reaction time including a 30 minute heating rate.

1.0 INTRODUCTION

1.1 Background

Prosopis juliflora is a perennial, fast-growing, often ever-green and drought resistant shrub or tree that grows in semiarid areas all over the world. This multipurpose dry land tree or shrub is native to South America, Central America and the Caribbean [1].

Scientific classification of Prosopis juliflora

Kingdom: Plantae, Division: Magnoliophyta,

Class: Magnoliopsida, Subclass: Rosidae,

Unranked: Eurosids Order: Fabales,

Family: Fabaceae, Subfamily: Mimosoideae,

Tribe: Mimoseae, Genus: Prosopis,

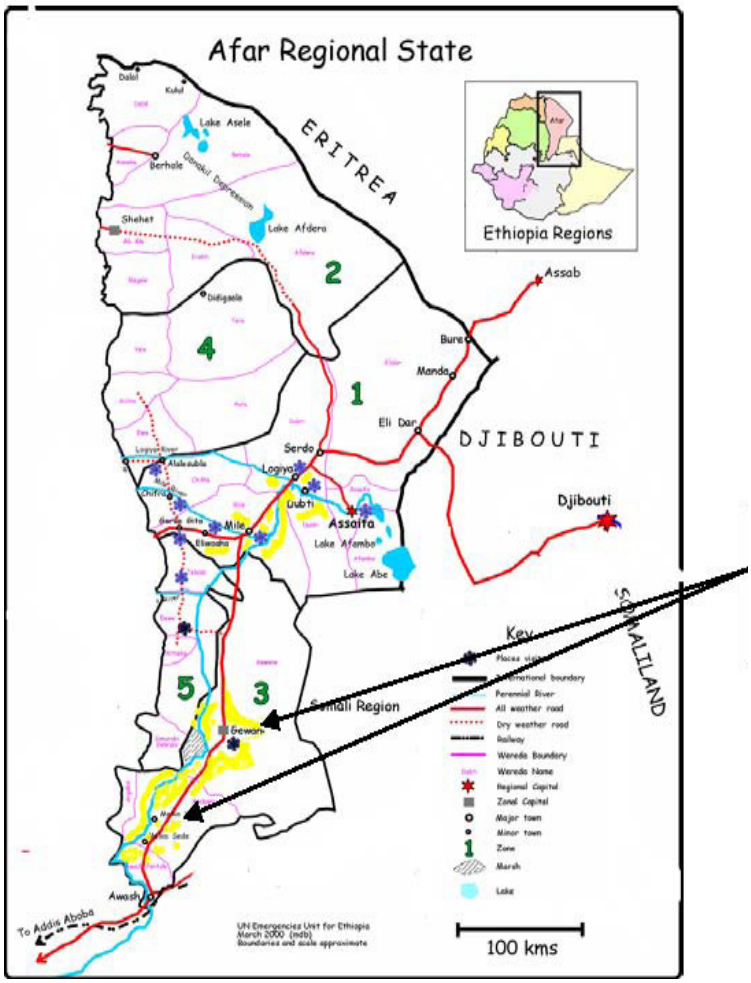
Species: Prosopis juliflora

Table 1.1 Category and families of Prosopis Juliflora, International Legume Database & Information Service.

Principal species	Prosopis africana
	Prosopis alba
	Prosopis articulata
	Prosopis caldenia
	Prosopis chilensis
	Prosopis cineraria
	Prosopis flexnosa
	Prosopis glandulosa
	Prosopis juliflora
	Prosopis nigra
	Prosopis pallida
	Prosopis tamarugo
	Prosopis velutina
Main common names	Mesquite (North America)
	Algarrobo (South America)
	Khejri (India)
	Weyane/Dergi-Hara, Biscuit (Ethiopia)

It has been introduced and naturalized in many parts of the world including Ethiopia and several arid and semiarid countries in Africa during the last 100-150 years [1]. It has also been planted successfully under desert like conditions where it is often used to halt shifting sand dune encroachment.

About three decades ago there was very little Prosopis juliflora in Afar Region. Prosopis Juliflora now occupies about over 700,000 hectares of prime grazing land and cultivable land following the Awash River in the Afar Region and this 2006 data and it is continually spreading [4].



The Yellow Color shows the area of coverage

Source of map: http://www.ocha-eth.org/Maps/downloadables/AFAR.pdf

Figure 1.1 Map of Afar National Regional State showing Prosopis coverage and area of spread.

This accounts for 15% of the region's productive land (4,670,316 hectares), excluding wetlands, water bodies, sandy and rocky areas (4,856,251 hectares).

When Prosopis juliflora infests grasslands, it continually robs grasses and other range forages of moisture, sunlight, and space. About two acres of good grassland, free of Prosopis juliflora is required to support one cow, whereas twelve to one hundred acres is required for Prosopis juliflora-infested land [6].

However, despite its qualities and uses in its natural range, Prosopis becomes a serious invading weed when introduced into non-native areas without proper management [2]. In Ethiopia and elsewhere, Prosopis has also caused considerable problems because of its rapid growth and damage to farmlands, pasture and especially the irrigated agricultural schemes. The shrub is dispersed in a many ways, including distribution of seeds from the pods via the faeces of goats and sheep. There are differing perspective in Ethiopia among policy makers and academics and such views range from total physical eradication of Prosopis to seeking alternative uses for it.

1.2 Rationale of the study

In order to provide an adequate diet for feeding the increasing world's population, many attempts have been made to increase the global food supply. One of the novel approaches is to produce animal feed from woods and agricultural waste materials, which in turn may relieve the grain being used by animals for human consumption. Wood and wood-based residues as a roughage substitute are not a new idea. As early as 1920, wood was being studied as the roughage component of animal feed [6]. Many researchers have been reported in improving the digestibility of woods and agricultural wastes such as straw through physical, thermal and chemical methods.

Livestock production plays an important role in Ethiopia's economy. Estimates indicate that livestock production contributed one-third of agriculture's share of GDP[5]. Ethiopia has great potential for increased livestock production, both for local use and for export. However, expansion was constrained by inadequate nutrition due low quality forages, cattle disease due to hygiene, and other reason [7].

Prosopis Juliflora is one of the most abundant plants in the Somali, Afar region and Awash River basin. Prosopis Juliflora has high amount (70-75 %) digestible carbohydrates and is a possible source of energy for ruminant animals. In addition, people in these regions are predominantly pastoralists dependent on livestock rearing for their survival. Untreated Prosopis is palatable that it is a possible source of bulk in rations for ruminant animals when either roughages are scarce; it may prove to be a dependable source of roughage during drought. However, at the present time, the low digestibility has been the primary factor for limiting greater feed utilization of P.juliflora.

1.3 Objective of the research

General Objective

The general objective was to develop sufficiently digestible cattle feed from Prosopis juliflora and accordingly to generate data for the Technology to be developed.

Specific Objective

The specific objective of this thesis was to investigate and apply treatment process: cooking P.juliflora with Sulfurous acid (SO_2-water mixture). The relative mass ratios of P.juliflora and Sulfurous acid were varied at specific reaction times and temperatures. Specifically to:-

- Investigate and analyze the chemical composition of the Prosopis juliflora forage (wood and leaf) in order to understand its potential for cattle feed.
- Assess the effect of Sulfurous acid treatment of the Prosopis juliflora and identify the optimal conditions in order to attain highest digestibility of treated Plant which is determined by In Vitro Dry Matter Digestibility (IVDMD).

2.0 LITERATURE REVIEW

2.1 The benefits of Prosopis juliflora

It is important to elaborate the usefulness of Prosopis and how people in other nations use this tree for multipurpose uses. Even though in Ethiopia this tree is consider to be invasive, many other countries where this tree is introduced and has been naturalized find the presence of Prosopis as a blessing for improving livelihoods.

2.1.1 Nitrogen fixation promoting soil fertility

Similar to many other leguminous plants, Prosopis has evolved a symbiotic relationship with nitrogen fixing bacteria. All Prosopis species have been found to fix atmospheric nitrogen, which plays an important role in arid ecosystems such as afar where soil nitrogen is naturally low. About eight strains of bacteria have been identified from Prosopis, and the strains identified were recognized to be tolerant to salinity, high temperatures and low soil moisture conditions or drought [8].

2.1.2 Benefits to biodiversity from faunal associations

Prosopis provides shade, protection and food not only for domesticated livestock, but also for wildlife [5]. The wide crown of the plant provides excellent shade for livestock during grazing and for wild animals in national parks. Leaves and pods are also an excellent food for many wild animals [9].

2.1.3 General Prosopis juliflora Applications

Much research has been devoted to the utilization of Prosopis juliflora. These methods include the conversion of Prosopis to useful products such as charcoal, ethyl alcohol, and plastics; the extraction of tannin from Prosopis; use as special wood products, such as block flooring and woodwork; use as raw material for pulp manufacturing and use as a livestock feed [10].

Special products can be produced or extracted from Prosopis. The wood has of cellulose and a high percentage of lignin, which is detrimental in the production of ethylalcohol but advantageous in the production of plastics [11]. The use of Prosopis as lumber has very little application. The Prosopis tree trunk is short and crooked, and the tree itself has many short branches. Prosopis could be ground into small pieces and used as fiberboard, or as a raw material in the pulp and paper industry, however, the abundance of other woods in forests makes these utilizations currently uneconomical [12].

2.2 Prosopis juliflora as a Cattle Feed

Ruminant animals such as beef cattle have the ability to digest cellulose and hemicellulose. These animals have four stomachs. Of these, the first three have the unique ability to digest cellulosic materials which humans cannot digest. This is possible because these animals have a symbiotic arrangement with cellulolytic microorganisms which habitat their rumen, a first stomach. Thus, the rumen provides ideal conditions for fermentation, and the microorganisms provide the animal with nutrients by enzymatically breaking down cellulose and hemicelluloses [14]. Pure cellulose is completely digestible and thus provides as much energy as the best feed grains [15]. The animals also obtain proteins from the bacteria and protozoa that pass into the digestive tract. Thus, in the overall process man gains because materials not suited for human consumption are converted to usable products [12].

In feedlots and dairy farms, the cattle are fed high carbohydrate grains to promote meat and milk production. However, some roughage material must be mixed with these high energy rations or else abnormalities in the liver and stomach occur. Roughage is also required to physically stimulate the rumen walls and promote chewing which increases salivation for the maintenance of rumen pH [15].

Most untreated wood can serve as a roughage substitute but contributes little to the dietary energy needs of ruminants. Table 2.1 compares the digestible percentages of numerous woods and grass forages.

Table 2.1 The digestible percentages of numerous woods and forages [42].

Species	Untreated Ground Ration % In Vitro Digestibility	Plant Type
Soft Maple	36	Twigs
Trembling Aspen	33	Buds
Bigtooth Aspen	31	Hardwood
Prosopis juliflora*	27	Hardwood
Soft Maple	17	Hardwood
Sugar Maple	7	Hardwood
Red Oak	3	Hardwood
Orchard Grass	64	Hardwood
Alfalfa	60	Grass
Hay	55	Grass

When the supply of conventional roughages (e.g., hay, corn cobs and cotton seed hulls) becomes limited and costly in a particular location, it is desirable to have alternative sources.

Prosopis juliflora is a nitrogen-fixing species and contains 70-75 percent digestible carbohydrates and crude protein in the wood as well as up to 100 percent digestible leaf. Therefore, it is a possible source of energy for ruminant animals. Prosopis wood can be ground and used as a roughage substitute without harmfu1 effects. But it is not first choice since the ground ration of raw P.juliflora is not a sufficiently digestible material to serve as a major component in cattle rations [15].

Several experiments have been conducted to determine the feasibility of using P.juliflora as an animal feed. Tests using P.juliflora as a roughage feed were conducted by the Texas Agricultural Station at Spur, Texas. In

this study, yearling steers were fed P.juliflora with molasses and P.juliflora without molasses. Approximately 10 pounds of P.juliflora without molasses were consumed per head per day, and 16 pounds were consumed per head per day when molasses was added to the feed. No palatability problems were encountered and acceptance of Prosopis was enhanced with the addition of molasses. A control group of steers were fed silage and at the end of the test, the carcass data of the control group differed little from the steers fed Prosopis [15]. This study would suggest that Prosopis, even untreated, is suitable as a roughage feed. Other research conducted has explored the potential uses of P.juliflora as an energy feed for ruminant animals. Instead of steers, pregnant lactating beef cattle were fed ground P.juliflora. In these studies, it was found that Propsopis without any supplements could not provide the nutritional or energy requirements for pregnant lactating beef cattle [16].

2.3 Chemistry of wood

In order to understand what chemical reactions are taking place when P.Juliflora is treated with sulfur dioxide, it is essential that the chemical composition of the wood be understood. Prosopis juliflora is a deciduous wood, and like all wood is heterogeneous in its chemical composition. Wood has three major chemical groups. Cellulose, hemicelluloses and lignin are the chemical components found in Prosopis and all other types of wood.

Cellulose is the most abundant polysaccharide found in wood. It is a linear with high Degree Polymer composed of 7000 to 10,000 glucose residues. It is a high molecular weight polymer with repeating 3-D-glucopyranose monomers composed of linear chains of D-glucose linked by ß-1,4-glycosidic bonds as shown Figure 2.1.

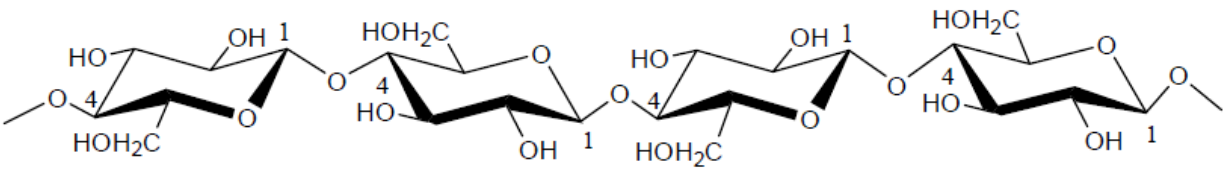
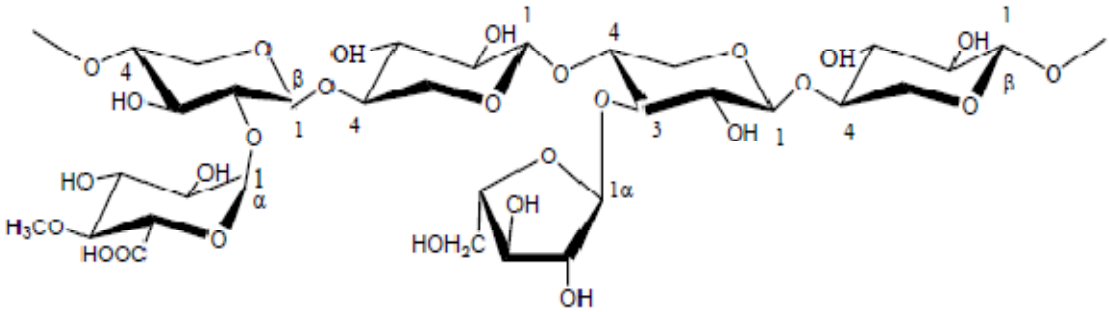


Figure 2.1 the Linear Structure of Cellulose in Wood, [29]

Each D-anhydroglucopyranose unit possesses hydroxyl groups at C2, C3, and C6 positions, capable of undergoing the typical reactions known for primary and secondary alcohols. The molecular structure imparts cellulose with its characteristic properties: hydrophylicity, chirality, degradability, and broad chemical variability initiated by the high donor reactivity of hydroxyl groups. Cellulose is, therefore, an anhydroglucose and can be converted to glucose by hydrolysis with dilute acid. From elementary analysis the monomer is best described by the empirical formula, [$C_6H_{10}O_5$], which suggests its close relationship to glucose, $C_6H_{12}O_6$ [18].

Wood also contains a second important group of polysaccarides, termed hemicellulose. Hemicellulose, like cellulose, is also a polymer, but differs in that it is a branched molecule composed of several different sugar units. It contains only about 150 to 200 of these sugar units. The hemicelluloses of wood are made up of five basic sugars; three hexoses, glucose,manose, and galactose, and two pentoses, xylose and arabinose.



Sugar units: β-D-xylopyranose (XylP); 4-O-methyl-α-D-glucopyranosyluronic acid (GlcpA); R = Acetyl group (CH3CO).

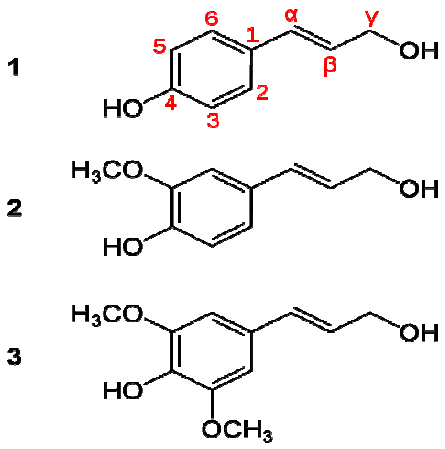
Figure 2.2 Principal Structure of Glucuronoxylan in Hardwood [29]

Hardwoods such as Prosopis contain a large amount of xylose in the form glucuronoxylan. All of the other sugars are only found in trace amounts. Like cellulose they are all insoluble in water, but are quite soluble in cold aqueous alkali.

Hydrolysis to simple sugars and some acetic acid is easily accomplished with hot mineral acid. Solubility in alkali and the ease of hydrolysis serves

to distinguish hemicellulose from cellulose. The combination of cellulose and hemicellulose in native wood is referred to as holocellulose.

Lignins are the major non-carbohydrate Components of wood. Lignin is an amorphous, polymetric material composed of propyl-benzene units, methoxyl groups, and hydroxyl groups. They are complex, cross linked polymers formed from phenolic units. A heterogeneous, branched and cross-linked polymer with phenylpropane units linked by C-C and C-O bonds



Common of monolignols: paracoumaryl alcohol (1), coniferyl alcohol (2) and sinapyl alcohol (3)

Figure 2.3 Principal Structure of Lignin [29]

Due to the structural differences in lignin, i.e. the type of base phenolic structure, softwood lignins are more easily degraded than hardwood lignins. Lignin is indigestible and resistant to many microorganisms. To isolate lignin from wood, 72 percent sulfuric acid can be used. The cellulose and hemicellulose will solubilize in the sulfuric acid, and the residue is designated as lignin [29].

The fourth component, termed extractives, includes a wide range of chemical types and a large number of individual compounds. The chemical types include terpenes, fatty acids, and aromatic compounds [20].

2.3.1 The association between lignin and the carbohydrates

If the digestibility of Prosopis could be increased, the processed feed would have potential for significant utilization on ranches. In order to meet these needs, a treatment would be necessary to make the cellulose more readily available to the rumen microorganisms. In order to increase the digestibility of wood, it is necessary to break down the lignin in the cell walls and to disrupt the structure of the cellulose.

The association between lignin and carbohydrate has not yet been fully established. The hemicellulose of wood is generally considered to consist of several types of amorphous, polymeric carbohydrates that can be associated with either cellulose or lignin. Three theories involving the association are hydrogen bonding between the constituents, covalent chemical bonds, and incrustation [20].

Hydrogen bonding is probably only a contributing factor in the ligno-cellulose bonding. Lignin has a considerable amount of polar alcoholic, phenolic, and ether-oxygen groups which can easily be involved with hydrogen bonding with hydroxyl groups in the carbohydrates [18]. However, hydrogen bonds are very weak unless systematically arrayed, which is unlikely with the amorphous character of lignin. It does not seem likely that hydrogen bonding explains the total resistance of wood from attack by cellulolytic enzymes.

Covalent chemical bonds also cannot explain the total resistance of wood to cellulolytic microorganisms' enzymes. Even if all of the lignin molecules were combined with carbohydrate, the wood would not be as resistant to cellulolytic attack as it is.

Incrustation, along with hydrogen and chemical bonding, is the most plausible explanation. The lignin polymer prevents the large enzyme molecules of ruminant bacteria from entering the structure.

2.4 Factors Affecting Digestibility

It has been demonstrated the relationship between the cellulose or lignin content of feeds and their digestibility [22]. Tomlin et al, they found that the digestibility of cellulose varied inversely with the lignin content as orchard grass matured.. In-vitro studies also found that the cellulose digestion and lignin content of grasses and legumes were inversely correlated [22].

It has been theorized that incrustation of lignin along with hydrogen bonding might explain the resistance of wood to cellulolytic enzymes.

This would suggest that lignin molecules position and bond to cellulose in such a manner that the cellulolytic enzymes are sterically hindered and cannot contact the cellulose. This would suggest that the lignin does not need to be removed from the wood but that it would be dissociated in such a manner as to remove its steric hinderance to cellulolytic enzymes.

Delignification removes this barrier completely. Swelling of the wood permits the entrance of enzymes [21].

2.4.1 General Treatments to Increase Digestibility

Many efforts have been made to increase the digestibility of wood and wood-based residues. Some of these treatments are electron irradiation, vibratory ball milling, gaseous and liquid ammonia, gaseous sulfur dioxide, and dilute sodium hydroxide. Each of these treatments can increase digestibility for particular wood wastes.

Mechanical processing, such as grinding and pelleting, usually increases daily intake but decreases digestibility of the dry matter. Vibratory milling can increase digestibility as much as 70% to 80%. However, the amount of increase for different woods varies. Tests have shown that in vitro digestibility results are higher than in vivo digestibility because finely ground feeds exhibit insufficient residence times in the rumen.

Generally, the responses to mechanical processing are generally greater with low- than with high-quality forages and with smaller, younger animals

than with larger, older ones. This is attributed to the greater degree of comminution necessary before the residue will pass from the retieulo-rumen of the smaller animals.

Electron irradiation destroys the lignin in order to increase the digestibility of wood. It is a very effective method of increasing digestibility of woods that are not initially very digestible. A high level of irradiation, however, also destroys the carbohydrates, and is very expensive [22].

Chemical processing has shown better improvement. Treatment with liquid anhydrous ammonia increases digestibility. Maximum digestibility can be reached in 30 minutes at 30°C. Hardwoods that have been treated with liquid ammonia have an increased swelling capacity which permits greater accessibility of cellulose to rumen microorganisms. Ammonia treatments also react with various woods to give different digestibilities [13].

Treatment with sodium hydroxide breaks by saponification the ester bonds that interlink the cellulose molecules, thus promoting the swelling of wood by water. This swelling degrades the wood further so that microorganisms can penetrate to the cellulose and increase digestion. Results show that this treatment affects various woods in different ways. For example, aspen and basswood respond extremely well to alkali treatment; ash, birch, and maple show an intermediate response; and fir and spruce show were little response to treatment. For hardwoods, the difference seems to be related to the lignin content [24].

In the treatment using Sulfurous acid, the lignin-cellulose association is broken chemically without removing the lignin. The lignin polymer is split apart when it is reduced by the H_2SO_3 and hydrolyzed to become lignosulfonic acid. This reaction is widely used in the pulp and paper industry. There is evidence that the association between lignin and cellulose can be broken by treatment with sulfur dioxide and water. The treatment of red oak sawdust with sulfur dioxide has been reported to increase its in vivo digestibility to a level comparable with that of a medium quality hay [25]. In these tests, red oak sawdust and water in a ratio of 1:2, were placed in a digestor and subjected to 30 psig of sulfur dioxide gas. The

samples were cooked at 110°C for 3 hours and then neutralized to pH of 8. An adequate amount of material was processed for feeding studies with goats being fed 0-50 percent rations. No palatability problems were encountered and in vivo digestibilities as high as 60 percent were reported.

From this study, it would seem possible that sulfur dioxide treatment of P.juliflora might yield results similar to the red oak sawdust.

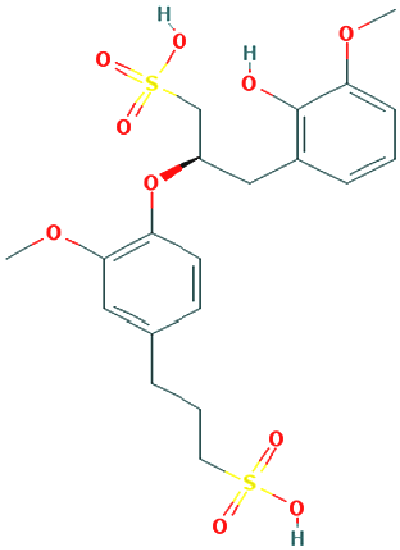
It has already been shown that if the association between lignin and cellulose is disrupted, an increase In vitro DMD occurs. This allows the cellulose of the wood to be digested by cellulolytic enzymes, and increases the amount of energy that can be utilized in animal feeds. If the bonding between lignin and the cellulose can be determined, this will lead to an understanding of how lignin can be displaced to allow bacterial enzymes to utilize the cellulose. Suphrous acid treatment is the most favorable treatment for P.Juliflora and detailed process will be discussed during experimental observations.

2.5 Techniques in Wood Pulping

In paper making, the commercial pulping process must remove lignin from wood. This delignification is accomplished by chemical agents which alter and solubilize the lignin. However, no known reagents are capable of dissolving lignin without attacking the carbohydrate components. The pulp and paper industry treats wood chips at elevated temperatures and pressures with sulfides under alkaline conditions (kraft process) or with sulfites under both alkaline and acid conditions to remove the lignin. The kraft process uses NaOH and Na_2S as the active chemicals in the cooking liquor. It evolved from the older soda process which used only NaOH. The addition of the sulfide ion accelerates the rate of delignification, with less damage to the cellulose and hemicellulose. The cooking temperatures range from 168°C to 175°C with corresponding pressures of from 95 to 115 psig. The cooking time varies from 45 to 90 minutes. The lower temperatures and longer heating times yield a more highly purified pulp [18][26].

In the sulfite pulping process, the cooking liquor consists of 4 to 9% SO_2 dissolved in water together with 2 to 3% SO_2 in the form of bisulfite. The total time of cook varies from 5 to 12 hours. Higher temperatures are used for shorter cooking times. The cooking times are relatively long in order to allow complete penetration of SO_2 into the wood chips. Thus, the temperature is slowly raised to 130°C with a pressure of 100 psi at the beginning of the cook [18][26]. The liquor to-wood ratio is usually around 10:1 by weight. The wood was heated up slowly and usually maintained at a maximum temperature of 130°C, and the pressure in the cooking vessel is in the range of 100 psi. There are three primary reactions which occur in the sulfur dioxide treatment of wood. These reactions are: sulfonation, hydrolysis, and condensation or polymerization [18][29].

In the sulfonation step, the bisulfite ion reacts with the lignin and partially disrupts the association of lignin and cellulose by forming lignosulfonate. The sulfonated lignin is easily hydrolyzed to lignosulfonic acid which is readily soluble in aqueous solution.



(IUPAC Name: (2R)-3-(2-hydroxy-methoxyphenyl)-2-[2-methoxy-4-(3-sulfopropyl) phenoxy] propane-1-sulfonic acid ($C_{20}H_{26}O_{10}S_2$).

Figure 2.4 Chemical Structure of Lignosulfonic Acid [14].

Hydrolysis and subsequent solubilization of lignin will only occur after a certain degree of sulfonation has taken place. The degree of cellulose degradation depends on the length of the cook and the acidity of the solution. The H^+ ion concentration controls hydrolysis and is favored at low pH. When a low pH is combined with high temperature, condensation or polymerization of the lignin molecules will occur. Condensation can be detected by the dark color of the cooking liquor. Although the condensation reactions which occur are not well understood, they tend to reduce the amount of lignin which can be hydrolyzed and broken away from the cellulose. When heating rates are fast and the sulfonation of lignin produces lignosulfonic acid which drops the pH and condensation occurs. Therefore, slow heating rate is important in order to favor hydrolysis of lignosulfonate.

As such, a small amount of condensation is tolerable. For this reason the chemistry regarding the sulfite paper process is useful, but the information must be interpreted with care when applied to the conditions appropriate for production of animal feeds.

2.6 Dry Matter digestibility (DMD)

A method called Dry Matter digestibility (DMD), will used as a design factor in order to analyze the effectiveness of thermochemical methods. There are two methods which are called In-vivo and In-vitro Dry Matter Digestibility. Both methods are used as a plant quality index for animal feed by animal nutritionists.

During In-vivo DMD analysis, there is real feeding to the cattle. Even if this test is expensive, it is more accurate and actual. The In-vitro Digestilbility is an anaerobic fermentation performed in the laboratory to simulate digestion as it occurs in the rumen. In-vitro Digestilbility which is also called the Apparent In-vitro DMD and OMD, was done according the modified Tilley and Terry method which was developed 1963. Although Apparent In-vitro DMD and OMD is one of the laboratory simulations of the cattle digestion system, it was proven by Tilley and Terry (1963) and Harris

(1970) that, the results In-vitro DMD and OMD were consistent with expected results based on existing information in the literature.

This observation further confirms the potential of this test to save time and effort since it is relatively simple and less expensive.

In this research Apparent In-vitro DMD was applied because the following reason.

- It is not possible to process enough material for actual feeding tests. This includes financial and time shortage.
- In-vitro DMD methods is fair enough for this research to fulfill the objective.

3.0 MATERIAL AND METHOD

3.1 Description of Prosopis juliflora Sample collection areas

Prosopis juliflora shrub was collected around Middle Awash river basin in methara which is the eastern region of Ethiopia. The land varies from 1000-1500m above sea level. The annual mean rainfall varies from 200-600 mm and mean temperature of 27^{o}C.

3.2 Sampling

Prosopis juliflora shrub was collected at their vegetative stage. It is known that the plant has two type appearance orientations. The tree types of Prosopis are more that 2 meter in which they were used for production of charcoal in some afar region and they are not suitable for harvest. On the other hand the shrub type of this plant has of 0.5 meter to 1.79 meter height and much branched which makes it a lot easier to harvest [1].

5 kilogram (72% wood and 28% leaf) of the vegetative stage shrubs are selected since the plant will have relatively less amount of lignin in there wood. These samples were taken from the targeted place with a random collection with four different plastic bags. The plants were cut with a handsaw. Immediately after collection, Prosopis juliflora originally had 34 percent moisture, but it was air-dried by spreading on the laboratory floor to approximately less than 7 percent moisture prior to storage to prevent spoilage. It is then ground through a cutter mill equipped with a 4 millimeter screen.

3.3 Thermochemical Treatment of Prosopis juliflora

Sulfur dioxide with 3.27-6.73 w/w percentage concentrations in form of sulfurous Acid was used for treatment of Prosopis. Sulfurous Acid was prepared in the laboratory much easily since sulfur dioxide is high soluble in water which is up to 22% especially in cold water. There is information on preparation and the gas solubility in relation with water on the Appendix-B. Ammonium Hydroxide was also used for Neutralization of Sulfurous acid treated Prosopis to neutral condition (pH= 7).

3.3 Methods for determination of cattle feed quality of P. juliflora.

The laboratory procedure for complete analysis of Prosopis sample is shown in Appendix-D.

3.3.1 Physical and Chemical Analysis

3.2.2.1 Determination of Dry Matter

The dry matter was determined by the weight loss of 2 gram samples exposed to 105°C for 24 hours which is approved by AOAC [43]. The dry matter content allows all other nutrient to be compared on a dry basis. Equipment used: Oven (Toschnival).

3.2.2.2 Determination of the Ash content

According to AOAC, ash was determined by the weight loss of 2 gram samples exposed to 600°C-800°C for 6-8 hours [43]. This measure represents the mineral content of the sample. Equipment used: Muffel-Furnace (Vecstar).

3.2.2.3 Acid and Neutral Detergent Fiber by refluxing Method

The acid detergent fiber (ADF) is composed of cellulose and lignin whereas the Neutral detergent fiber (NDF) is composed of cellulose, hemicellulose and lignin. It is used to determine the cellulose and hemicelluloses content of Prosopis. Laboratory was done based the official method of AOAC [44].

3.2.2.4 Determination of Soluble carbohydrate

Soluble carbohydrates were estimated by hydrolyzing is water them into monosaccharide or simple sugars [31].

3.2.2.4 Determination of Crude Protein

The crude protein was determined by multiplying the amount of total nitrogen which is by 6.25. Nitrogen is in all amino acids that are the building blocks of proteins and meat, which were determined by Semi Kjeldahl Method [44].

3.2.2.5 Determination Crude Fiber

The estimation of crude fiber is done by treating the moisture and fat- free samples successively with dilute acid and alkali. The residue is collected and the loss of weight on ignition is called Crude fiber [44].

3.3.2 Apparent In Vitro Dry Matter Digestibility Analysis

Each sample was run in duplicate according to the Moore modification of the Tilley and Terry method of Apparent In-vitro rumen digestibility which is explained briefly in Appendix-D.

Standard procedure of Apparent In-Vitro DMD analysis used in Holeta Research Institute is crossbred ruminant animals. The Apparent In Vitro Dry Matter Digestibility (IVDMD) Method involve ruminant microorganism, pepsin, hydrochloric acid incubator, furnace, Glass bottle, measuring cylinder and weighting scale will used during the experiment.

Percentage of Total Dry Matter Digestibility

$$\%\ \text{IVDMD} = \frac{\text{W1} - (\text{W2} - \text{W3})}{\text{W1}}\ \text{X}\ 100$$

Where: W1 = mass of Dry Matter sample
W2 = mass of Dry Matter residue
W3 = mass Dry Matter blank

The equation above shows how the percent total in vitro dry matter digestibility was determined for each sample after the rumen analysis is complete. During each analysis, duplicates of the blank were also run and a reference was run. In order to accept the rumen analyses as valid for a given lot of thermochemically-treated P. juliflora samples, the Hay and alfalfa reference must fall within a range of 50%-55% and 58% - 72% digestible respectively.

The percent total IVDMD values were reported in the following manner for the different Prosopis samples. Consider one member sample of a duplicate taken from a particular thermochemically-treated Prosopis batch. For this member, factors W1, W2, and W3 in the above equation are determined but note that W3 has two values because the blank was also run in duplicate. Each W3 value, and the average of the W3 values, is inserted into the equation along with the other factors to yield an average, high, and low percent IVDMD for the member. If the difference between the high and low values was approximately greater than 5%, the result was rejected.

When a Prosopis sample was analyzed for digestibility, because a given sample and the blank are analyzed in duplicate, four percent IVDMD values were available (two separate high and low values). If the results were acceptable, a mean percent IVDMD was calculated using these four values. The mean values of the highest and lowest percent IVDMD value were reported for a given compositional ratio and reaction conditions.

3.3.3 Analysis Lignin value

According to the 72 percent Sulfuric Acid method which is explained in the Appendix-D, to isolate lignin from wood, 72 percent sulfuric acid can be used. The basic principle is that cellulose, hemicellulose and crude protein will solubilize in the acid, and the residue is designated as lignin and ash [32].

Equation below shows how the lignin value is determined for each sample after the analysis is complete.

$$\text{Weight of Lignin} = \text{W residue} - \text{W ash}$$

Analysis of lignin value was determined in duplicate for each sample. Assuming that the difference between lignin values of the duplicates are less than 5%. The same error allowance for these means of duplicate had also been given.

3.4 Study Design

As briefly explained in Appendix-C, The surface response methodology (RSM) has been used throughout to design the experiment run. The data that are to be analyzed are processed and manipulated with the help of *Design Expert 7.0.0* software by *Stat-Ease Inc.*

The experiment was carried out based on the random selection of different runs using *design expert 7.0.0*. The following effects were considered: Concentrations of sulfurous Acid, Cooking temperature and Reaction time.

Therefore, in this study we will employ the most popular response surface method (RSM) design which is the central composite design (CCD). Here the effects of these three factors varied in five levels are to be determined.

3.2.1 Study variable

The experiment was designed to investigate the effects the various composition ratios of Prosopis to cooking liquor reacted at different reaction times and temperatures have on increasing its digestibility. Parameters such as amount of water and sulfur dioxide, Operating temperature, Processing time, the ratio of vapor space to the amount of Prosopis charged, the amount of neutralization required for processed Prosopis were studied.

3.4.1 Experiment Conducted

Table 3.3 Total number of Experiment chosen using Expert design 7.0.0

Std	Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours
10	1	Fact	4	100	2.5
16	2	Fact	6	120	2.5
3	3	Fact	6	100	1.5
15	4	Fact	6	120	2.5
13	5	Fact	4	120	2.5
1	6	Fact	4	100	1.5
4	7	Fact	6	100	1.5
17	8	Center	5	110	2
8	9	Fact	6	120	1.5
18	10	Center	5	110	2
12	11	Fact	6	100	2.5
6	12	Fact	4	120	1.5
5	13	Fact	4	120	1.5
14	14	Fact	4	120	2.5
2	15	Fact	4	100	1.5
19	16	Center	5	110	2
20	17	Center	5	110	2
9	18	Fact	4	100	2.5
7	19	Fact	6	120	1.5
11	20	Fact	6	100	2.5
26	21	Axial	5	110	2.866025404
24	22	Axial	5	127.3205081	2
22	23	Axial	6.732050808	110	2
21	24	Axial	3.267949192	110	2
23	25	Axial	5	92.67949192	2
25	26	Axial	5	110	1.133974596

3.4.2 Statistical Design

Analysis of variance (ANOVA) was used to investigate the statistical significance of the results obtained in order to compare main and interaction effects of each factors and multiple comparisons was conducted with the help of *Expert Design 7.0.0* Software. A 95% confidence limit is commonly used, meaning that, if the probability is less than 0.05, the difference is 'significant' and not caused by chance [36].
Model equation was also developed using regression method and adequacy of the model was analyzed from statistical point of view, with the following:

a) Test of significance of factors and interactions,
b) R-squared test, and
c) The lack-of-fit test

Test of significance means leaving out insignificant factors of a model, which in turn produces a simpler mathematical model and easier interpretation [41]. When p-value (probability value) of one term is more than 0.05, it indicates that this term is insignificant at the 95% confidence level and therefore that term must be discarded.

R-square is the relative predictive power of a model and is in the range of 0 to 1. If it is much closer to one, the model can better predict real data.

The test of lack-of-fit is used to determine whether discrepancies between measured and expected values can be attributed to random or systematic error. The lack-of-fit test compares the residual error to pure error from replicated design points. If p-value for lack-of-fit is less than 0.05, there is statistically significant lack-of-fit at the 95% confidence level.

3.5 Experimental setup and description

3.5.1 The 35-G Reactor System

Figure 3.1 is a schematic representation of the Laboratory system arrangement. To determine preferred operating conditions for the Prosopis pilot plant, it is necessary to know the temperature, heating rate other parameter during the reaction of Prosopis with sulfurous acid. In order to measure these parameters, a small, bench-scale, batch reactor was assembled. In addition to measuring temperature, 35 g of Prosopis was processed per batch, so that reasonable amounts of treated Prosopis are available for laboratory testing.

This reactor is referred to as the 35-G reactor and a description follows. The reactor can best be described as a cylindrical neck, round bottom, Borosilicate, and glass vessel. The reactor vessel is 14.33 cm in diameter, 27 cm high and has a capacity of 1 liter. A 3.5cm opening in the top provides for convenient removal and addition of sample and to assemble the stirrer and motor. The reactor has a rating up to 1 bar and more than 150°C. The reactor is heated indirectly with heat transfer oil using thermostat heaters. The thermostat heaters consist of on electrical resistance coil, thermocouple, digital reading and oil pump. Another thermometer, placed near the reactor body, permit the wall temperature of the vessel to be measured. This is to insure that temperature excesses which might damage the reactor walls can be avoided, and to help prevent the Prosopis from being overcooked and consequently burn up on the inside reactor wall. The temperature inside the reactor is calibrated by measuring the temperature of the heating oil around the reactor. The reactor is agitated by revolving stirrer which is inserted inside the reactor and installed using a closure gasket. They are used to assure that uniform heat-up of the entire content in the reactor is occurring.

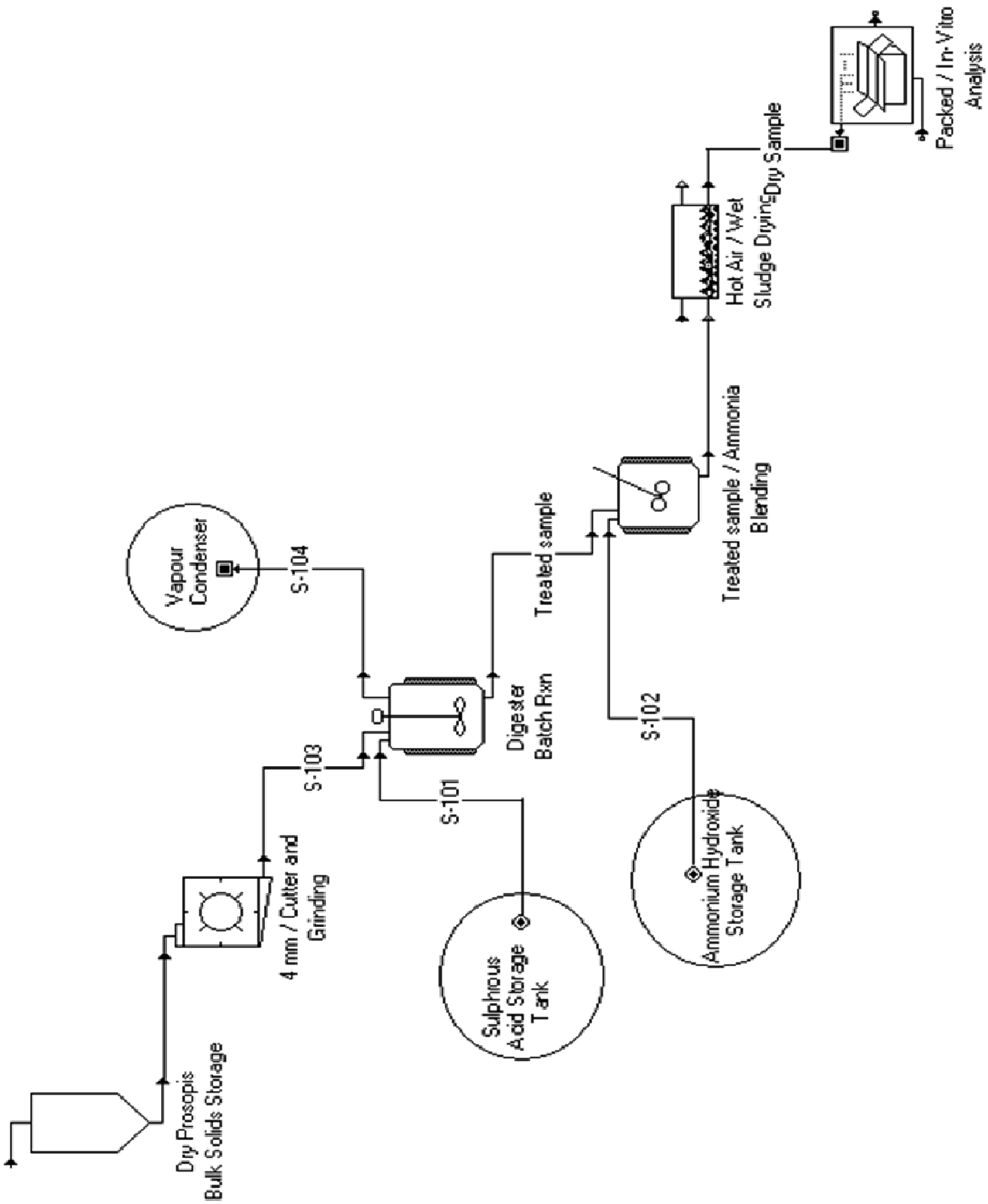


Figure 3.1 Schematic Diagram of Experiment Setup for Prosopis juliflora treatment.

The reactor has two 4.9 cm nozzles called U- Tube to permit attachment of condenser in order to cool down vapor consequently decrease the vapor pressure inside the reactor. All temperatures during heating rate are recorded manually. The 35-G Reactor and attachments are shown below.

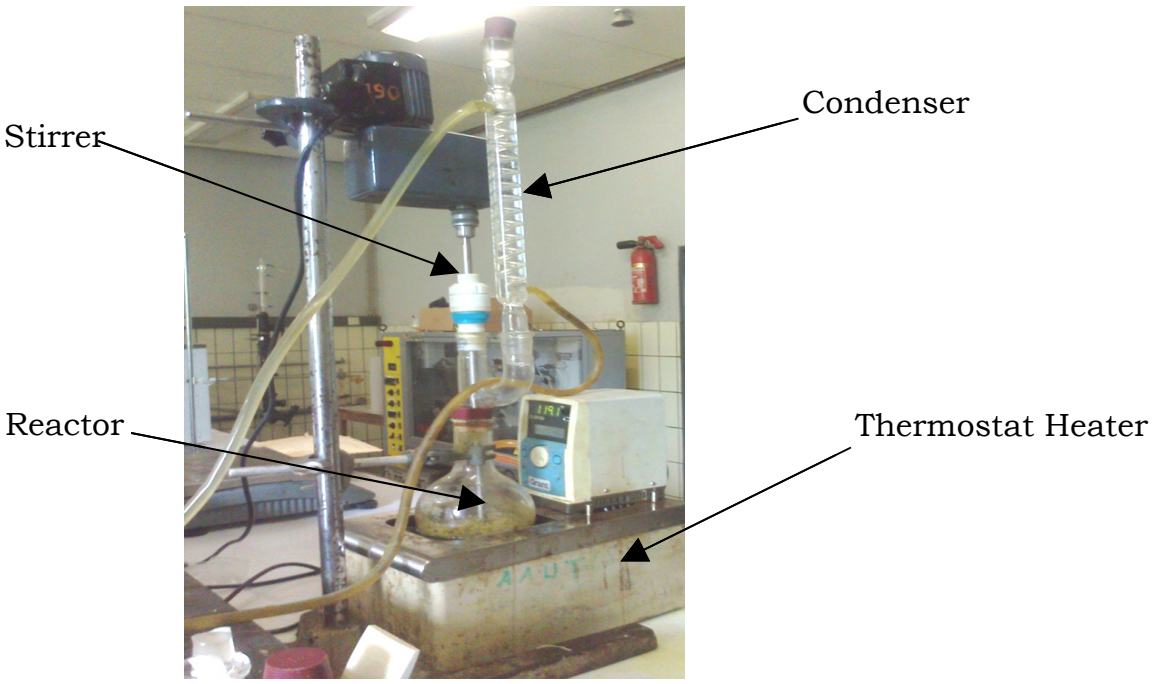


Figure 3.2 Photo of the reactor set up (Photo shot: Author)

In particular, the vapor space volume with respect to the amount of Prosopis reacted and water is important, the ratio of vapor space to amount of Prosopis and liquor charged is tabulated below:

Reactor	Reactor Volume, ml	Batch Size, gm	Ratio of Volume To Batch Size, ml/g
35-G	1000	87.5	11.4285

The 35-G Reactor was not entirely filled with Prosopis so that the Prosopis would mix when the reactor was being agitated.

3.5.2 Heating rates in the reactor

To estimate optimum reaction temperature and time for treating Prosopis in large reactors, it was desirable to know the actual time and temperature history inside the reactor being used for the further tests. Using a thermocouple attached and the reading on the thermostat and the time-temperature data were observed and registered. These data are also plotted in Figure 3.3. After thirty minutes in the thermostat, the reactor temperature was about 10-14 degrees below the heating oil temperature, and after one hour, the reactor was within 5-7 degrees of oil temperature.

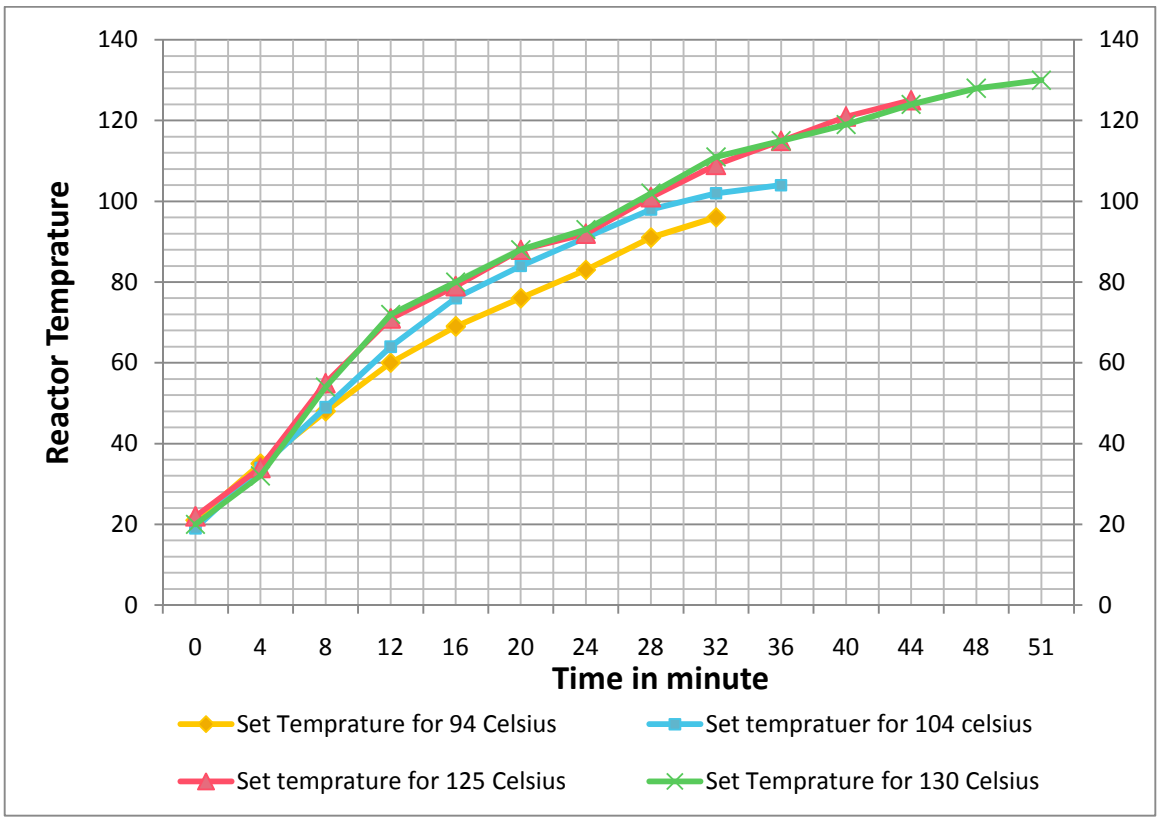


Figure 3.3 Heating rate in the Reactor at set temperature of 94°C, 104 °C, 125 °C and 130 °C.

3.6 Experimental Procedure

3.6.1 Size Reduction

Grounding of Prosopis involved a cutter mill equipped with a 4 mm screen. It was very difficult to quickly characterize the particle size of a very fibrous material.

Figure 3.4 Cutter mill for size reduction (photo shot : Author)

The smallest dimension of a particle fiber or particle was speculated to be the dimension which controls the rate at which diffusion can transport sulfur dioxide into the interior of the particle for reaction. Figure 3.5 illustrates particle distribution of the ground, raw Prosopis. The air-dry material was mixed, bagged and stored in a cool, dry location.

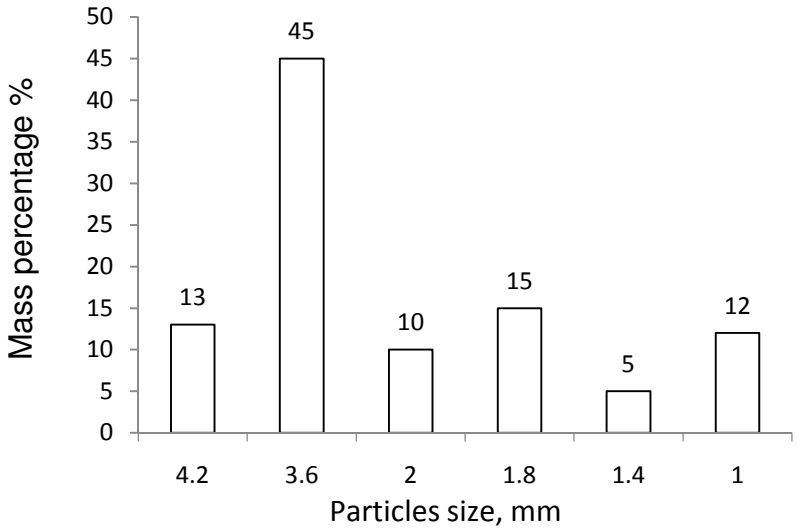


Figure 3.5 A Typical Prosopis Sample Particle Distribution before Treatment.

3.6.2 Treatment with Sulfurous Acid

Shredded Prosopis, usually 35 g, and sulfur dioxide as sulfurous acid, and additional distilled water added so that the final sample contained 60% water by weight. For low concentrations, it is convenient to add the sulfur dioxide as a 6-8 percent. This is commonly called sulfurous acid. Sulfurous Acid was prepared in the laboratory much easily since sulfur dioxide is high soluble in water which is up to 22% especially in cold water. There is information on preparation and the gas solubility in relation with water on the Appendix-B.

Heat was supplied by means of the heat transfer oil from thermostat heaters in which the reactor is immersed in the hot oil bath while the reactor is agitated at 60-70 revolution per minute. A constant stirring was maintained to approximate uniform heating of the slurry. Heat input rate is limited to prevent burning the sample on the sides of the reactor and to prevent overheating the heaters on the outside wall of the reactor.

The temperature of the thermocouples placed adjacent to the heater resistance coils was operating automatically and feedback controlling the heat input based the set desire temperature. The reactor can be adjusted to reach maximum temperature with the desired heating rate. The time required to bring the contents to a specified temperature was about 31-52 minute and Figure 3.3 illustrates the typical heating rates. When the reactor reached a preselected temperature, 120°C in the experiments reported here, the heat input was automatically reduced to maintain a steady temperature. Once the desired temperature was reached, the reaction is maintained at this T for a specific length of reaction time. At the end of the reaction period, agitation was stopped and the heaters turned off. Steam and sulfur dioxide were then vented. The PH this solution depends on the concentration of sulfur dioxide in the reactor system.

3.6.3 Product Neutralization

After the P. juliflora was treated with sulfrous acid, the product was at acidic media in which pH range from 4.5 to 6 for varying concentrations of sulfur dioxide used and reaction efficiency. Vaporization of water in the P. Juliflora quickly cools it to the boiling point at atmospheric pressure.

$$H_2SO_3 + NH_4OH \longrightarrow NH_4SO_3 + H_2O$$

This neutralizing base was choosen for the following reason:

- Weak base is favourable for neutralization of sensitive material inorder to avoid further reaction with the product.
- Ammonia in form ammonium sulfite could be as source nitrogen because there are microorganism available in ruminant which have an ability to change it into digestible protein.

The treated product was needed tobe neutralized to PH= 7 by using an average 1.3608 ml per batch of Ammonium Hydroxide (35% of NH_4OH solution). Amount of Ammonia solution added for each treated Prosopis is exhibited in Appendix-B.

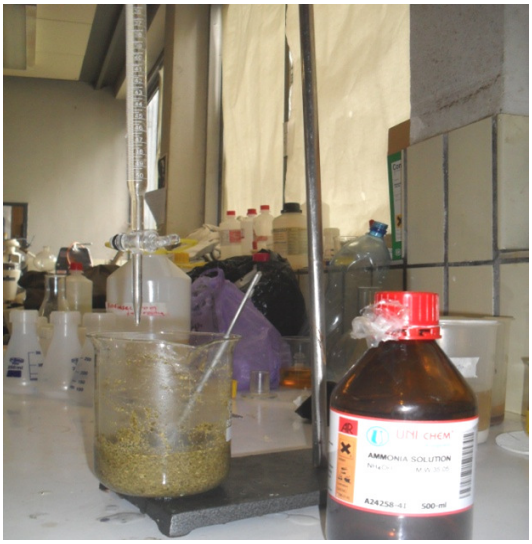


Figure 3.6 Neutralization of treated Prosopis (Photo shot: Author)

3.6.3 Drying of Final Product

After, the treated Prosopis was neutralized to pH 7 with 0.5N of ammonium hydroxide and then the product slurry was poured into a large, rectangular, flat-bottom, stainless steel pan. The pan was then placed in an air drying oven and dried at 50°C until the product contained 1 to 7 (w/w) % moisture content using weight differences. After completion of the drying process, the solid product was placed in a sample bag, labeled, and stored at 4°C in a refrigerator to prevent spoilage until it was delivered to the Animal Science Department of Ethiopian Agricultural Research Institute for In vitro DMD and OMD rumen analysis.

4.0 RESULT AND DISCUSSION

A complete analysis of Prosopis sample is shown in Table 4.1 and 4.2. The cell walls contain lignin, cellulose, and hemicelluloses. The cell soluble which include crude protein and soluble carbohydrates, are highly digestible. The acid detergent fiber (ADF) is composed of cellulose and lignin whereas the Neutral detergent fiber (NDF) is composed of cellulose, hemicellulose and lignin. Cellulose, Crude protein, Carbohydrates are very digestible and Crude Protien is partially digestible in the rumen and they are a valuable energy source for cattle. Lignin is not digestible.

If the cell wall contains a high quantity of cellulose and a low amount of lignin, then the sample is very useful as a ruminant feed. Laboratory procedure for analysis the plant is explained briefly in the Appendix-D. Mentioning the data on table below the nutritional value of Prosopis makes potential candidate for cattle feed and comparative to commercial roughages such as alfalfa and hay grass.

Table 4.1 Nutritional content analysis of Prosopis juliflora Leaves.

ITEMS	PERCENTAGE, %
DRY MATTER	53
COMPOSITION, PERCENTAGE OF DRY MATTER	
ORGANIC MATTER	94.9
ASH	5.1
Lignin	0
CELL SOLUBLES	
CRUDE PROTEIN	22
CRUDE FIBER	22.8
NITROGEN FREE EXTRACT(NFE) + ETHER EXTRACT	47.9

Table 4.2 Nutritional content analysis of Prosopis juliflora wood

ITEMS	VALUES IN PERCENTAGE, %
DRY MATTER	94.4
COMPOSITION, PERCENTAGE OF DRY MATTER	
ORGANIC MATTER	92.5
ASH	7.5
CELL WALLS	62.4
ACID DETERGENT FIBER	46.7
CELLULOSE	28
LIGNIN	17.7
NEUTRAL DETERDENT FIBER	63.4
HEMICELLULOSE	16.7
CELL SOLUBLES	37.5
CRUDE PROTEIN	22.6
ESTIMATED SOLUBLE CARBOHYDRATES	14.9

NDF = Hemicelluloses + Cellulose + Lignin + Minerals

ADF = Cellulose + Lignin + Minerals

Cellulose = ADF – Residue after extraction with 72% sulphuric acid

Hemicellulose = NDF- ADF

Lignin= Residue after extraction with 72% sulphuric acid – ash

% NFE = % DM - (% EE + % CP + % ash + % CF)

Where: NFE = nitrogen free extract

DM = dry matter, EE = ether extract or crude lipid

CP = crude protein, CF = crude fiber

About 26 productions of sulfur dioxide treated samples were done. Quality of the roughages was measured with their nutritional value and the exposure of the nutrition to cellulose assimilating microorganism inside cattle's stomach. As shown on table 3.1, 3.2, and discussed on the literature part that P. juliflora has high nutritional value.

The next most important issue that makes cattle feeds most primary choice even at commercial level are their high digestibility to mean that having high percentage of In-vitro DMD. Therefore, In-Vitro DMD Analysis is the response for optimization of this experiment. Lignin loss (lignin value) was also discussed since they have effect on the quality of treated sample and helps to explain somehow the reaction that was going on during treatment.

4.1 Experiment Data In-vitro DMD Analysis

The overall In-vitro dry matter digestibility analysis of SO_2 treated samples' were done in Ethiopia Agricultural Research Institute, Holetta Genet. The experiments took place on random order based on *Expert Design 7.0.0* software suggestion. The design model was designed with quadratic response surface and a modified quadratic model equation was also used to define the trend of In-vitro DMD response data as it was suggested by *Expert Design* software. As you can see in Appendix-A, the model was adequate to represent the true value and it can be used to navigate the design. The total digestibility of the Prosopis juliflora, which had no treatment, was 47% and the improvement was discussed referencing from the untreated sample DMD.

GROSS ENERGY MCAL/KG (DM)	4.22
IN-VITRO TOTAL DIGESTIBILITY OF UNTREATED PROSOPIS	
DRY MATTER	47
ORGANIC MATTER	40

4.1.1 Main Effects

Effect of reaction temperature

In the literature, Sulfite pulping has diffusion barrier before the SO_2 and Lignin reaction process. As reaction temperature increases, Diffusions of SO_2, H^+ and water as well as product like Lignosulfonate will increase which crucial since it will avoid barrier to reaction. This facilitates delignification due to the fact that sulfonation and hydrolysis reactions have direct relation with temperature. Increasing temperature also favour swelling of Prosopis wood takes place. Despite higher temperature might have a negative effect on favoring condensation reaction which binds cellulose and lignin, it is not severe within the set temperature range. As it is shown in Figure 4.1 (a)-(c), reaction temperature has an effect on In-vitro DMD analysis. In figures, In-vitro Analysis was increased when reaction temperature was increased to from 100°C to 120 °C in quadratic relation. Even if the figures have more resemblance; the effect temperature as observed on the slope of the figure has decrease as the other factors level are increasing close to flat shape. This shows that there is interaction effect of the other factors.

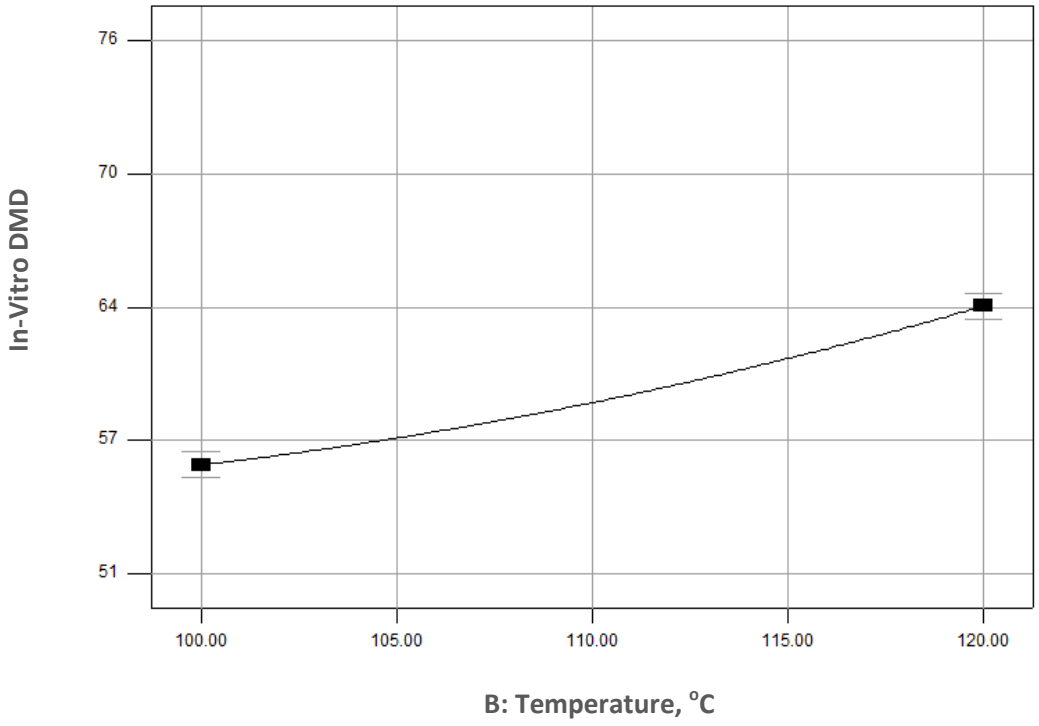


Figure (a) Conc. of SO_2 = 5.00 and Reaction time = 1.50

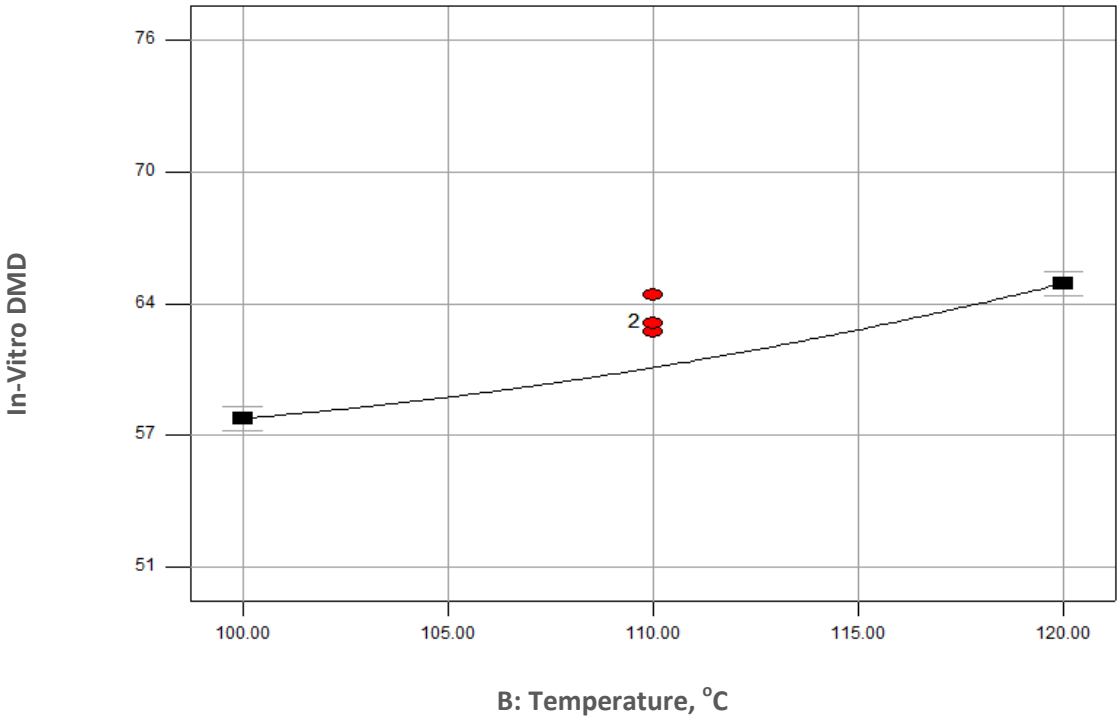


Figure (b) Conc. of SO_2 = 5.00 and Reaction time = 2.00

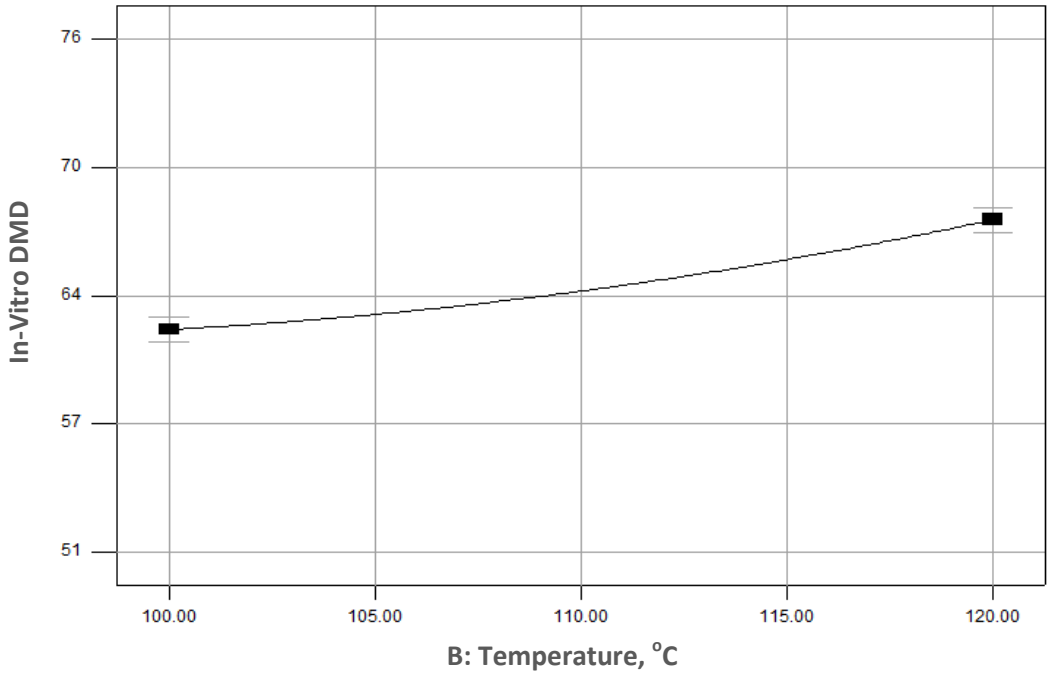


Figure (c) Conc. of SO_2 = 5.00 and Reaction time = 2.50

Figure 4.1(a)-(c) Effect of temperature on In-Vitro DMD at different Conc. of SO_2 and Reaction time.

Effects of SO_2 Concentration

Figures 4.2 of (a) up to (c) show similar pattern on the effect of SO_2 concentration on In-Vitro DMD. As Sulfurous acid (SO_2 + H_2O) concentration increased, Total Digestibility values were increased. According to literature, in sulfite pulping, when concentration of Sulfurous acid (SO_2 + H_2O) increases, there is high amount of sulfite ion and H^+ ion which favor sulfonation and hydrolysis reaction.

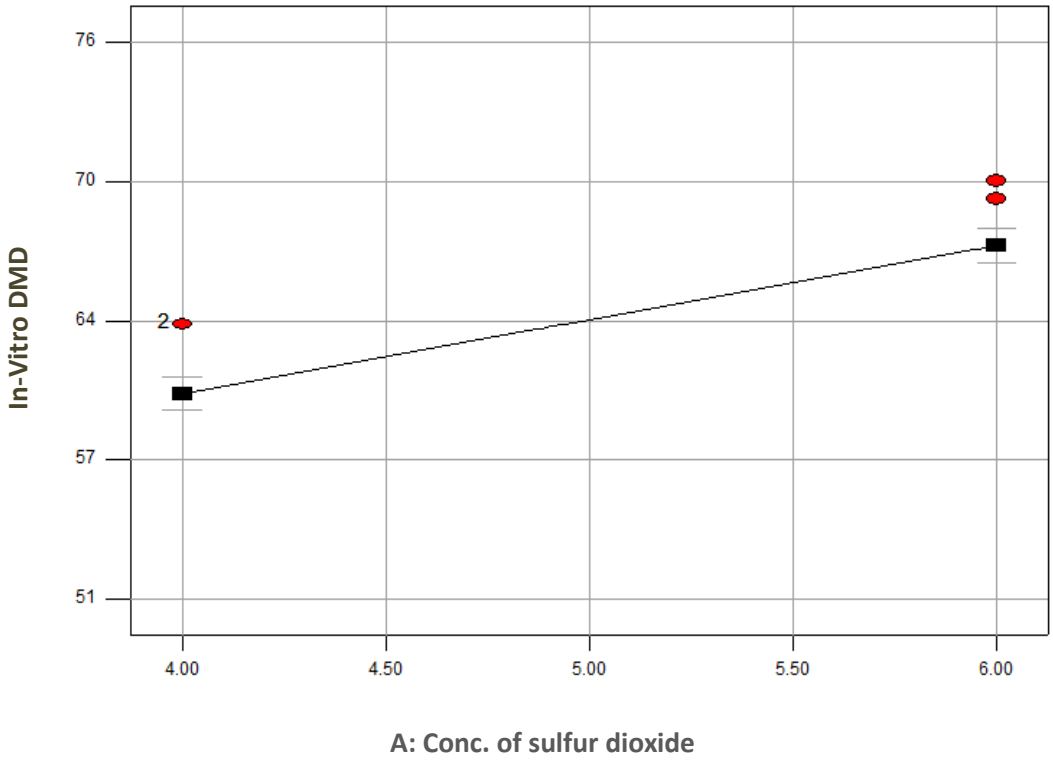


Figure (a) reaction temperature = 120 °C and Reaction time = 1.50

Therefore sulfonation reaction increase with concentration increase and The H+ ion concentration controls hydrolysis and is favored at low pH. The In-vitro DMD response has a linearly relation with the concentration. In addition, the response increase to maximum value when the temperature and reaction time increase as seen at the graphs especially in graph 4.2 (b) and (c) this indicates that there is an interaction effects. This shows that other two factors has an effect of avoid barrier that limits the two important reactions. According to the statistics analysis, it also support the above explanation. It is shown that the reaction is most significant factor ($p<0.0001$) next to Concentration of SO_2 ($p<0.0001$).

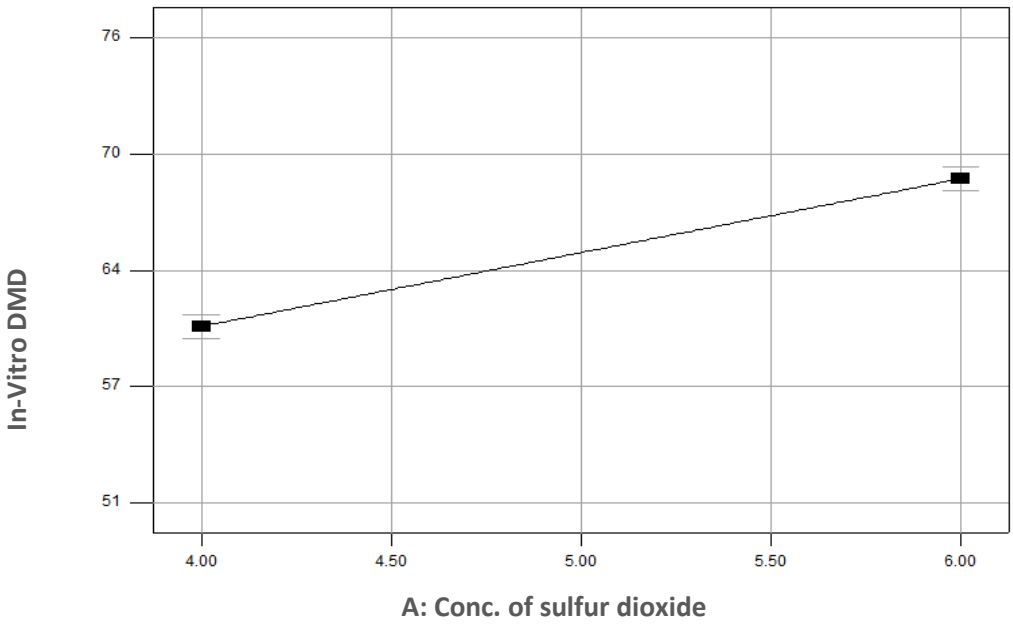


Figure (b) reaction temperature = 120 °C and Reaction time = 2.00.

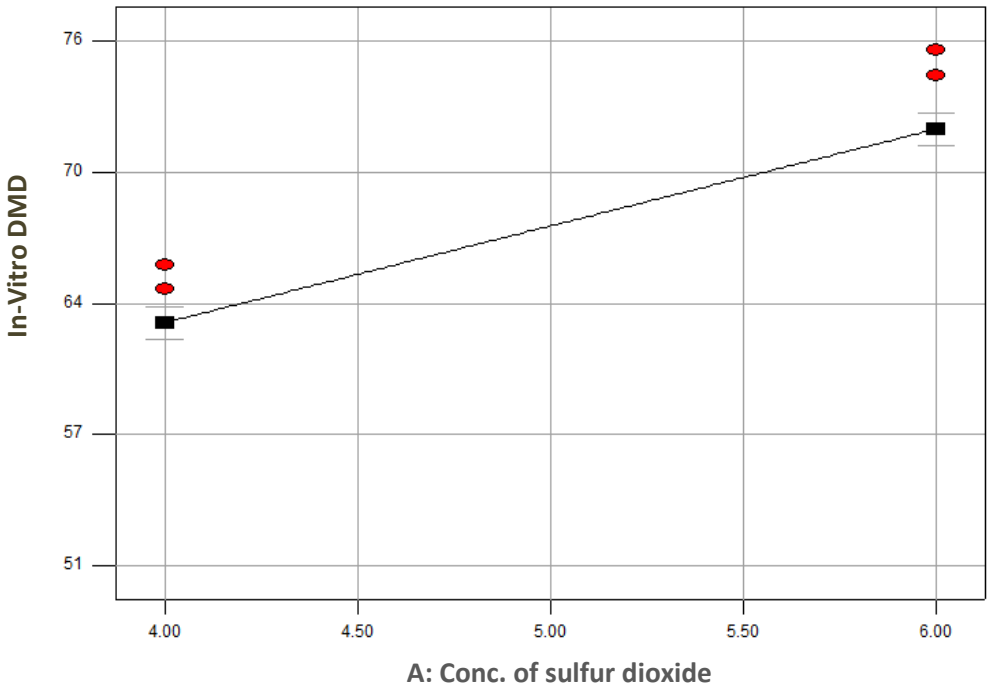


Figure (c) reaction temperature = 120 °C and Reaction time = 2.50.

Figure 4.2(a)-(c): Effect of Conc. of SO_2 on In-Vitro DMD at different Reaction temperature and time.

Effects of Reaction time

As reaction times are relatively long, it allows better swelling consequently penetration of SO_2 into the processed Prosopis since there is plenty of residence time and the reaction is irreversible. It was shown in the following figures 4.3(a) up to (c), the graph is less sloppy, in fact it very to close horizontal in most figure show between 1.50 hrs and 2.00 hrs. Beyond 2.00 hrs, when the time increases, the In-Vitro DMD increases as well. There is quadratic relation between the time and the response.

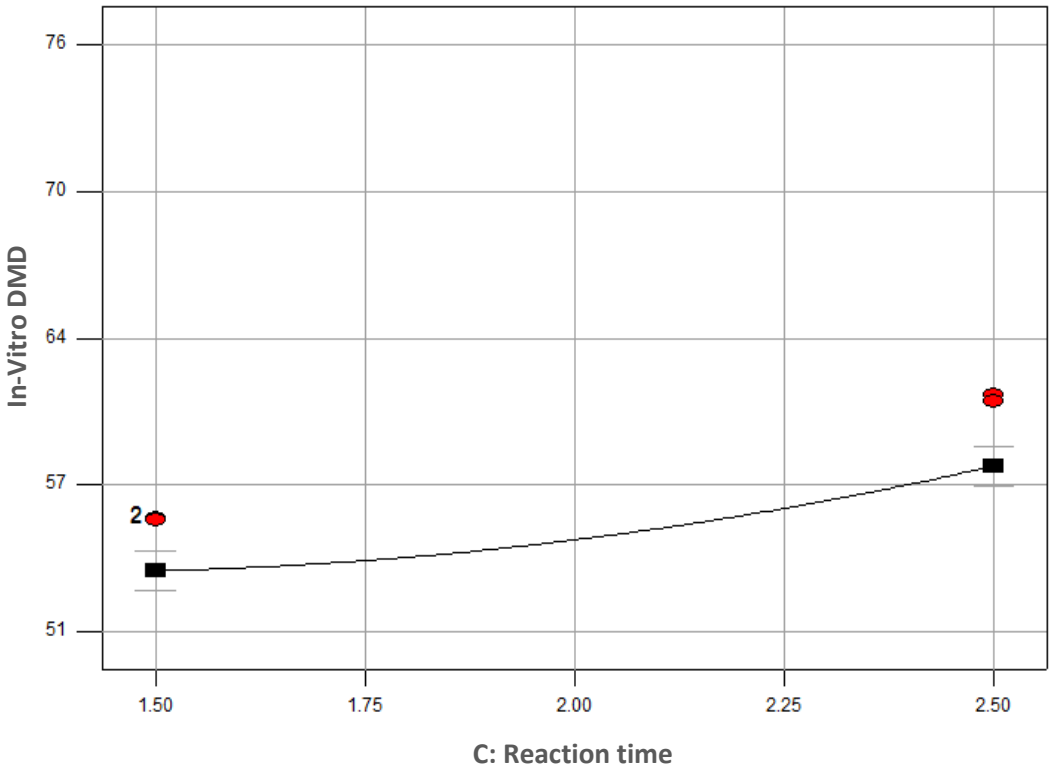


Figure (a) reaction temperature = 100 °C and Conc. of SO_2 = 4.00

It can probable be concluded that residence time is relatively important. It also important to mention that reaction time is less significant compare to temperature and concentration of SO_2 especially after two and half hours it has no effect and figure 4.3 (c) shows this. This observation is also proven by the *Expert design* output of the ANOVA analysis as it is observed in the appendix-A.

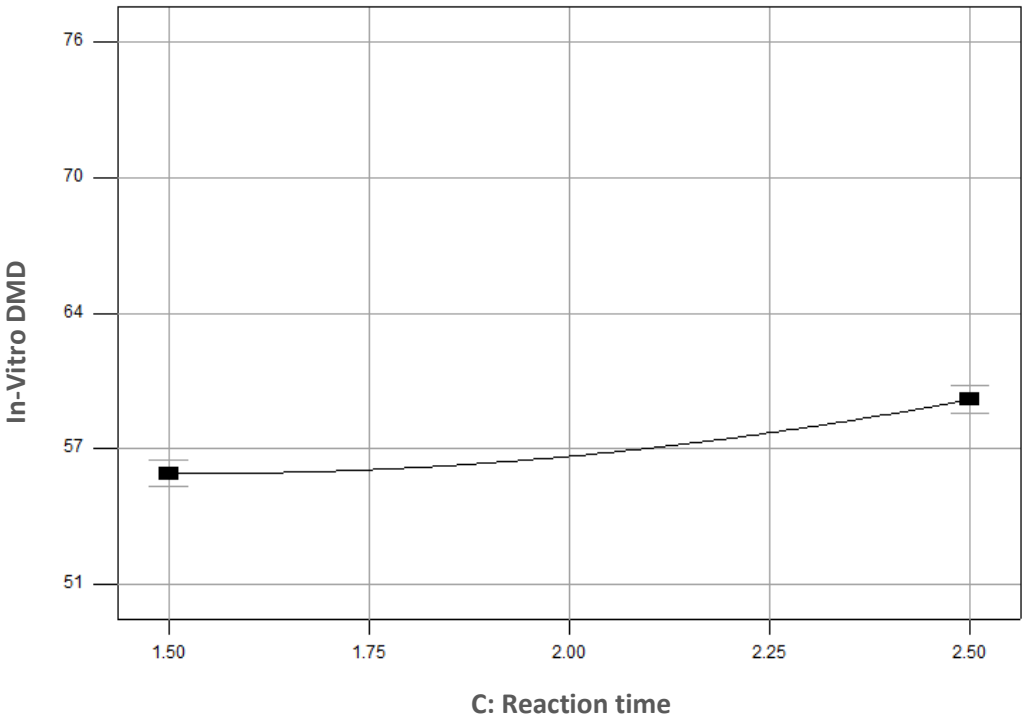


Figure (b) reaction temperature = 110 °C and Conc. of SO_2 = 4.00.

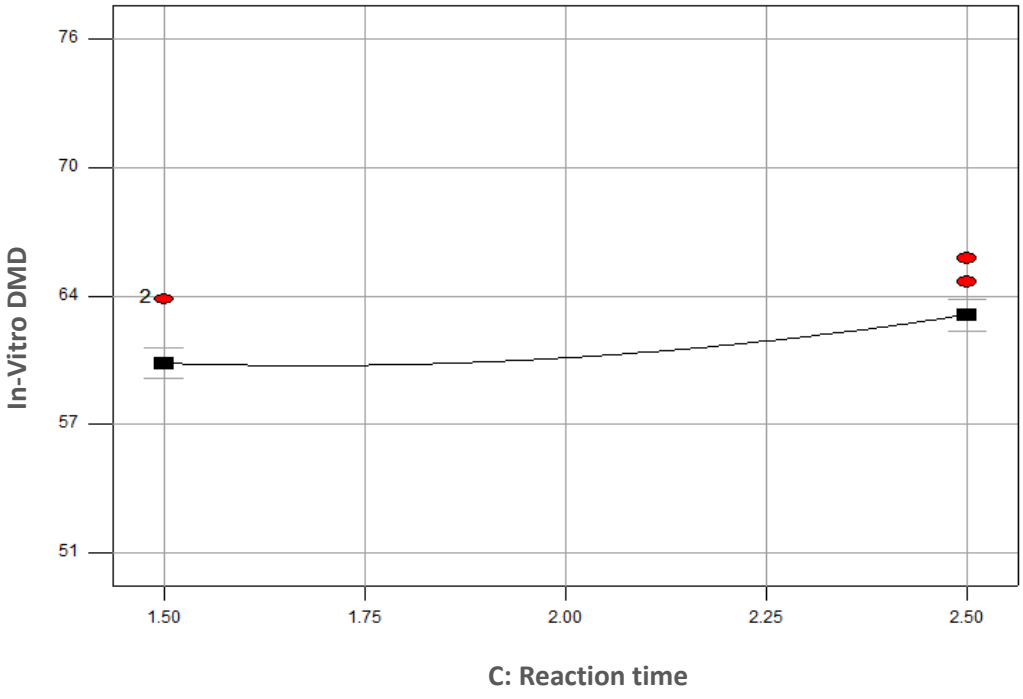


Figure (a) reaction temperature = 120 °C and Conc. of SO_2 = 4.00

Figure 4.3(a)-(c): Effect of Reaction time on In-Vitro DMD at different Conc. of SO_2 and temperature.

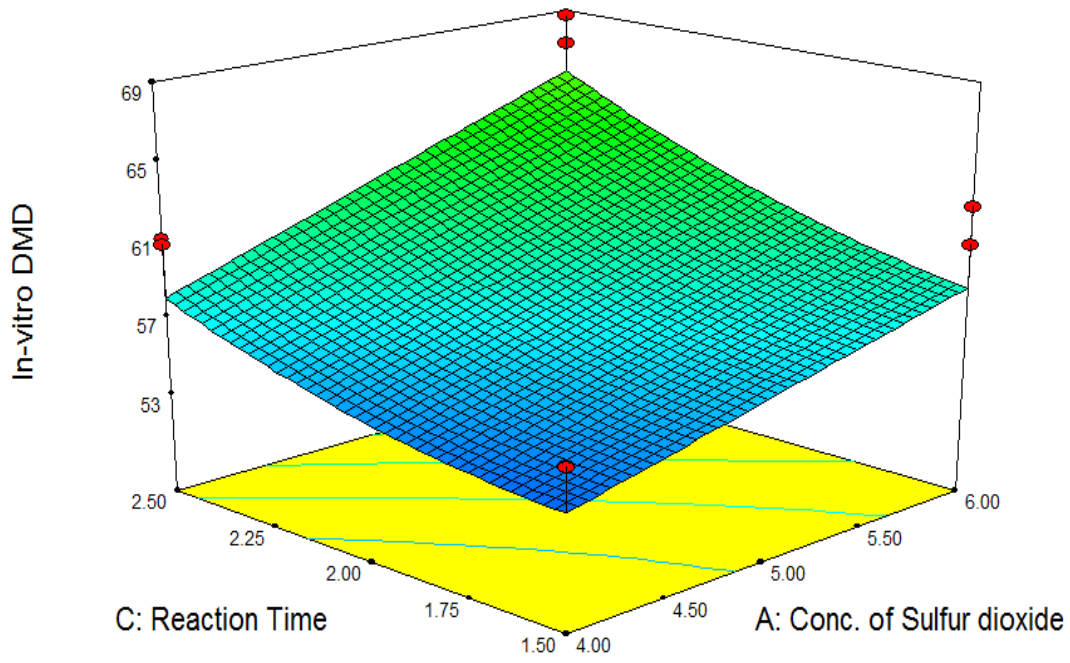
4.1.2 Interaction Effects

Discussion of the single factors tells that even if each of the three factors has significant effect on the response of total digestibility. But it was also observed the dependence of one another or their interaction.

Setting the temperature at 100 °C different In-Vitro DMD value was observed for five level SO_2 concentration and Cooking time. As shown in Table 4.1, the highest digestibility attained among the samples submitted was the sample with 6% sulfur dioxide and a two and a half hour heating time. Its IVDMD was determined to be 67-68%.

Table 4.1 In-Vitro Dry Matter Digestibility at 92.68 °C and 100 °C

Std	Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours	Response In-vitro DMD m/m %
23	26	Axial	5	92.67949192	2	51
10	1	Fact	4	100	2.5	60.8
3	3	Fact	6	100	1.5	62.775
1	6	Fact	4	100	1.5	55.84
4	7	Fact	6	100	1.5	60.83
12	11	Fact	6	100	2.5	68.68
2	15	Fact	4	100	1.5	55.77
9	18	Fact	4	100	2.5	61.1
11	20	Fact	6	100	2.5	67.15



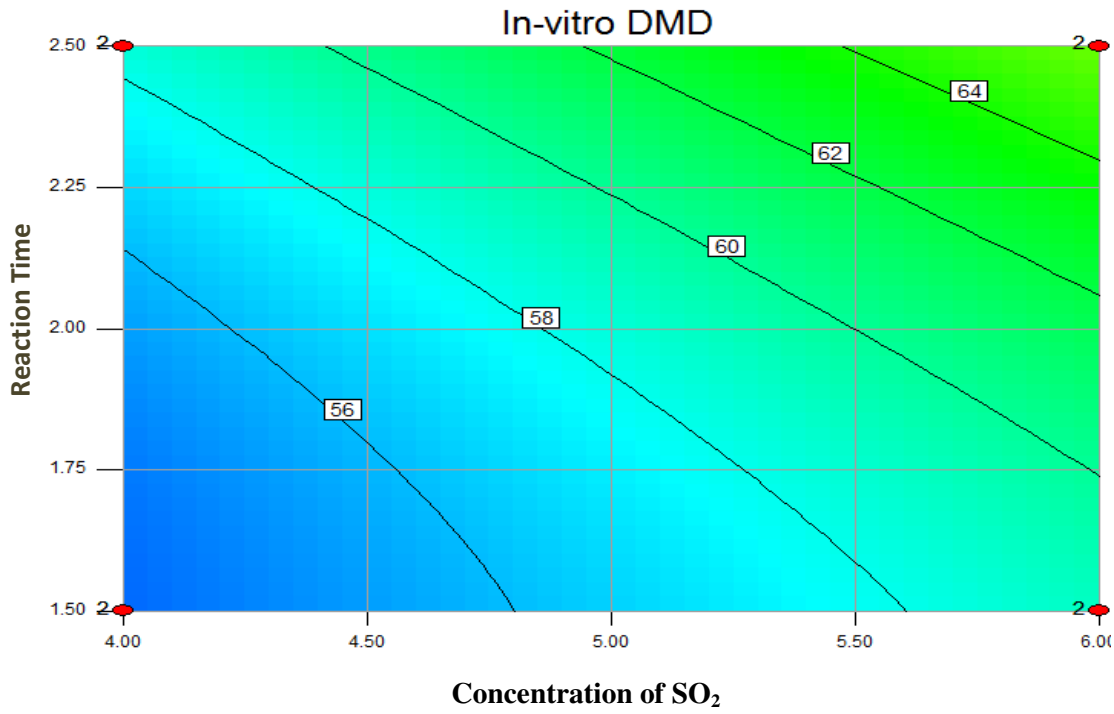


Figure 4.4 Surface response curve of In-Vitro DMD of treated Prosopis juliflora as concentration of SO_2 and reaction time varies at temperature 100 °C.

The trend the In-Vitro DMD as seen on graph 4.1 shows that the graph more stiff on concentration of SO_2 side. This can explain that concentration sulfur dioxide has an effect more significant than compare to the reaction time. According to literature evidence indicates that if the sulfur dioxide concentration is high for a given temperature, lignin condensation decreases and sulfonation increase which facilitate delignification process. Even if the temperature is relative lower, as reaction time is longer, there a plenty of time for penetration of SO_2 into the swelling woods.

The temperature in the reactor was then increased to 110°C and samples were prepared in the same manner. The two higher digestibility attained were 64.7 and 62.6 for the sample that was treated with 5% and 6.73% sulfur dioxide and heated for 2.87 hours and 2 hours respectively as shown in Table 4.2. The highest digestibility is attained at high SO_2 concentration and long reaction time.

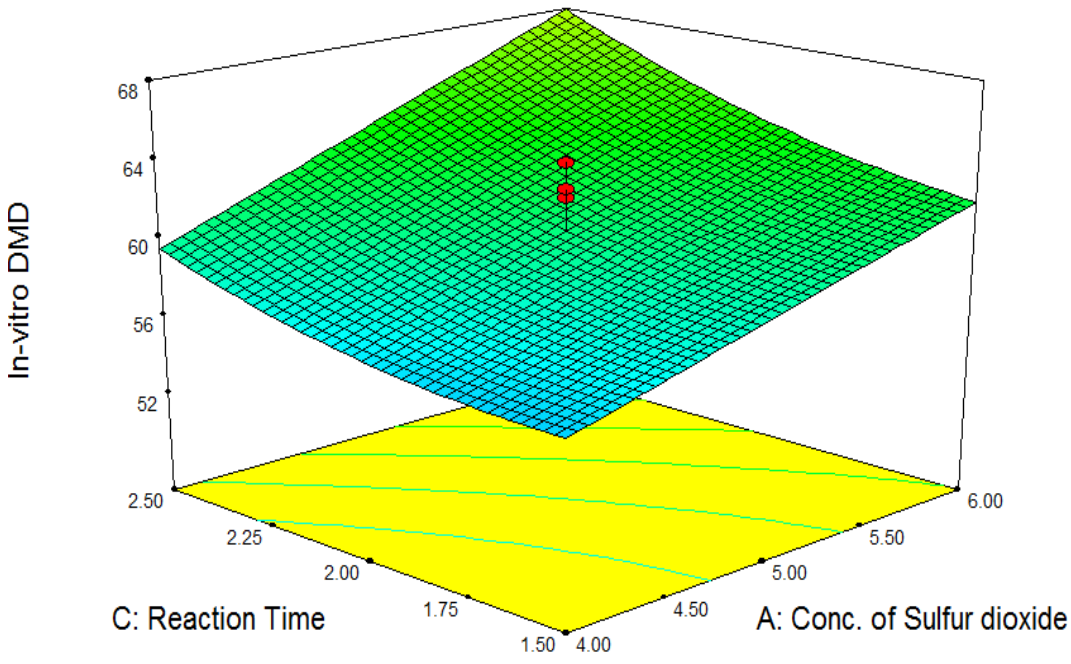
The trend the In-Vitro DMD on graph 4.2 shows that the graph more stiff on concentration of SO_2 side. This can explain that concentration sulfur dioxide has an effect more significant than compare to the reaction time.

This is supported also in the ANOVA analysis in Appendix-A. Longer time digestion of the 2.87 hours case show that more digestibility occur because delignification goes on for long time cooking consequently increase.

Table 4.2 In-Vitro Dry Matter Digestibility for different Reaction time and SO_2 concentration at 110 °C

Std	Run	Type	**Factor 1** A:Conc. of SO_2 w/w%	**Factor 2** B:Temprature Degree centigrade	**Factor 3** C:Reaction Time Hours	**Response** In-vitro DMD m/m %
17	8	Center	5	110	2	62.13
18	10	Center	5	110	2	62.55
19	16	Center	5	110	2	63.91
20	17	Center	5	110	2	62.55
26	22	Axial	5	110	2.86	64.75
22	24	Axial	6.73	110	2	63.96
21	25	Axial	3.26	110	2	52.48
25	26	Axial	5	110	1.13	56.04

Samples of grinded wood and leaf Prosopis were prepared with 3.27% to 6.7% sulfur dioxide and heated from 1.13 to 2.87 hours at temperatures of 100°C to 127°C.



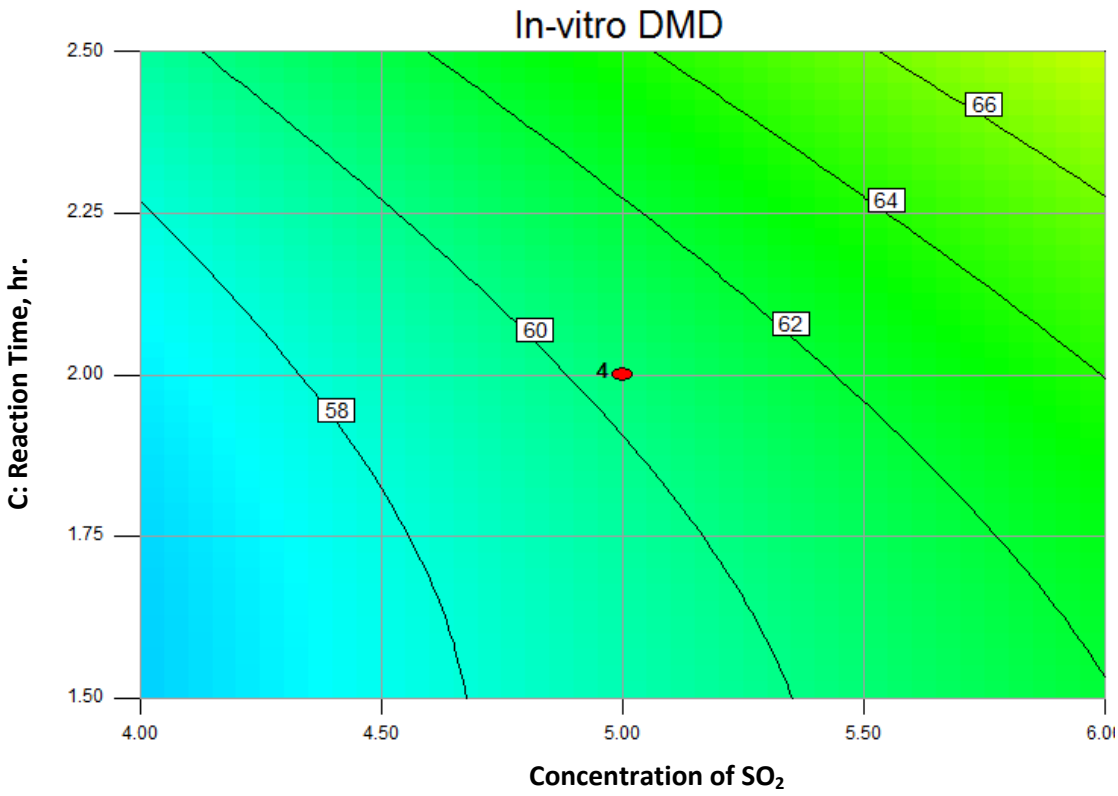


Figure 4.5 Surface response of In-Vitro DMD of treated Prosopis juliflora as reaction time and SO_2 concentration varies at temperature 110 ^oC.

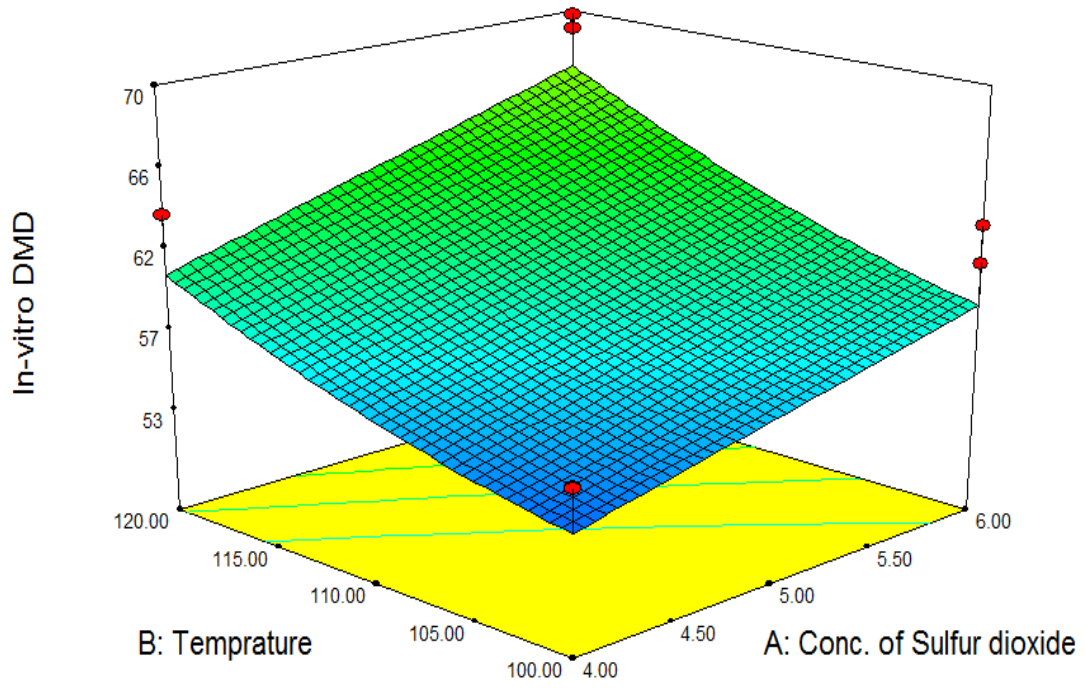
It is also observed that, as both Concentration of sulfur dioxide and reaction increase, the response In-Vitro DMD also increase as well and vice versa. It also indicates that there is somewhat an interaction between these two factors which is not significant as shown on the ANOVA analysis as well.

Setting the reaction time at one and a half hour, different In-Vitro DMD value is observed for five level SO_2 concentration and temperature. As shown in Table 4.3, the highest digestibility attained among the samples submitted was the sample with 6% sulfur dioxide and 120 ^oC temperature. Its IVDMD was determined to be 68-69%.

Table 4.3 In-Vitro Dry Matter Digestibility for different Reaction temperature and SO2 concentration at 1.5 hours

Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours	Response In-vitro DMD m/m %
5	Axial	5	110	1.13	56.04
1	Fact	6	100	1.5	55.83724
7	Fact	4	100	1.5	55.84
16	Fact	4	100	1.5	61.68
21	Fact	6	100	1.5	67.1507
2	Fact	4	120	1.5	63.32807
6	Fact	4	120	1.5	63.33
24	Fact	6	120	1.5	69.7516
25	Fact	6	120	1.5	68.9498

The highest digestibility is attained at high SO_2 concentration and higher temperature. The response also increases as both temperature and concentration increase. The trend the In-Vitro DMD on graph 4.3 shows that the response surface more stiff on concentration of SO_2 side decreasing when moving to the temperature. This can explain that concentration sulfur dioxide has an effect bit more significant than compare to the reaction temperature.



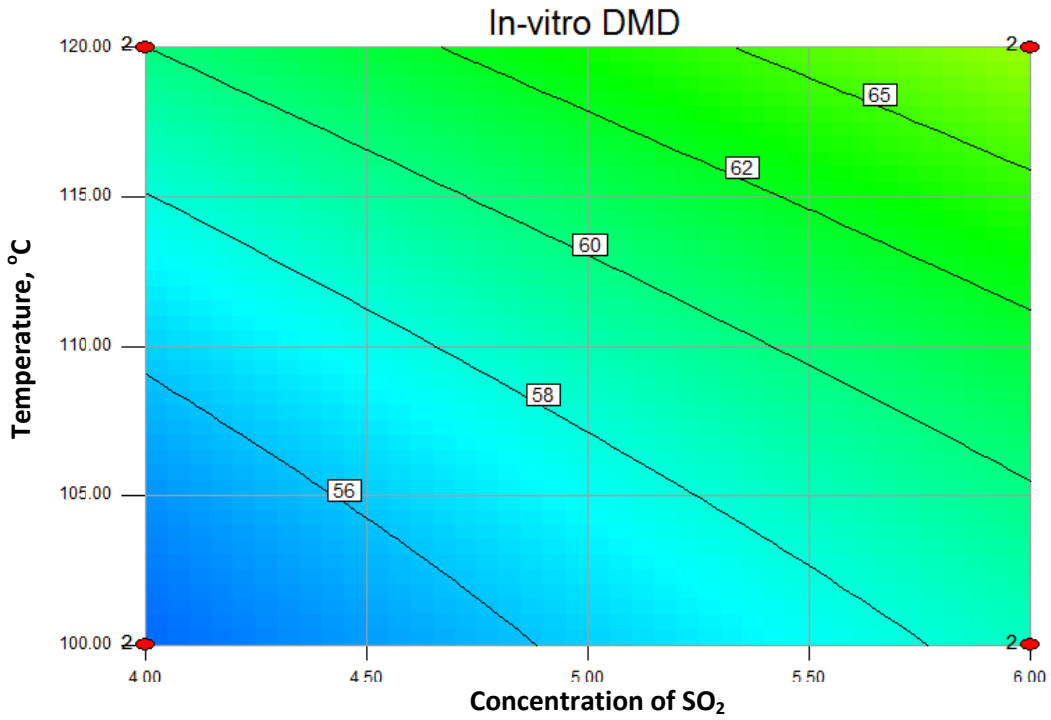


Figure 4.6 Surface response of In-Vitro DMD of treated Prosopis juliflora as reaction temperature and SO_2 concentration varies at cooking time 1.5 hours

Analyzing the interaction between temperature and reaction time and their effect of the In-vitro DMD. At 4 % w/w SO_2 concentration different In-Vitro DMD value is observed for five levels of reaction time and temperature.As shown in Table 4.4, the highest digestibility attained among the samples submitted was the sample with 2.5 hours and 120 °C temperature. Its IVDMD was determined to be 65%.

Table 4.4 In-Vitro DMD responses for different Reaction temperature and time at 4 % w/w SO_2 concentration

Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours	Response In-vitro DMD m/m %
25	Axial	3.26	110	2	52.48
1	Axial	4	100	2.5	60.8
6	Axial	4	100	1.5	55.84
15	Axial	4	100	1.5	55.77
18	Axial	4	100	2.5	61.1
5	Axial	4	120	2.5	65.32
12	Axial	4	120	1.5	63.33
13	Axial	4	120	1.5	63.3
14	Axial	4	120	2.5	64.19

From the figure 4.7 below, the response surface of In-vitro DMD has shown a lesser change compare to the reaction time. Higher In-vitro values were registered on the temperature side of the 3D figure and Table 4.4.

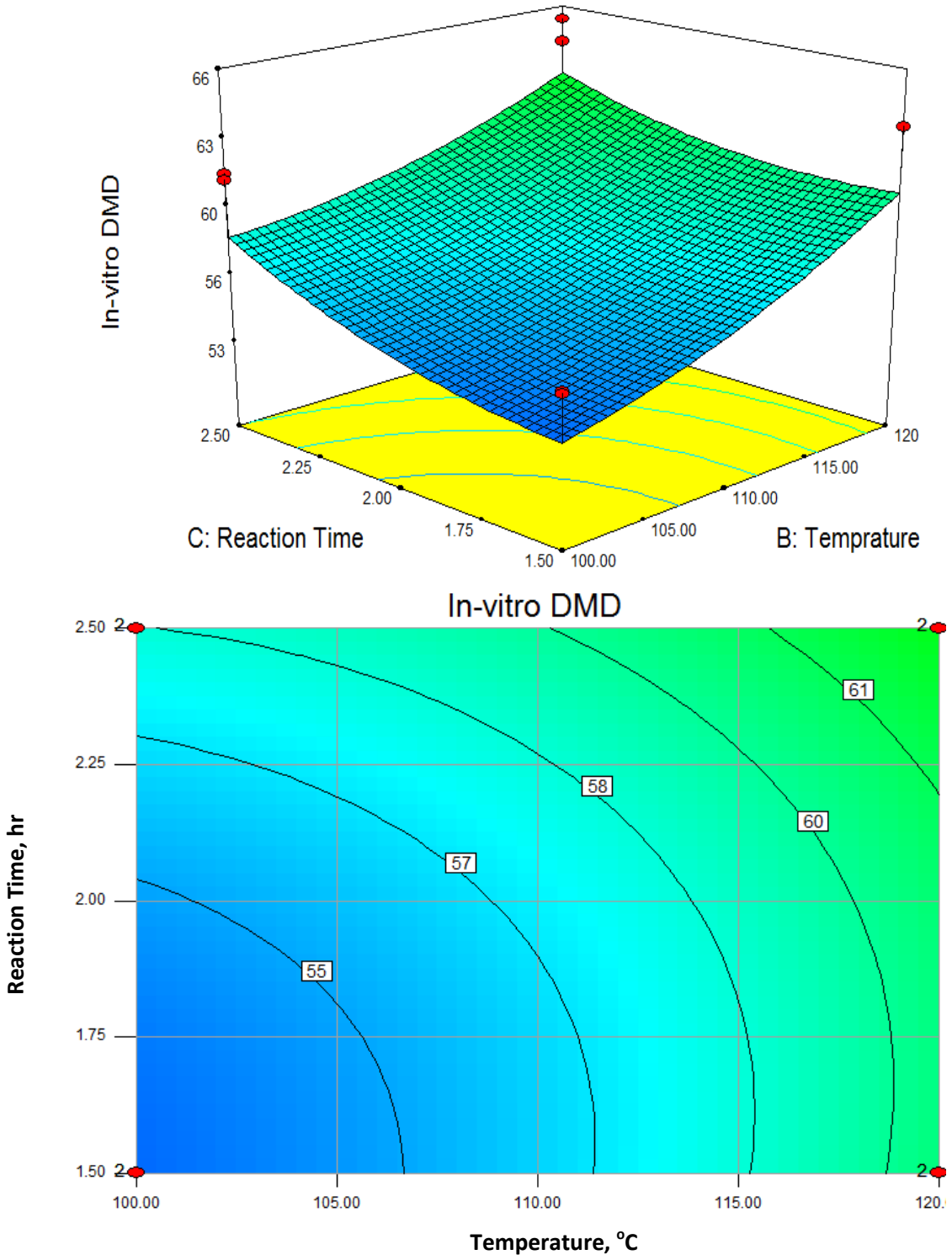


Figure 4.7 Surface response of In-Vitro DMD of treated Prosopis juliflora as reaction temperature and cooking time varies at 4 % w/w SO_2 concentration.

The lowest digestibility is observed in area where the lowest temperature and time is existed as in the figure 4.7. The graph is stiffer in the temperature side which means that even higher IVDMD can be achieved at lower reaction time.

Generally, the digestibility of the Prosopis shown an improvement under the above set condition however it is not satisfying compare to the responses set at relatively higher SO_2 concentration. This is because sulfonation reaction may take place in a limit range due to the fact that the ratio of Prosopis amount to SO_2 is smaller.

When concentration had increased to 6 % w/w SO_2, the highest and most satisfying digestibility had been observed. Because the lignin content the untreated Prosopis is 12.39 % which represent the non digestible, the In-vitro DMD can be reach a maximum of 88 %. These highest values observed were very reasonable, as it looks below in figure 4.8.

All the experimental runs and their In-Vitro analysis results are shown in Table 4.5 below. As shown in the table, the highest digestibility attained among all the samples submitted for In-Vitro Analysis was the sample with 6% sulfur dioxide and 2.5 hrs heating time at 120 ^oC. Its IVDMD was determined to be 75.5 %.

The conditions are favorable to facilitate the sulfonation and hydrolysis of lignin since Sulfur dioxide is high enough and the condensation reaction is favorable at higher temperature of between 168°C to 175°C as mentioned in pulping chemistry. Therefore, there was no severe condensation under at 120 ^oC. The greatest digestibilities attained in all cases were with a 6% sulfur dioxide treatment. This observation is also supported by ANOVA results which show that SO_2 influence is higher than the other studied factors

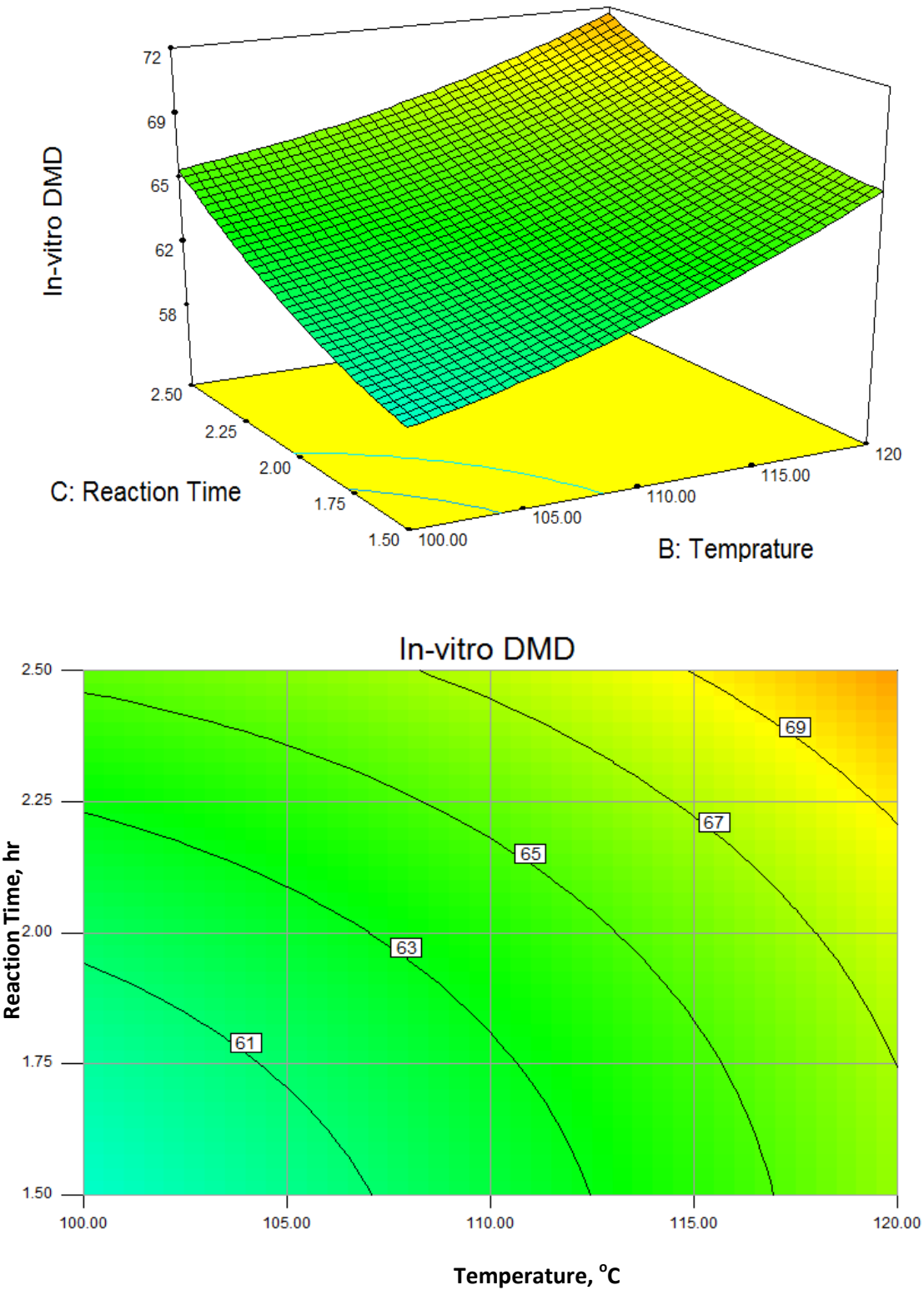


Figure 4.8 Surface response of In-Vitro DMD of treated Prosopis juliflora as reaction temperature and cooking time varies at 6 % w/w SO_2 concentration.

Table 4.5 the response In-vitro DMD with 26 runs set with Expert Design.

Std	Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours	Response In-vitro DMD m/m %
21	25	Axial	3.267949192	110	2	52.48
10	1	Fact	4	100	2.5	60.8
1	6	Fact	4	100	1.5	55.84
2	15	Fact	4	100	1.5	55.77
9	18	Fact	4	100	2.5	61.1
13	5	Fact	4	120	2.5	65.32
6	12	Fact	4	120	1.5	63.33
5	13	Fact	4	120	1.5	63.32807
14	14	Fact	4	120	2.5	64.19
23	26	Axial	5	92.67949192	2	51
17	8	Center	5	110	2	62.13
18	10	Center	5	110	2	62.55
19	16	Center	5	110	2	63.91
20	17	Center	5	110	2	62.55
26	22	Axial	5	110	2.866025404	64.75
25	21	Axial	5	110	1.133974596	56.04
24	23	Axial	5	127.3205081	2	65.39
3	3	Fact	6	100	1.5	62.775
4	7	Fact	6	100	1.5	60.83
12	11	Fact	6	100	2.5	68.68
11	20	Fact	6	100	2.5	67.15
16	2	Fact	6	120	2.5	74.3655
15	4	Fact	6	120	2.5	75.587
8	9	Fact	6	120	1.5	69.7516
7	19	Fact	6	120	1.5	68.9498
22	24	Axial	6.732050808	110	2	63.9644

Prosopis was also treated with additional concentration of 6.732 % sulfur dioxide. As shown in Table, no appreciable increase in digestibility above 6% sulfur dioxide was realized fact that hydrolysis of lignin retarded at high SO_2, it is possible that the SO_2 dose have created undesired condition for the crude protein and cellulose assimilating bacteria in ruminant fluid during the In-Vitro DMD.

4.2 Experiment Data of Lignin Analysis

Lignin loss is another way of knowing that to what sulfonation and Hydrolysis reaction has takes place. In-vitro Dry Digestibility has an inverse relation with lignin amount in the treated Prosopis sample. These analyses consider only the wood part the sample since the leaf has no lignin.

If was found out that the lignin value untreated Prosopis juliflora was to be 17.7 percent. Following the sort experiment run, setting the reaction time at two and an half hour, different Lignin value was observed for five level SO_2 concentration and temperature. As shown in Table 4.6, the lowest lignin attained among the samples submitted was the sample with 6% sulfur dioxide and 120 ^oC temperature. Its lignin was determined to be 7.77 %.

Table 4.6 Lignin Amount of treated P. juliflora for at 1.50 hours.

Type	A:Conc. of SO_2 w/w%	B:Temprature Degree centigrade	C:Reaction Time Hours	Wood lignin m/m %
Fact	4	100	2.5	15.2
Fact	4	120	2.5	12.88
Fact	4	100	2.5	14.97
Fact	4	120	2.5	14.16
Fact	6	100	2.5	10.55
Fact	6	120	2.5	7.91
Fact	6	100	2.5	10.27
Fact	6	120	2.5	7.77

Figure 4.9 has also shown that as the Concentration of sulfur dioxide increase and temperature increase, amount of lignin decrease. This is due to that fact that sulfonation reaction. It does not guarantee that after the lignin transforms to lignosulfonate whether hydrolysis (lignin dissolves) takes place or not.

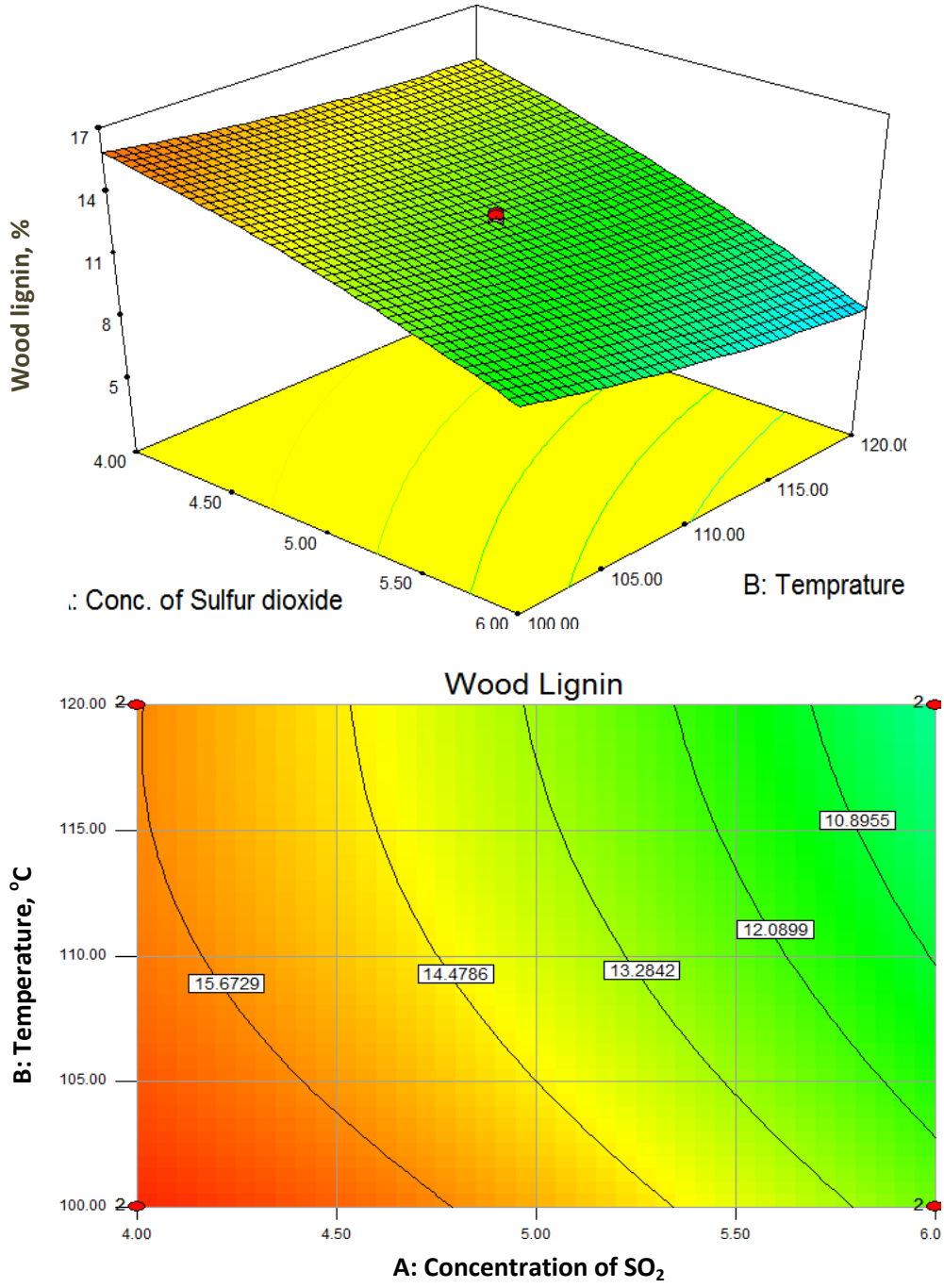


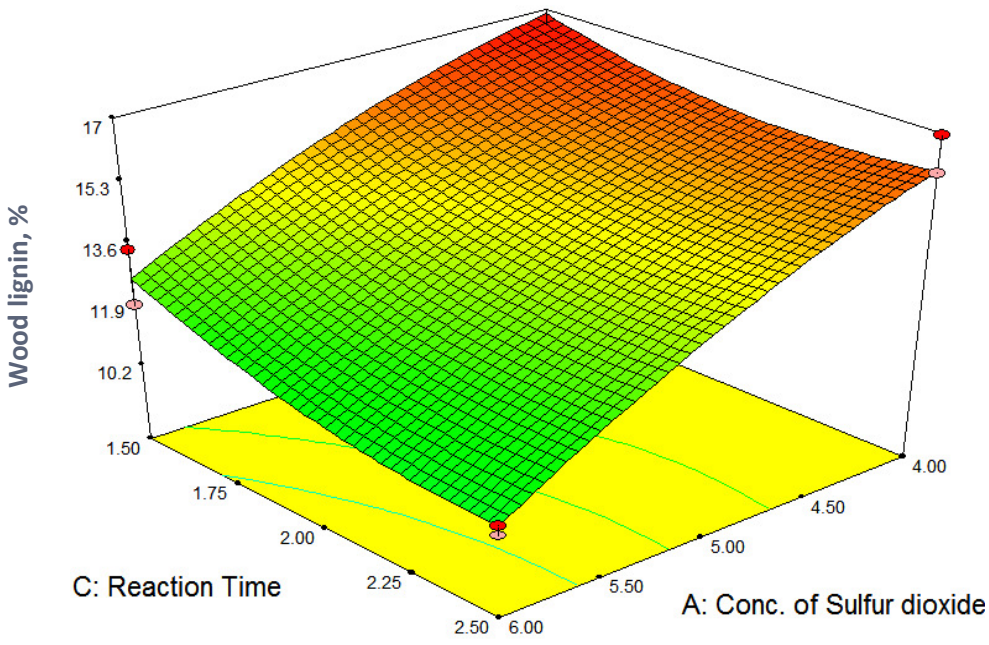
Figure 4.9 Surface response Lignin amounts of treated Prosopis as SO_2 conc. and reaction temperature varies at cooking time 1.5 hours.

The figure also indicates that lower lignin values were registered at highest value SO_2 concentration relative to that of temperature. The graph is oriented as it is steeper to low lignin value on the SO_2 concentration side. Even if their interaction has also crucial effect, SO_2 concentration is more significant factor. It is also worth to show Reaction Temperature effect on lignin value and its relation to lignin loss (Digestibility).

Table 4.7 Lignin amount of treated P. juliflora at 100 °C

Run	Type	A:Conc. of SO2 w/w%	B:Temprature Degree centigrade	C:Reaction Time Hours	Wood lignin percentage m/m %
21	Fact	4	100	1.5	14.69
25	Fact	4	100	1.5	14.82
4	Fact	6	100	1.5	11.51
18	Fact	6	100	1.5	11.47
3	Fact	4	100	2.5	13.40
20	Fact	4	100	2.5	13.57
5	Fact	6	100	2.5	9.55
24	Fact	6	100	2.5	9.27

Setting the 100 °C the rest condition are varies, the minimum lignin value attain was 9.27. This indicate that even if SO_2 concentration has relative more effect of wood delignification as shown in figure 4.10, Sulfonation and hydrolysis reaction was not takes place that much. Therefore, these reactions are more effective when temperature is higher.



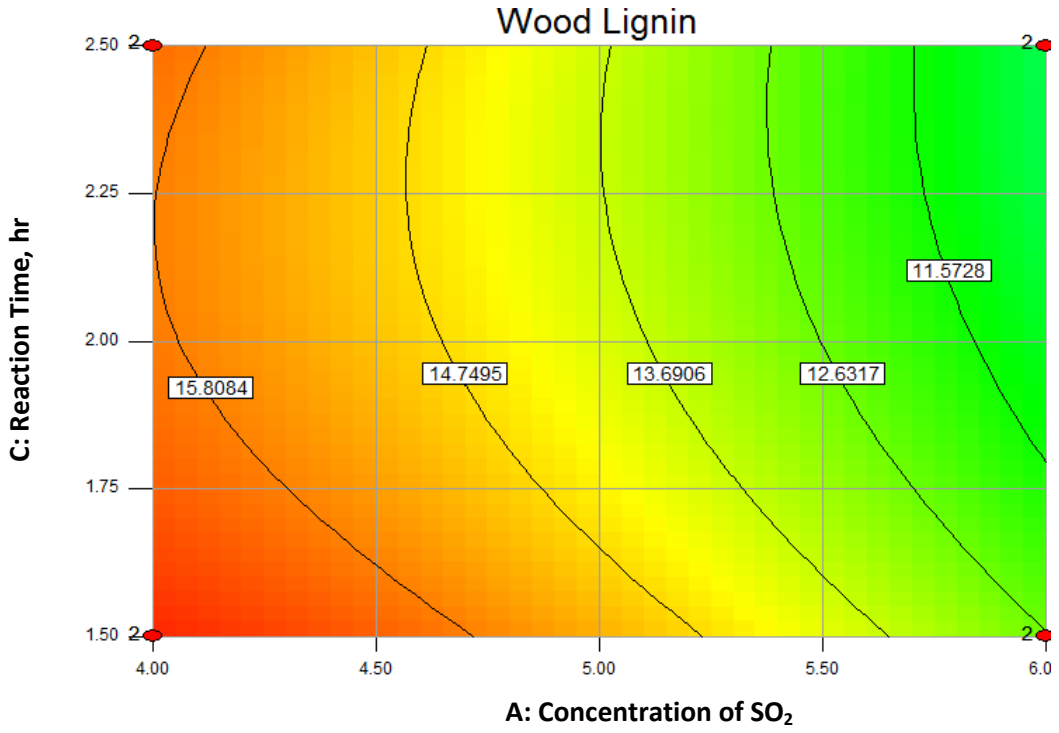


Figure 4.10 Surface Response Lignin amount of treated Prosopis as SO_2 concentration and reaction time varies at temperature 100 °C.

Again experiment run was observed increasing the temperature to 120°C, in which a very satisfying result was found. A minimum of 7.77-7.9 % lignin value was observed. It is probability close to the highest lignin loss that had been attained.

From this result, it can be conclude that highest possible lignin loss or indirectly higher Digestibility was favored at temperature of 120°C.

Table 4.8 Lignin amount of treated P. juliflora at 120 °C

Run	Type	A:Conc. of SO_2 w/w%	B:Temprature Degree centigrade	C:Reaction Time Hrs	Lignin percentage m/m %
8	Fact	4	120	1.5	13.77
9	Fact	4	120	1.5	13.77
14	Fact	6	120	1.5	9.3
19	Fact	6	120	1.5	9.166
15	Fact	4	120	2.5	13.88
27	Fact	4	120	2.5	14.16
16	Fact	6	120	2.5	7.91
28	Fact	6	120	2.5	7.77

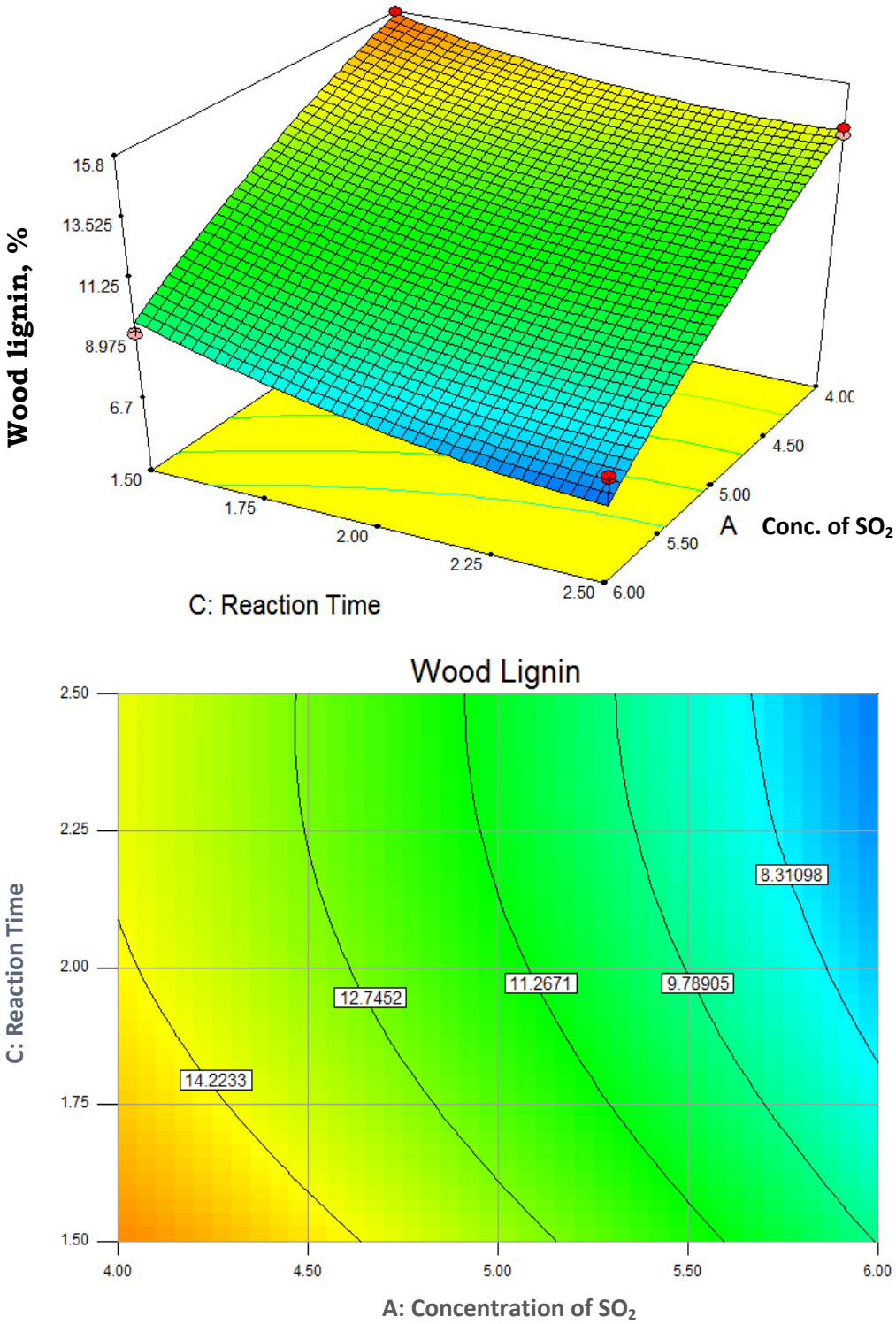


Figure 4.11 Surface Response Lignin amount of treated Prosopis as SO_2 concentration and reaction time varies at temperature 120 °C

Table 4.9 Lignin values of 27 runs at 3.27%, 4.00%, 5.00%, 6.00% and 6.73% SO_2 Concentration.

Run	Type	Factor 1 A:Conc. of SO_2 w/w%	Factor 2 B:Temprature Degree centigrade	Factor 3 C:Reaction Time Hours	Response 2 Lignin percentage, m/m %
24	Fact	4	100	1.5	12.3
20	Fact	4	100	1.5	11.8
19	Fact	6	100	1.5	8.1
16	Fact	6	100	1.5	9.7
17	Fact	4	120	1.5	11
8	Fact	4	120	1.5	11
23	Fact	6	120	1.5	6.8
4	Fact	6	120	1.5	6.6
9	Fact	4	100	2.5	12.2
28	Fact	4	100	2.5	12.1
26	Fact	6	100	2.5	7.6
5	Fact	6	100	2.5	7.4
18	Fact	4	120	2.5	10
25	Fact	4	120	2.5	10.2
27	Fact	6	120	2.5	5.6
21	Fact	6	120	2.5	5.7
3	Axial	3.26	110	2	12
13	Axial	6.73	110	2	3.6
22	Axial	5	92.67	2	11.8
14	Axial	5	127.32	2	7.6
1	Axial	5	110	1.13	12.5
11	Axial	5	110	2.86	7.8
2	Center	5	110	2	8.7
12	Center	5	110	2	8.9
10	Center	5	110	2	9.1
6	Center	5	110	2	8.8
15	Center	5	110	2	8.8
7	Center	5	110	2	8.7

All the experimental runs and their Lignin value results are shown in Table 4.9 above. As shown in Table, the lowest lignin value attained among the samples being analyzed using 72% sulfuric acid method was the sample with 6% sulfur dioxide and 2.5 hrs heating time at 120 ^{o}C. Its Lignin value was determined to be 5.7%. The treatments are the most effective level at these conditions.

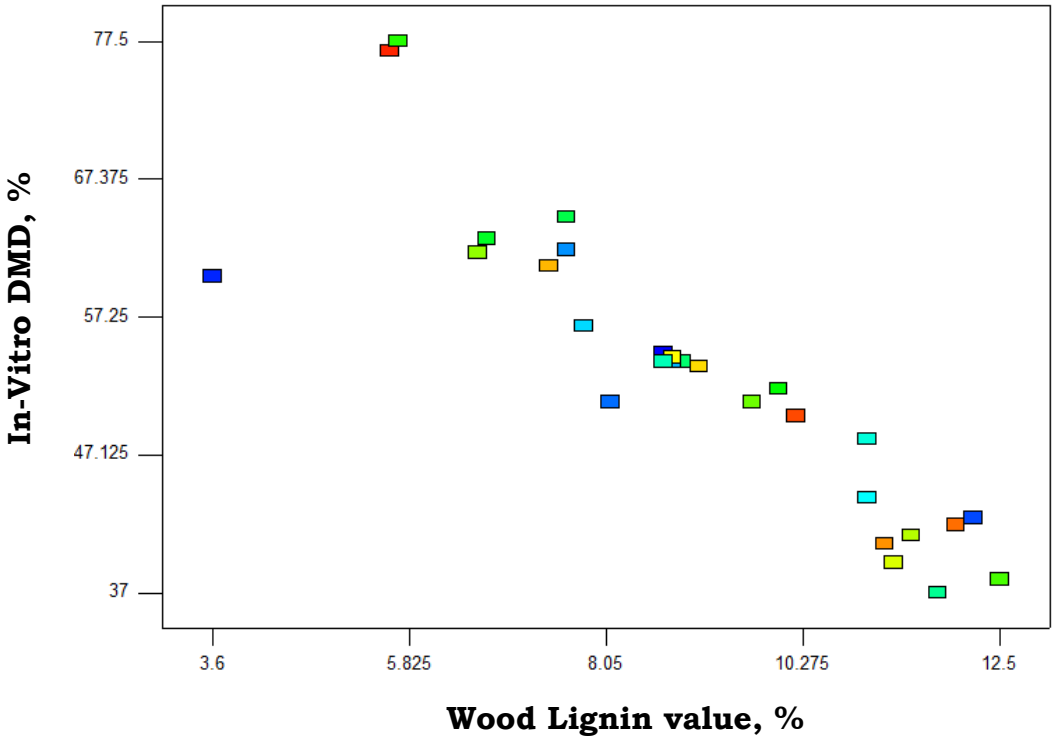


Figure 4.12 Relationship between lignin content of Prosopis and dry matter disappearance (rumen digestibility)

The results on figure 4.12 show that as the lignin content of the treated Prosopis decreases, the dry matter disappearance increases accordingly. This suggests that the utilization of Prosopis by rumen organisms was improved by delignification by Sulfurous acid treatment.

5.0 CONCLUSION AND RECOMMENDATION

Conclusion

This study shows that treatment of Prosopis juliflora with Sulfurous acid has better potential in improving digestibility of this plant. Beside the findings from the experiment, In-vitro DMD and OMD digestibility as well as Lignin value testing, show that there are several parameters important in determination of the optimal condition to have highest digestibility. These parameters are particle size, amount of water, amount of Sulfurous acid, Operating temperature, Processing time, the ratio of vapor space to the amount of Prosopis charged and the amount of NH_4OH required for neutralization.

Based on the accumulated data it would seem that the best treatment using sulfurous acid is one that operates at the 110-120 ^oC temperature, has 6 percent SO_2, moisture content of approximately 2:3 on a dry weight of Prosopis batch, and is contacted for 2 hours or more. As mentioned earlier, the temperature favors the sulfonation and hydrolysis reaction and does not favor condensation reactions which bind the lignin and cellulose. The 60% moisture content appears to be favored because it is just sufficient to swell the woody structure, yet not provide a diffusion barrier for the SO_2 or reaction products and enough to avoid burning of the sample in the reactor due the high temperature which will lower in vitro DMD digestibility. The treatment should have a 2 hour processing time with a 30 minute heating rate for better output.

For the SO_2 treated Prosopis, the SO_2 residues exist in the treated samples. The Prosopis should be neutralized to pH 7 after the sulfur dioxide reaction and an average of 1.715 ml of 35% NH_4OH solution was used.

The apparent dry matter disappearance has an inverse relation with the Prosopis treated lignin content. There was an inverse relationship between the SO_2 level and the lignin content of Prosopis.

Recommendations for further studies

The following recommendations are made for further study of the Sulfur dioxide reaction with Prosopis.

1. The In-vivo DMD analysis of higher percentage of Sulfur dioxide treated Prosopis diets must be studied to increase the percentage of treated Prosopis in diet means to increase the Prosopis consumption. Therefore, if the Sulfurous acid treatment Prosopis plant can be enlarged, the cost of production for Prosopis can be reduced.
2. It is recommended to conduct mathematically model the sulfur dioxide reaction with Prosopis wood. In order to reach this goal,
   - Analysis of the composition of reaction products might be useful.
   - Determination of the significant chemical reactions takes place between sulfur dioxide and Prosopis.
   - Chemical kinetics - The rate and mechanism of reaction of sulfite and bisulfite ions and lignin must be known.
   - Diffusion - The rate and mechanism which sulfur dioxide will diffuse into particles of Prosopis must be known.

   This information will be critical in scale-up to a commercial size operation.

3. The processed Prosopis plant has high amount of digestible carbohydrates (energy). Prosopis feed is deficient to some extent in protein and minerals such as calcium and phosphorus. In order to overcome the resulting nutrient limitations (protein and mineral deficiencies), dietary supplementation are the solution.It is adviced to formulate a complete feed additive.

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APPENDIX

APPENDIX –A: *Design Expert 7.0.*0 Software output of statistical Analysis.

A.2. ANOVA result and Residual plot for the In-Vitro DMD Analysis.

Response	**In-Vitro DMD Analysis**				
Analysis of variance table [Partial sum of squares - Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Block	61.48029	1	61.48029		
Model	629.3262	8	78.66578	108.1758	< 0.0001
A-Conc. of Sulfur dioxide	278.6939	1	278.6939	383.2409	< 0.0001
B-Temprature	168.775	1	168.775	232.0879	< 0.0001
C-Reaction Time	121.5147	1	121.5147	167.0988	< 0.0001
AB	2.690281	1	2.690281	3.699491	0.0736
AC	6.674692	1	6.674692	9.178583	0.0084
BC	4.422577	1	4.422577	6.081628	0.0262
B^2	6.023601	1	6.023601	8.283248	0.0115
C^2	11.56686	1	11.56686	15.90596	0.0012
Residual	10.90804	15	0.727203		
Lack of Fit	4.287606	4	1.071901	1.780987	0.2029
Pure Error	6.620437	11	0.601858		
Cor Total	701.7146	24			

Table A.2. ANOVA results

Values of "Prob > F" less than 0.0500 with 95% of confidence level indicate model terms are significant. In this case A, B, C, AC, BC, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The "Lack of Fit F-value" of 1.78 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good.

Std. Dev.	**0.852762**		**R-Squared**	**0.982962**
Mean	63.66125		Adj R-Squared	0.973876
C.V. %	1.33953		Pred R-Squared	0.946111
PRESS	34.50175		Adeq Precision	42.19255

The model for the In-Vitro DMD was obtained (see Eq. A.1) after performing 26 experiments, and discarding the insignificant effects.

Final Equation in Terms of Actual Factors:

IVDMD = 121.6071756 -3.534913384 * (CS) -1.430131459 *T
-4.859118241 * t + 0.041005188 * CS* T + 1.29177125 * CS * t
-0.105149625 * T * t + 0.007979408 * T^2 + 3.66677547 * t^2 (A.1.)

Where:

IVDMD: Invitro Dry Matter Digestibility

CS: Conc. of Sulfur dioxide

T: Temprature

t : reaction time

Residual Plots for In-vitro DMD Analysis

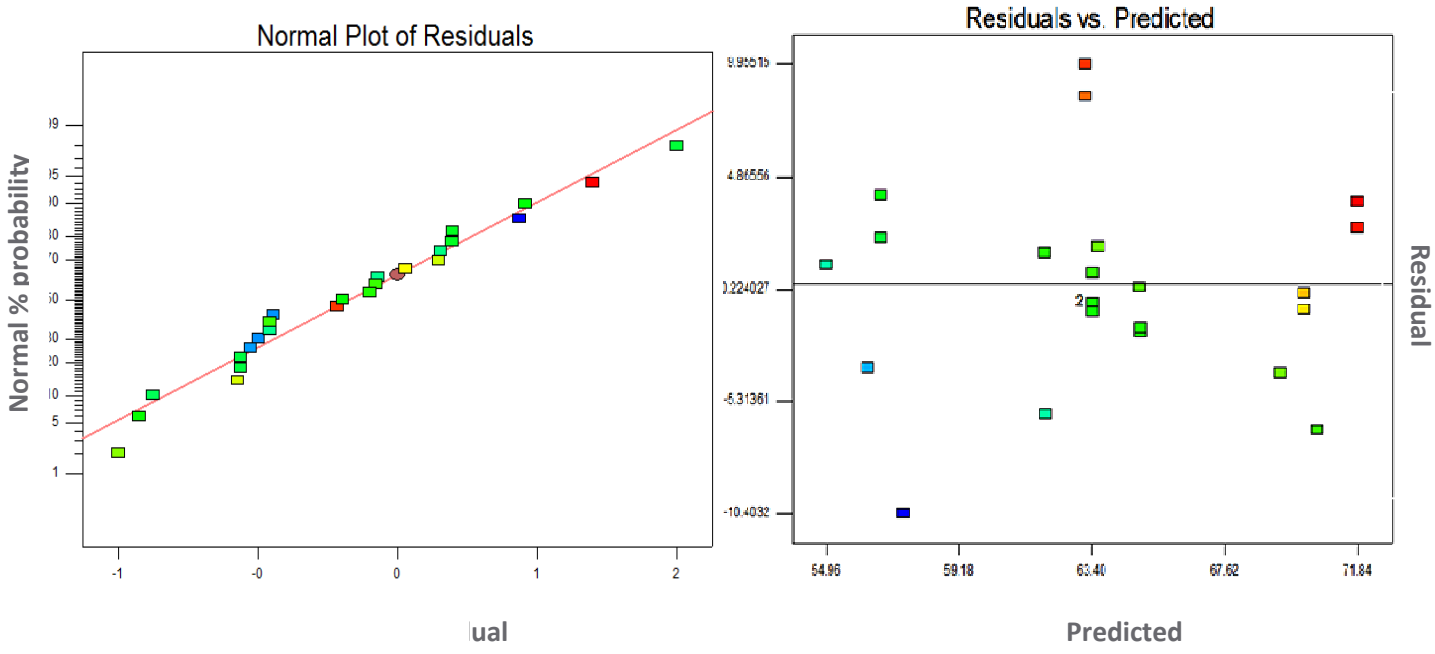


Figure A.1 Residual Plots for In-vitro Analysis

Table A.2. Diagnostics Case Statistics

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Run Order
1	55.84	56.20843	-0.36843	0.402443	-0.55889756	-0.54565783	6
2	55.77	56.20843	-0.43843	0.402443	-0.66508697	-0.65222379	15
3	62.775	61.21495	1.56005	0.402443	2.366579073	2.888266089	3
4	60.83	61.21495	-0.38495	0.402443	-0.58396517	-0.57068831	7
5	63.32807	62.84112	0.486954	0.389952	0.731102546	0.719243181	13
6	63.33	62.84112	0.488884	0.389952	0.734000207	0.722199823	12
7	68.9498	69.48785	-0.53805	0.389952	-0.80781367	-0.79797274	19
8	69.7516	69.48785	0.263752	0.389952	0.395991828	0.384579926	9
9	61.1	60.66853	0.431468	0.402443	0.654532772	0.641566959	18
10	60.8	60.66853	0.131468	0.402443	0.199435982	0.192929424	1
11	67.15	68.2586	-1.1086	0.402443	-1.68173231	-1.80361533	20
12	68.68	68.2586	0.421401	0.402443	0.639261269	0.626173591	11
13	65.32	65.19823	0.12177	0.389952	0.18282358	0.176821474	5
14	64.19	65.19823	-1.00823	0.389952	-1.51373446	-1.58878348	14
15	75.587	74.4285	1.158496	0.389952	1.7393417	1.880687529	4
16	74.3655	74.4285	-0.063	0.389952	-0.09459294	-0.0914127	2
17	62.13	63.07364	-0.94364	0.163462	-1.20986234	-1.23041346	8
18	62.55	63.07364	-0.52364	0.163462	-0.67137143	-0.65857659	10
19	63.91	63.07364	0.836359	0.163462	1.07231492	1.078099881	16
20	62.55	63.07364	-0.52364	0.163462	-0.67137123	-0.65857659	17
21	52.48	51.67263	0.807369	0.482517	1.316123193	1.351950464	25
22	63.9644	64.00206	-0.03766	0.482517	-0.06139162	-0.05931739	24
24	65.39	65.77485	-0.38485	0.85 #	-1.15060259	-1.16414752	23
25	56.04	56.51678	-0.47678	0.597902	-0.88170289	-0.87477586	27
26	64.75	64.65808	0.091923	0.597902	0.16999339	0.164387641	22

A.3 ANOVA result and Residual plot for the of Lignin Value

Table A.3. ANOVA result of Lignin Value analysis.

Response	**Wood Lignin**				
Model ANOVA for Response Surface Reduced Quadratic					
Analysis of variance table [Partial sum of squares - Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	248.091161	6	41.34852684	44.27801421	< 0.0001
A-Conc. of Sulfur dioxide	182.404333	1	182.404333	195.3274341	< 0.0001
B-Temperature	33.11637734	1	33.11637734	35.46262803	< 0.0001
C-Reaction Time	19.5828931	1	19.5828931	20.97031467	0.0002
AB	3.25261225	1	3.25261225	3.483055442	0.0760
A^2	4.420453613	1	4.420453613	4.733636791	0.0411
C^2	4.565733613	1	4.565733613	4.889209683	0.0383
Residual	19.61061441	21	0.933838781		

Residual Plots for Lignin Analysis

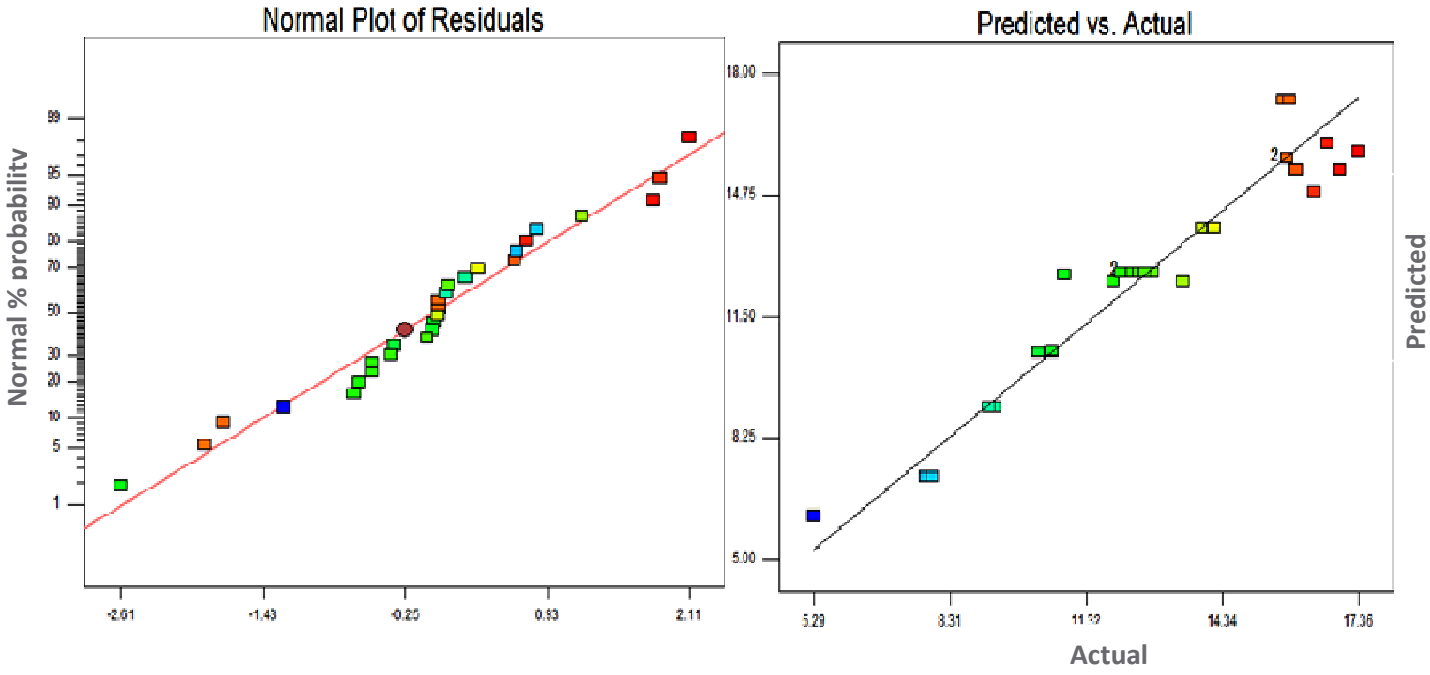


Figure A.3 Normal plots for Lignin value Analysis

APPENDIX- B Preparation of Sulfurous Acid

It is very convenient to add sulfur dioxide together with Prosopis sample in form Sulfurous acid (liquid form) for lower concentration which is up to 22% as you seen on the graph below.

B.1. Laboratory procedure

First proper ratio of sulfur powder with 40% allowance is added together with potassium per manganent (oxidizing agent) is added to the burner as in figure-B2 below. Potassium per manganent ($KMnO_4$) is used to facilitate the oxidation process. Concentration of Sulfurous acid is controlled my PH meter.

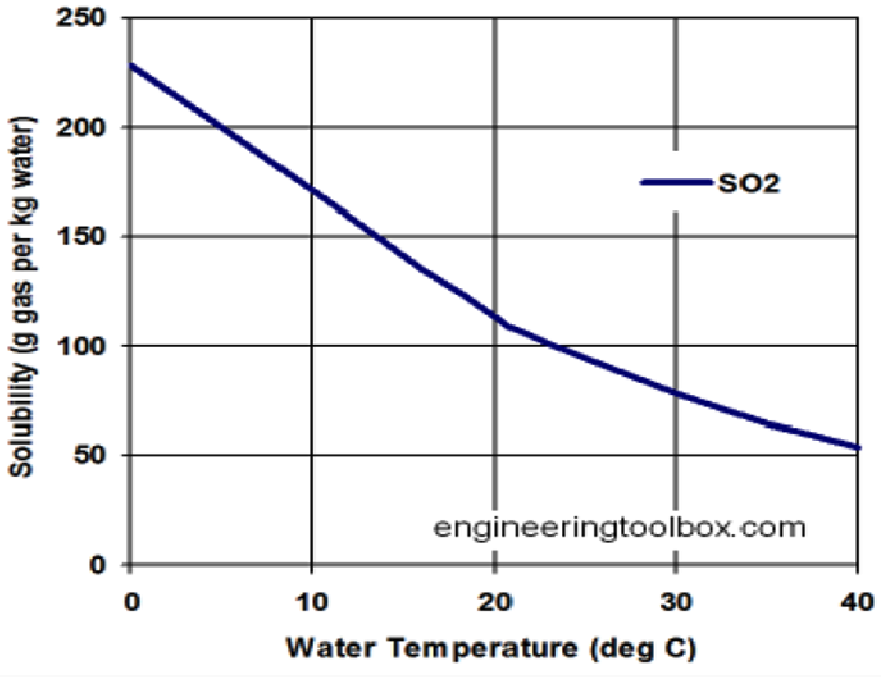


Figure B-1 Solubility of Sulfur Dioxide - SO_2 - in Water

Heat is supplied using stove and melting and burning of sulfur into SO_2 takes place.

$$S + O_2 \longrightarrow SO_2$$

The gas is transferred using a tube by immersed into a beaker containing cold water (3-10^oC) since the process is exothermic.

$$SO_2 + H_2O \longrightarrow H_2SO_3$$

Figure B-2 Laboratory set for preparation of Sulfurous acid from sulfur powder

APPENDIX –C: Experimental Design

The Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [39].

A CCD has three groups of design points:

(a) Two-level factorial

(b) Axial points (sometimes called "star" points) [- Alpha, + Alpha]

(c) Center points [mid way between -1 and +1]

According to the range of each variable, the independent variables are coded to the (-1, 1) interval as shown in Table C-1. The low and high levels are coded -1 and +1, respectively.

Figure C-1 shows the layout provided by CCD for experiments needed to study a 3-parameter process. In this Figure, the factorial portion is represented by the points forming a box and the star (axial) points are located at the end of the sphere that come from the center point. The axial points are located at (± α, 0, 0), (0, ±α, 0), (0, 0, ±α) where α is the distance of the axial point from center and makes the design rotatable.

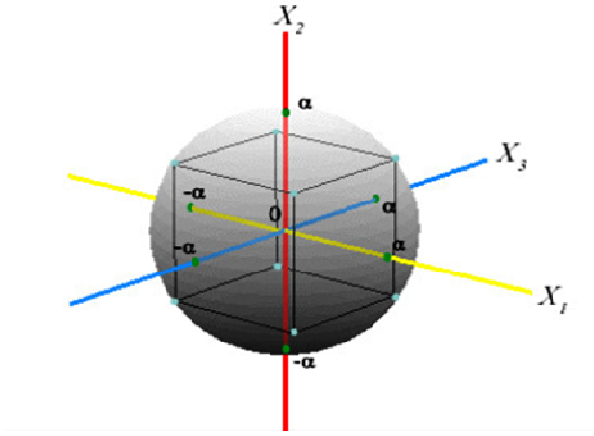


Figure C-1 rotational study with the radius of α value from center point

Rotatability is a reasonable basis for the selection of a response surface design. Because the purpose of RSM is optimization and the location of the optimum is unknown prior to running the experiment. It makes sense to use a design that provides equal precision of estimation all directions [39].

Name of Factor	**Unit**	**- level**	**+ level**
Concentration of H_2SO_3	w/w %	4	6
Temperature	^oC	100	120
Reaction Time	Hours	1.5	2.5

Table C-1 the factorial design three factors with two levels.

Alpha for rotatability α, the distance of the axial points from center in fact, $\alpha = (nF)^{1/4} = (2^k)^{1/4}$ yields a rotatable central composite design where nF, is the number of points used in the factorial portion. Alpha= $(2^3)^{1/4} = 1.68179$.

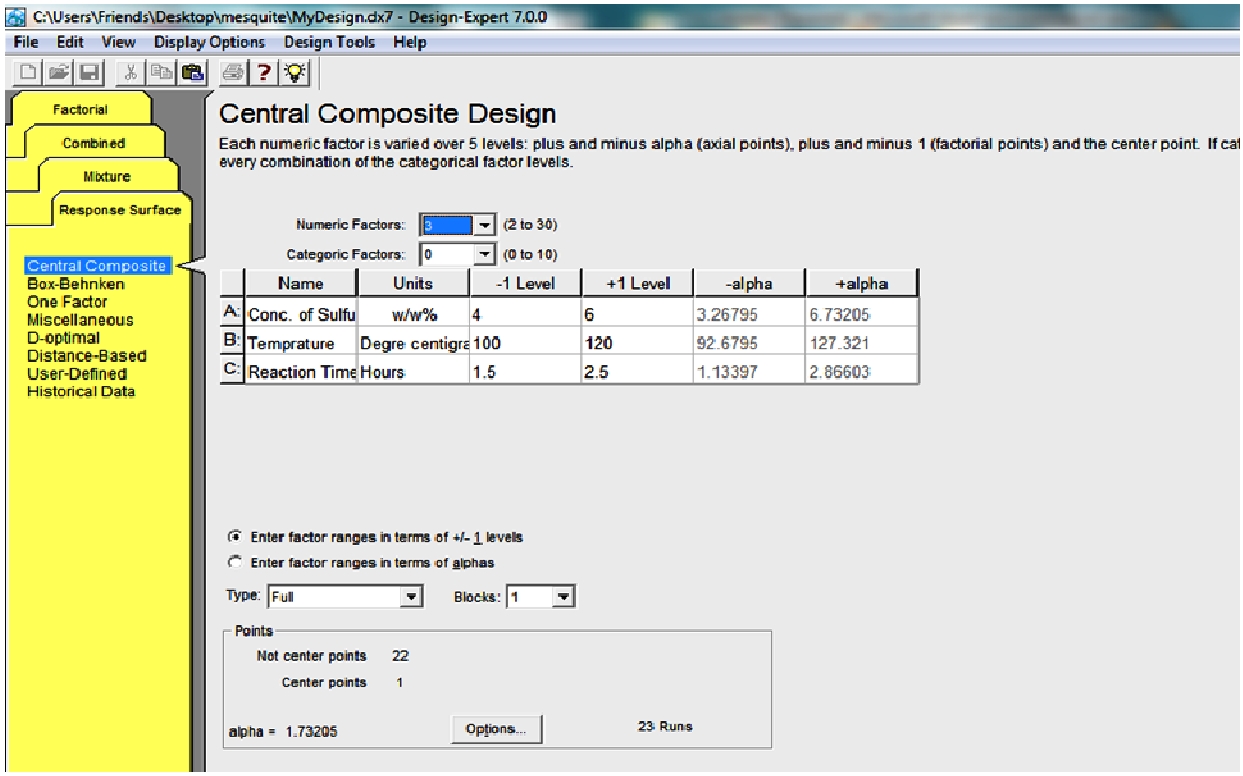


Figure C-2 Surface response displays on *design expert 7.0.0* software.

Name of factor	Unit	- level	+ level	- Alpha	+ Alpha
Concetration of SO2	w/w%	4	6	3.26795	6.73205
Temprature	^{o}C	100	120	92.6795	127.321
Reaction Time	Hours	1.5	2.5	1.13397	2.86603

Table C-2 the final experiment with its five level values of the three factors including the factorial and axial and center points.

Center runs include 4 replications which are performed by setting all factors at their midpoints to estimate the residual error. They can be considered as a barometer to evaluate the variability in the system [41] and to estimate the residual error. The central runs are at 5 w/w %, 110 ^{o}C, 2 Hours.

Generally, the CCD consists of a duplicate $2*2^{k}$ of factorial runs with 2k axial or star runs and Nc center runs (4 replicates) [39], where k is the number of parameters. The effects of three parameters were studied as follows: reaction temperature, Concentration of SO_2 and Reaction time. The responses were In-Vitro DMD and wood Lignin value. Four replications were performed at the centre point. Therefore, the total number of experiments (N) required is as follows;

$$N = 2 * 2^{K} + 2K + Nc = 2 * 2^{3} + 2 * 3 + 4 = 26$$

APPENDIX- D: Analytical Techniques for evaluation and analyses the ruminant feed.

D.1. In- vitro Dry Matter Digestibility Procedure

The analytical procedure used to study the effect of the chemically treated P.Juliflora was the Moore modification of the two stage Tilley-Terry procedure [30]. The method includes two consecutive digestion phases.

During the first digestion phase in this study, plant materials were incubated under anaerobic conditions.

1. Rumen fluid was collected from a fistulated steer four hours after being fed sorghum hay. The rumen fluid was strained through cheesecloth and then filtered through glass wool.
2. A buffer solution saturated with carbon dioxide was added to the filtered rumen fluid in a 7:3 ratio. The buffered rumen solution was added to two grams of each of the treated P.Juliflora samples and placed in a flask with a carbon dioxide environment. The samples were incubated at 39°C for 48 hours; then

Figure D-1 Flask where the rumen fluid and plus P.Juliflora placed and sealed to create anaerobic environment inside the flask

3. HCl and pepsin were added and the samples were incubated for another 48 hours. Following this 72 hour incubation, residual plant materials were collected and oven dried (105°C for 12 hours). Ash contents were determined by combustion (550°C for 2 hours).

Figure D-2 filtration of the residue from the digested fluid

4. The above data used and the samples were then weighed, and the difference in weight before and after incubation determined the in-vitro DMD.

Reference forage was also included in the test to assure the reliability of the results, these tests are conducted in the Animal Science Department, Ethiopia Agricultural Research Organization, Holeta Genet. All in vitro DMD were determined in duplicate to insure the accuracy of the results.

D.2. Procedure for Determination of Lignin in P. Juliflora

1. Approximately 6 grams of air-dried and less 2 mm size sample are weighed. The crucible and its contents are dried to constant weight at 105°C,-cooled, and weighed .
2. The material is then extracted for 4 hours with alcohol-water in a round bottom flask in which the boiled vapour is refluxed using condenser apparatus.
3. It is then filtered, washed with hot water, then with alcohol, and finally dried. (Washing the residue with alcohol aids in the removal of the residue from the crucible after drying).
4. The dried residue is transferred to a glass- stoppered weighing bottle, and is well mixed with 25 ml of 72 percent sulfuric acid at 20°C. and maintained at that temperature for 2 hours.
5. The resulting mixture is transferred to an Erlenmeyer flask, diluted with 927 ml of water to make a 3-5 % acid solution, and then boiled for 4 hours under a reflux condenser.
6. The hydrolyzed residue is filtered with vacuum filter, washed free of acid by means of hot water, dried, and weighed.
7. The lignin content is calculated on the basis of the oven-dry sample. In case a correction : for ash; is desired, transfer the lignin residue to a metal dish into a furnance and ash.

(a)

(B)

(C)

Figure D-4 Photo Courtesy of Laboratory Activity in lignin determination, (a) Sample socked in H_2SO_4, (b) Vacuum Filtration of lignin, (C) Sample oven dried Lignin

D.3. Proximate Analysis: Moisture

Procedure

1. Accurately weigh a moisture dish of appropriate size.
2. Add approximately 10 g of the comminuted sample and reweigh.
3. Place the container in oven at 100 ^{o}C approximately 24 hours.


Figure D-5 Photo of Hot Air drying Oven and drying metal plate.

4. Remove dish from the oven, cover, cool in desiccator, and weigh.
5. Re-dry 1 hr and repeat process until constant weight has been achieved, i.e., change in weight between successive dryings at 1 hour intervals is < 5 mg.

Calculation

Calculate the percentage moisture (wet weight basis) as follows:

$$Percent\ Moisture = \frac{(W1 - W2)}{W1} * 100\%$$

W_1 = weight in g of sample

W_2 = weight in g of dried sample

D.2. Proximate analysis: Ash

Principle

The ash fraction contains all the mineral elements jumbled together. It would be more useful to know the amounts of different individual elements. This method consists of oxidizing all organic matter in a weighed sample of the material by incineration and determining the weight of the ash remaining. Note that the high temperature may cause the volatilization of certain elements (particularly K, Na, Cl, and P) and may also cause the mineral matter to melt and fuse.

Procedure

1. Accurately weight 5 g of sample in a crucible.
2. Place crucible in drying oven at 105 ^{o}C for 24 hours.
3. Transfer to cool muffle furnace and increase the temperature step wise to 550 ^{o}C ± 5 ^{o}C.
4. Maintain temperature for 8 hours or until a white ash is obtained.
5. If white ash is not obtained after 8 hours, moisten ash with distilled water, slowly dry on a hot plate, and re-ash at 550 oC to constant weight. Repeat if necessary.
6. Remove crucible to a desiccator and weight soon after cool.

Figure D-6 Photo courtesy of Crucible inside the furnace.

Calculation

Calculate the percentage ash content (wet weight basis) as follows:

$$\% \text{ ASH (wet)} = \frac{\%\text{wt. crucible and ash} - \text{wt. crucible ash (wet)}}{(\text{wt. crucible and sample} - \text{wt. crucible})} * 100$$

Calculation of ash content on dry basis (when moisture content is known) as follows:

$$\% \text{ ASH (dry)} = \frac{\% \text{ ash (wet)}}{100 - \% \text{ moisture}} * 100$$

Determination of Crude Fiber

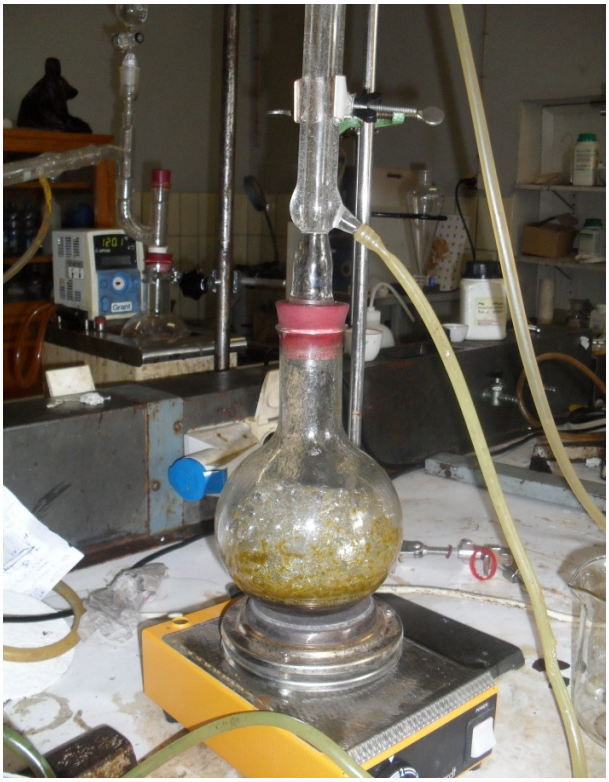
Crude Fiber consists of cellulose, variable pro portion of hemicelluloses and highly variable proportion of lignin along with some minerals.

Principle: The estimation of crude fiber is done by treating the moisture and fat- free samples successively with dilute acid and alkali. The residue is collected and the loss of weight on ignition is called Crude fiber.

Laboratory Methods

Transfer 3-4 gram P.Juliflora leaf Sample to 1000ml flask to which 100 ml of distilled water and 1N sulfuric acid are added. Immediately, the digestion flask is connected to condenser and heated. It is necessary to rotate the flask until the sample is wetted. The boiling is continued briskly and afterward the contents are filter through filter paper. The residue is washed with water until free from acid. The filter paper is then pierced with glass rod and 100 ml of 1N NaOH are added so that the residue is transferred to digestion flask. Next it is refluxed for exactly 30 minute and then filtered through filter paper, wash repeatedly to remove traces of NaOH and dried at 110^{o} C for 4 to 15 hrs to constant weight.

$$\% \, Crude \, Fiber = \frac{\text{Weight of } dried \text{ residue}}{Weight \, of \, dry \, sample} \text{ x } 100$$



D.5. Estimation of Carbohydrate

Carbohydrates are normally classified as mono-, oligo- and polysachrides based on the number of monomer unit present in the molecule. Carbohydrates are major constituents of plant material. Soluble Carbohydrates are estimated hydrolyzing or socking in hot water them into mono-saccharides or simple sugars [36].

APPENDIX-E: Figure of Apparent In-vitro DMD response trend as main factors varies.

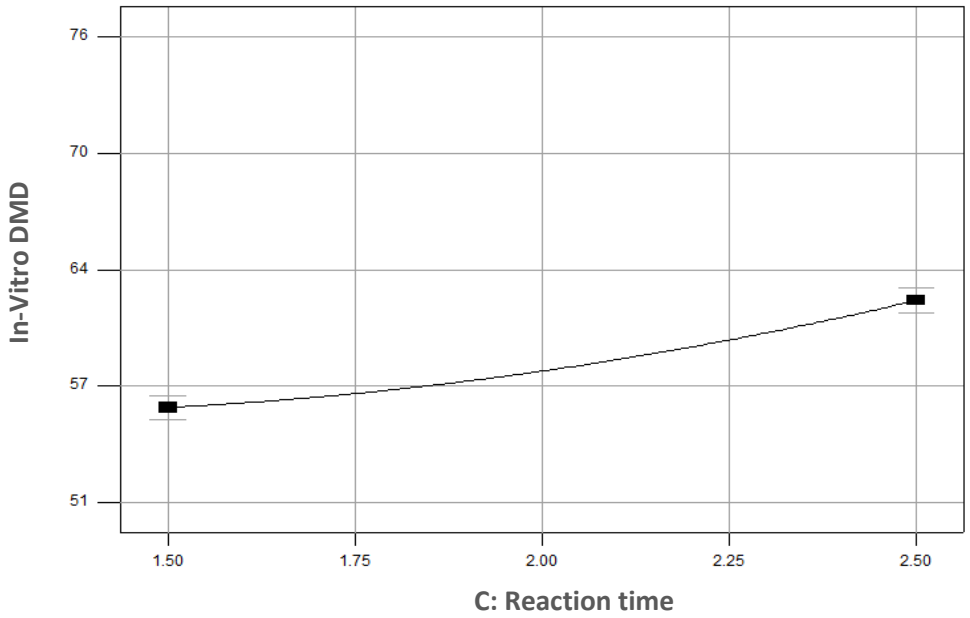


Figure (a) reaction temperature = 100 °C and Conc. of SO_2 = 5.00

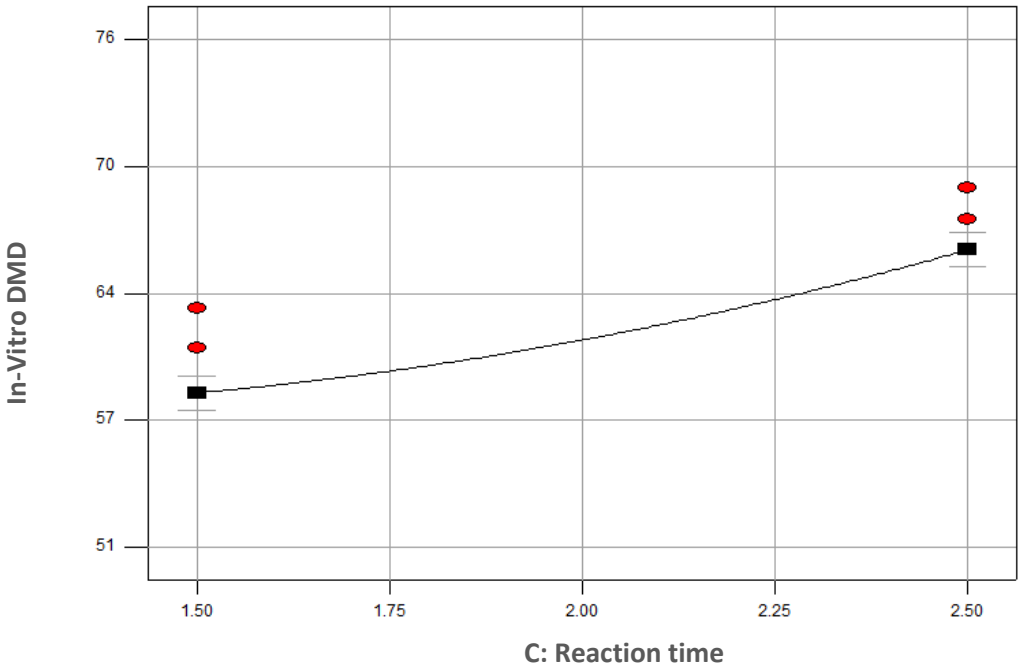


Figure (b) reaction temperature = 100 °C and Conc. of SO_2 = 6.00

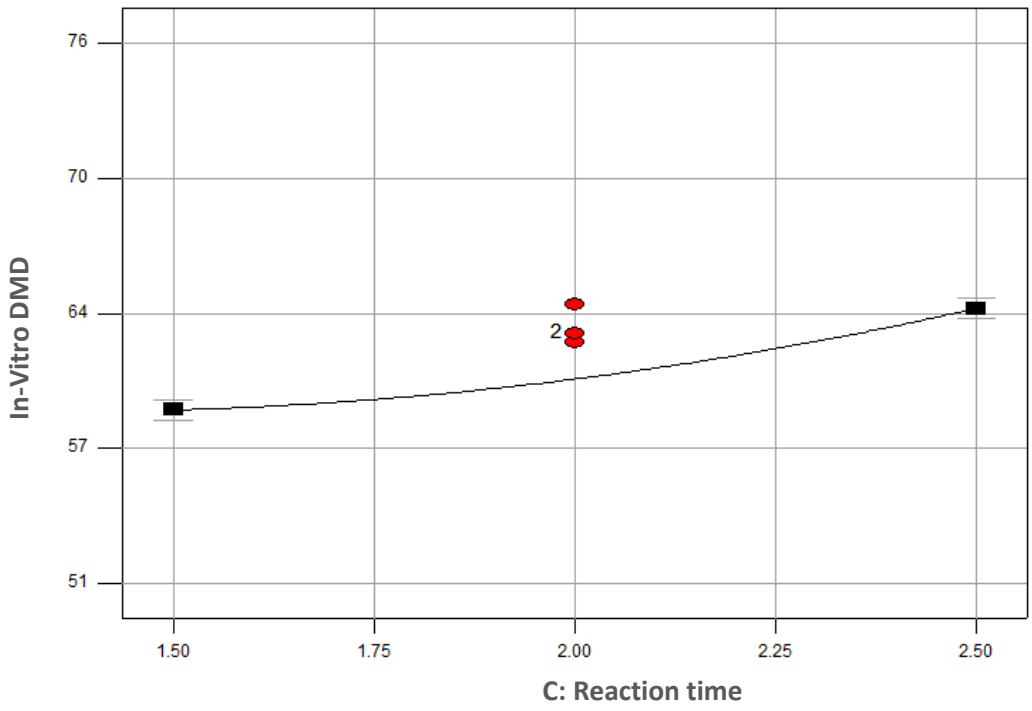


Figure (c) reaction temperature = 110 °C and Conc. of SO_2 = 5.00.

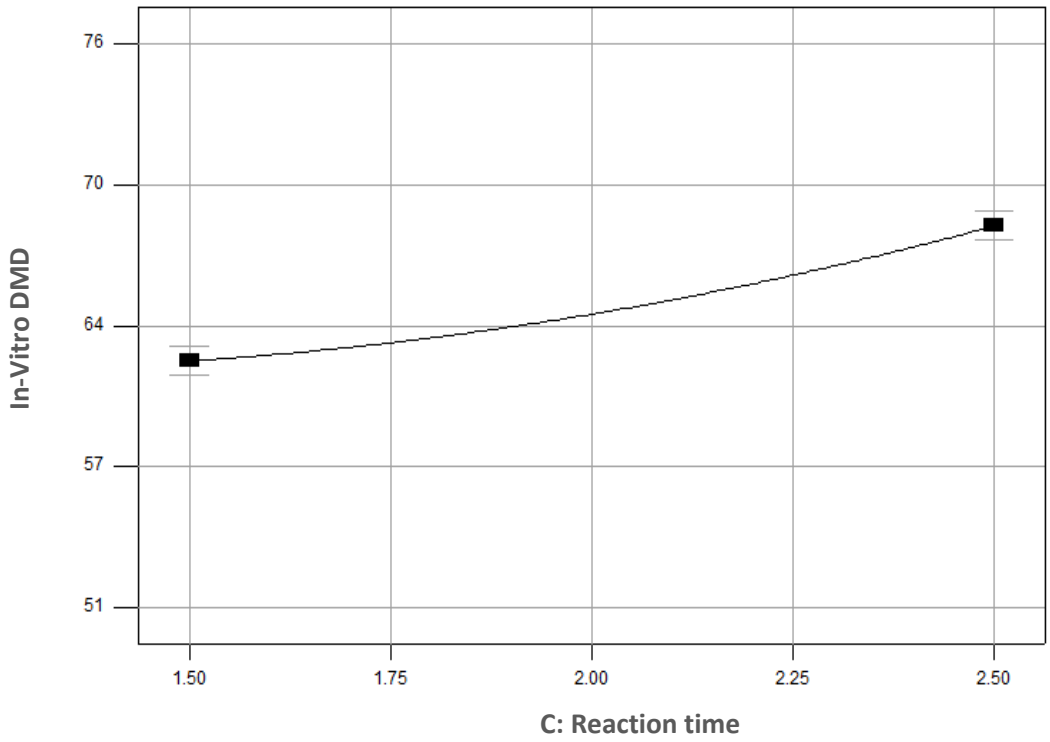


Figure (d) reaction temperature = 110 °C and Conc. of SO_2 = 6.00

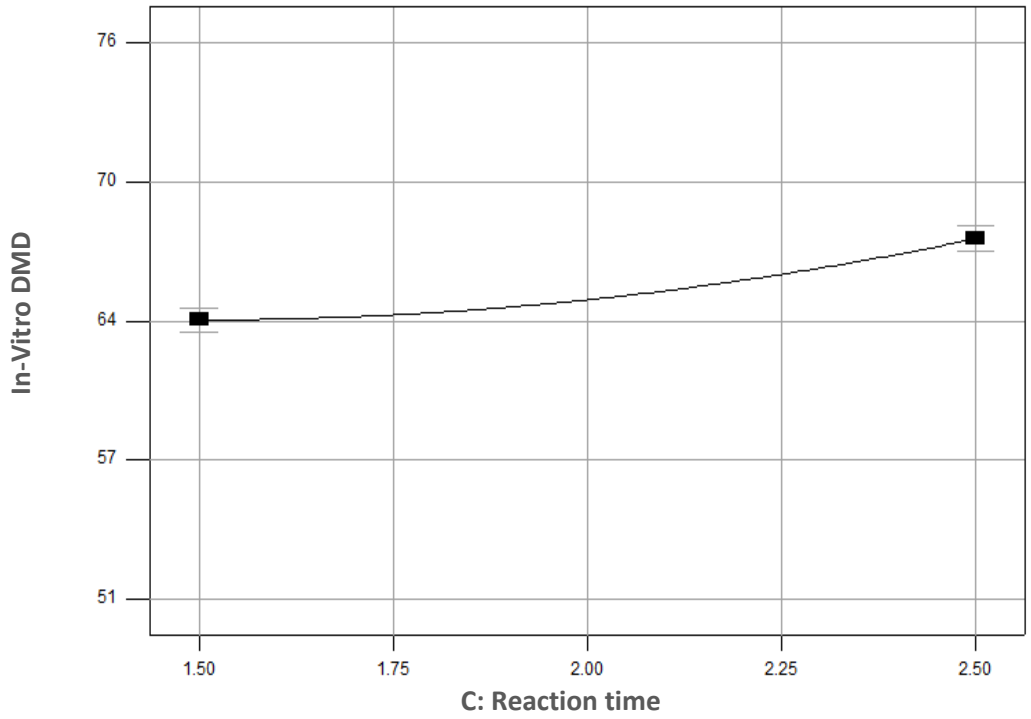


Figure (e) reaction temperature = 120 °C and Conc. of SO_2 = 5.00

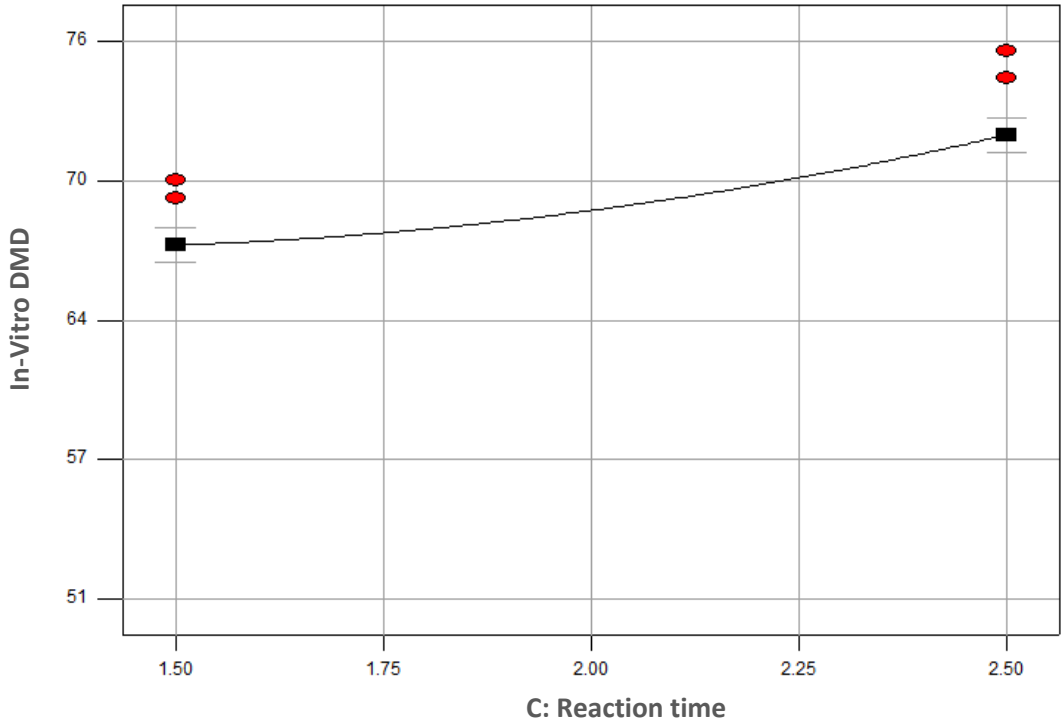


Figure (f) reaction temperature = 120 °C and Conc. of SO_2 = 6.00

Figure E-1(a)-(f): Effect of Reaction time on In-Vitro DMD at different Conc. of SO_2 and temperature.

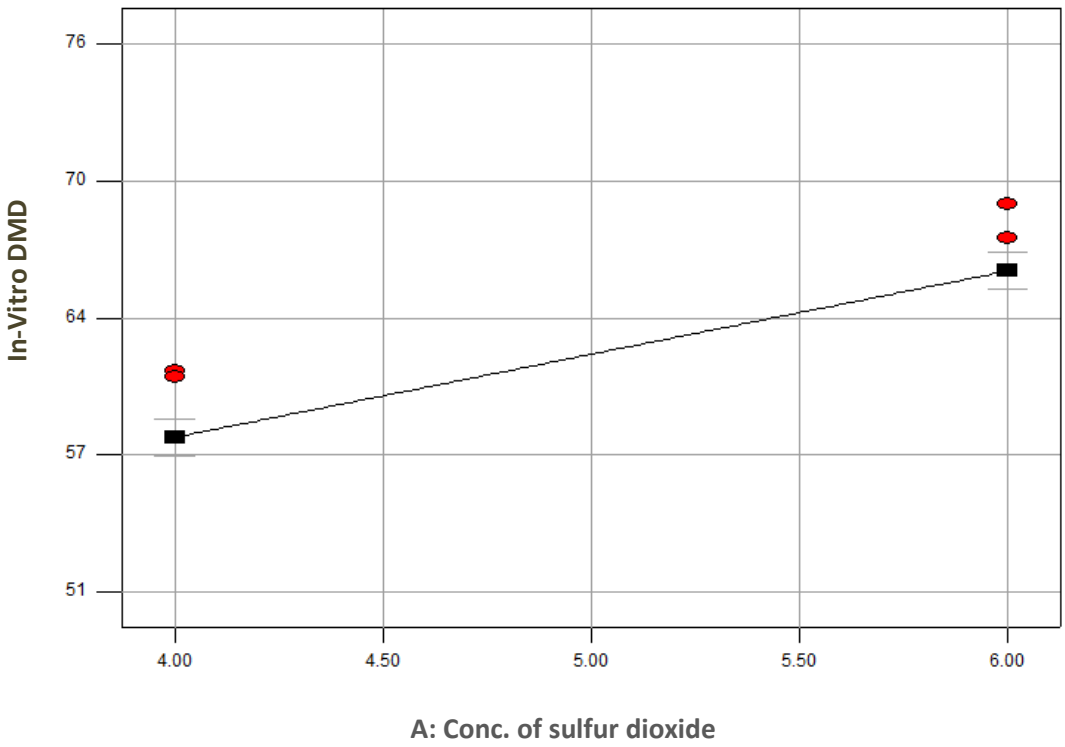


Figure (a) reaction temperature = 100 ◦C and Reaction time = 2.50.

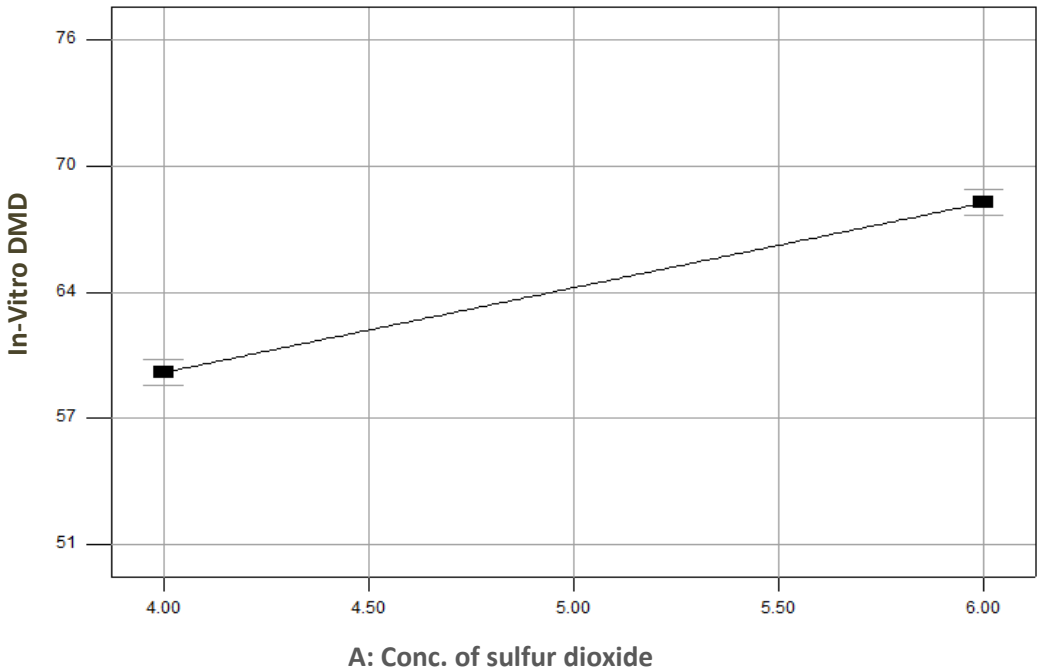


Figure (b) reaction temperature = 110 ◦C and Reaction time = 2.50.

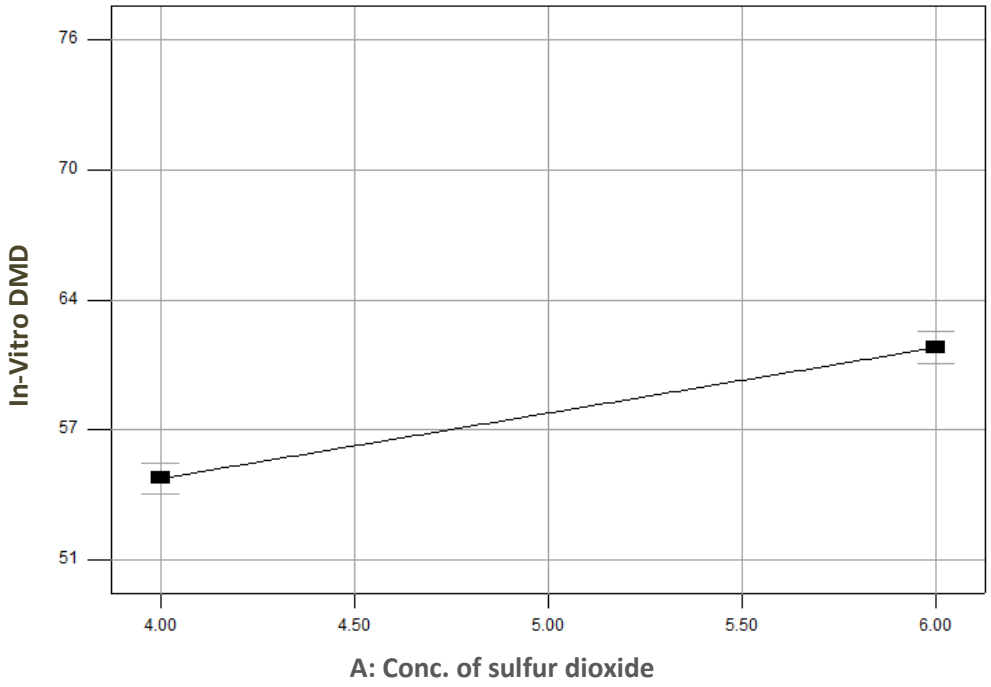


Figure (c) reaction temperature = 100 ◦C and Reaction time = 2.00

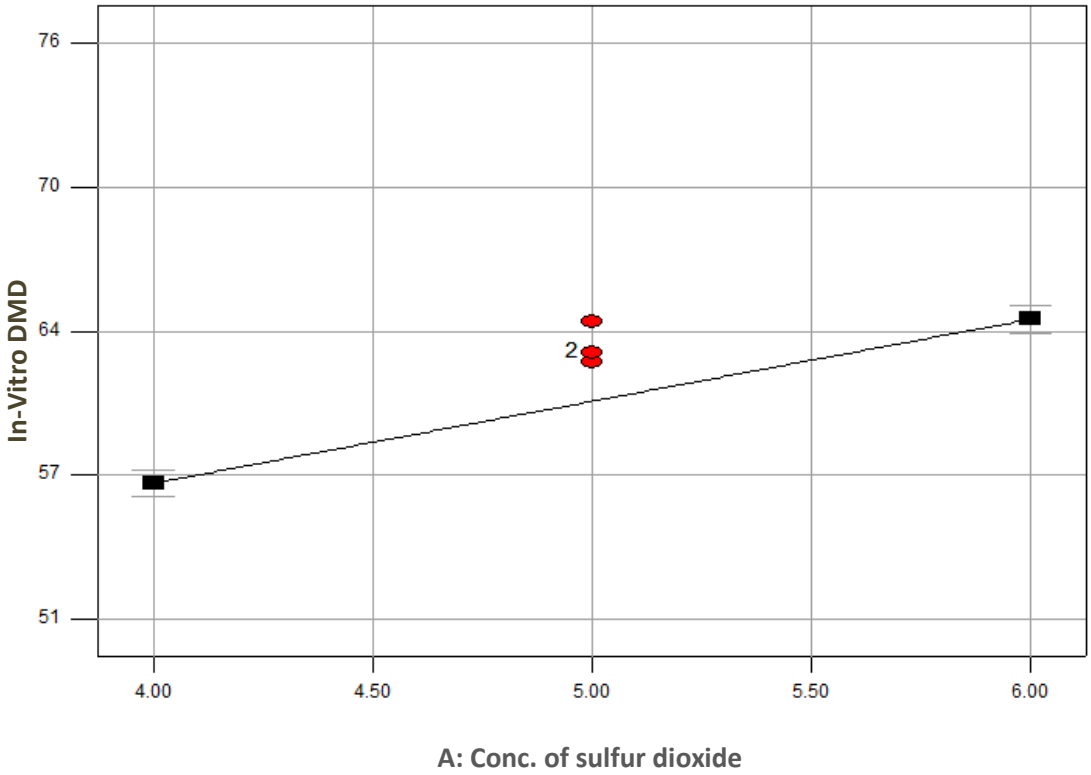


Figure (d) reaction temperature = 110 ◦C and Reaction time = 2.00.

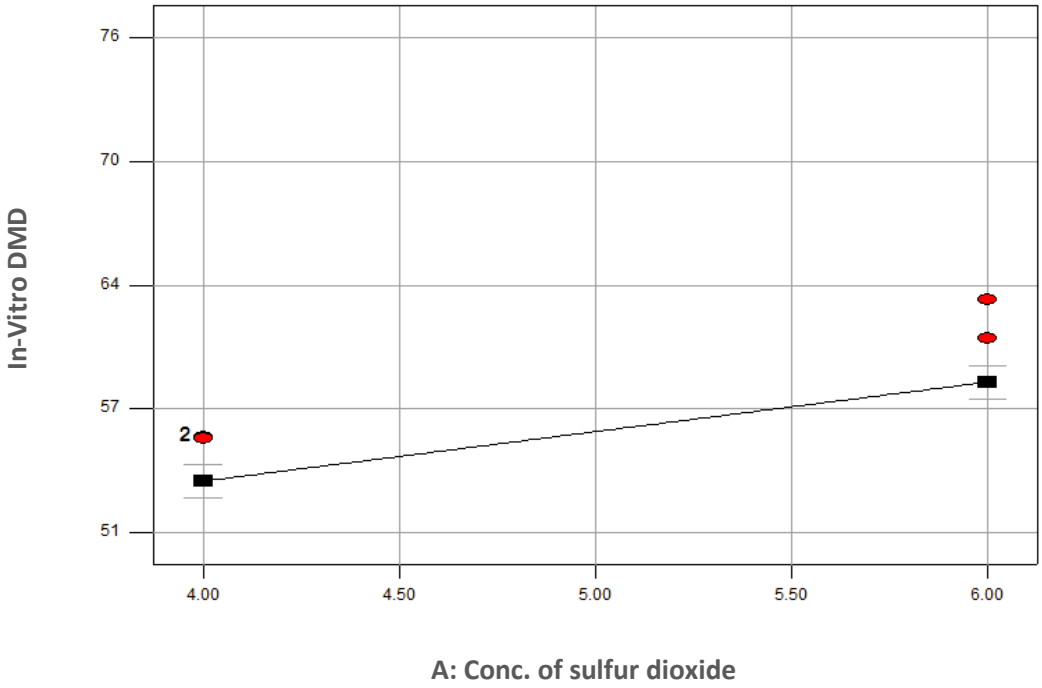


Figure (e) reaction temperature = 100 ◦C and Reaction time = 1.50

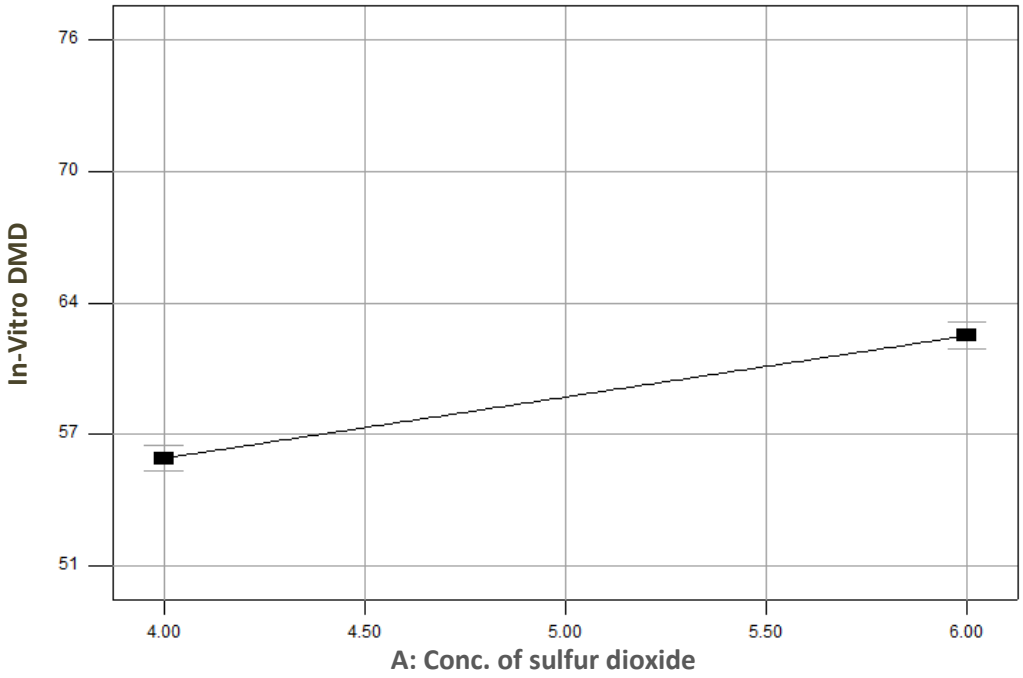


Figure (f) reaction temperature = 110 ◦C and Reaction time = 1.50

Figure E-2 (a)-(f): Effect of Conc. of SO_2 on In-Vitro DMD at different Reaction temperature and time.

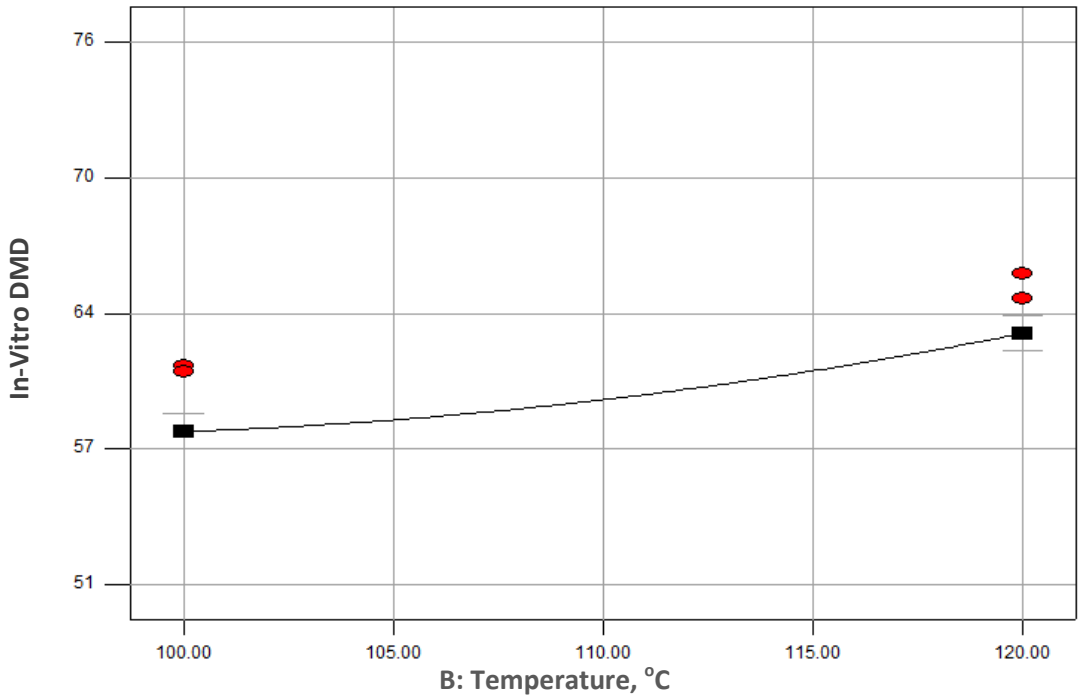


Figure (a) Conc. of SO_2 = 4.00 and Reaction time = 2.50

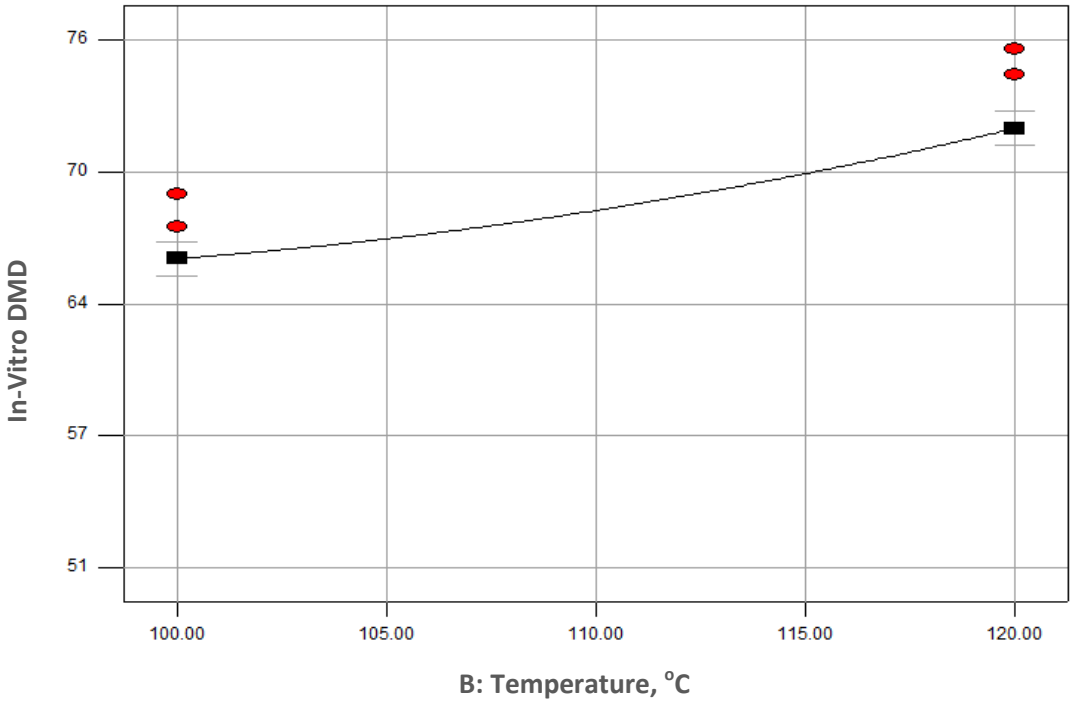


Figure (b) Conc. of SO_2 = 6.00 and Reaction time = 2.50.

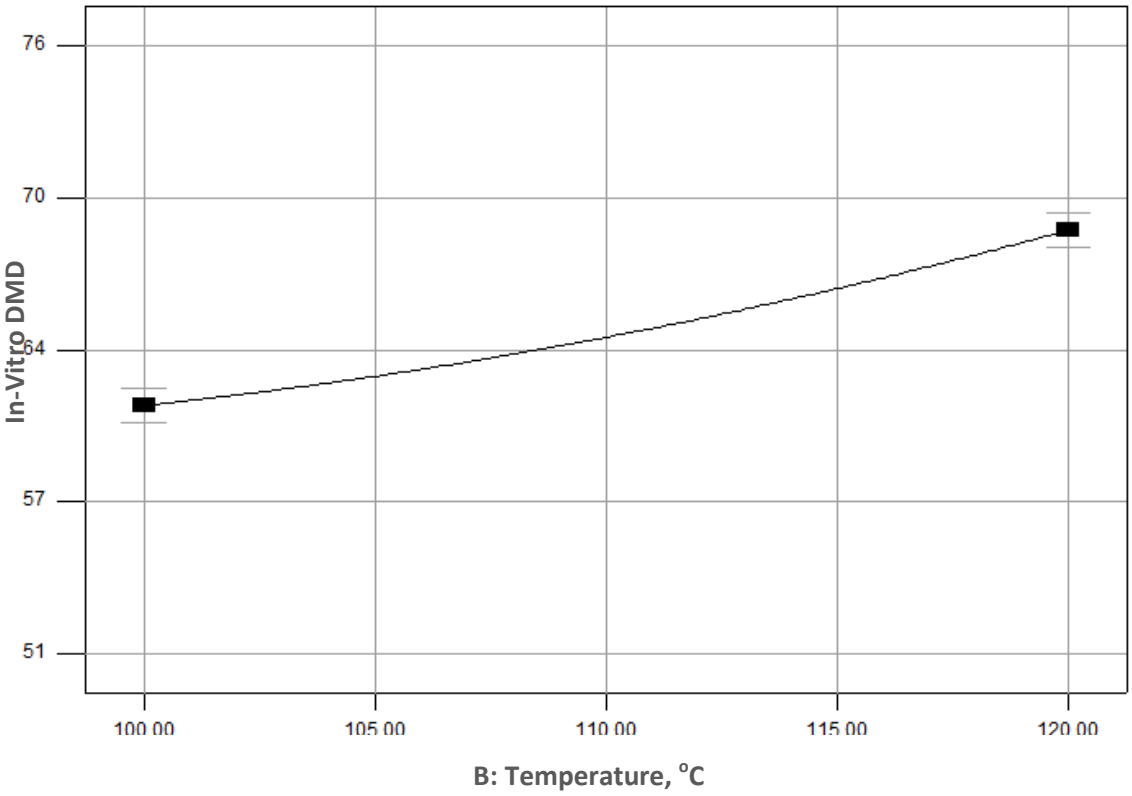


Figure (c) Conc. of SO_2 = 6.00 and Reaction time = 2.00

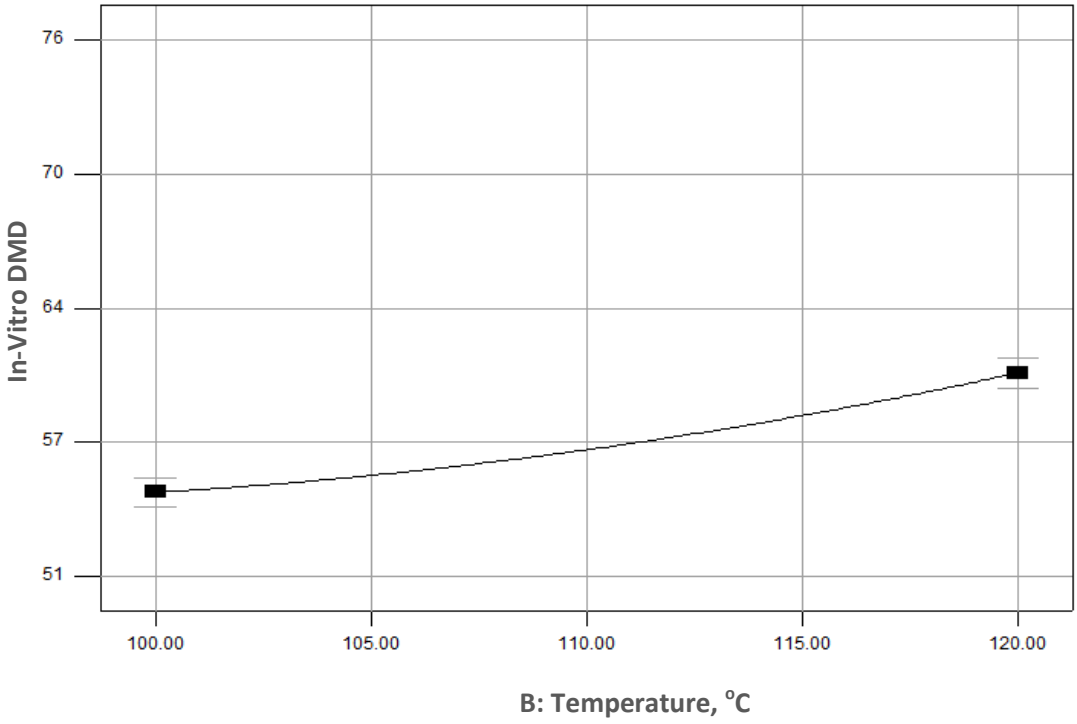


Figure (d) Conc. of SO_2 = 4.00 and Reaction time = 2.00

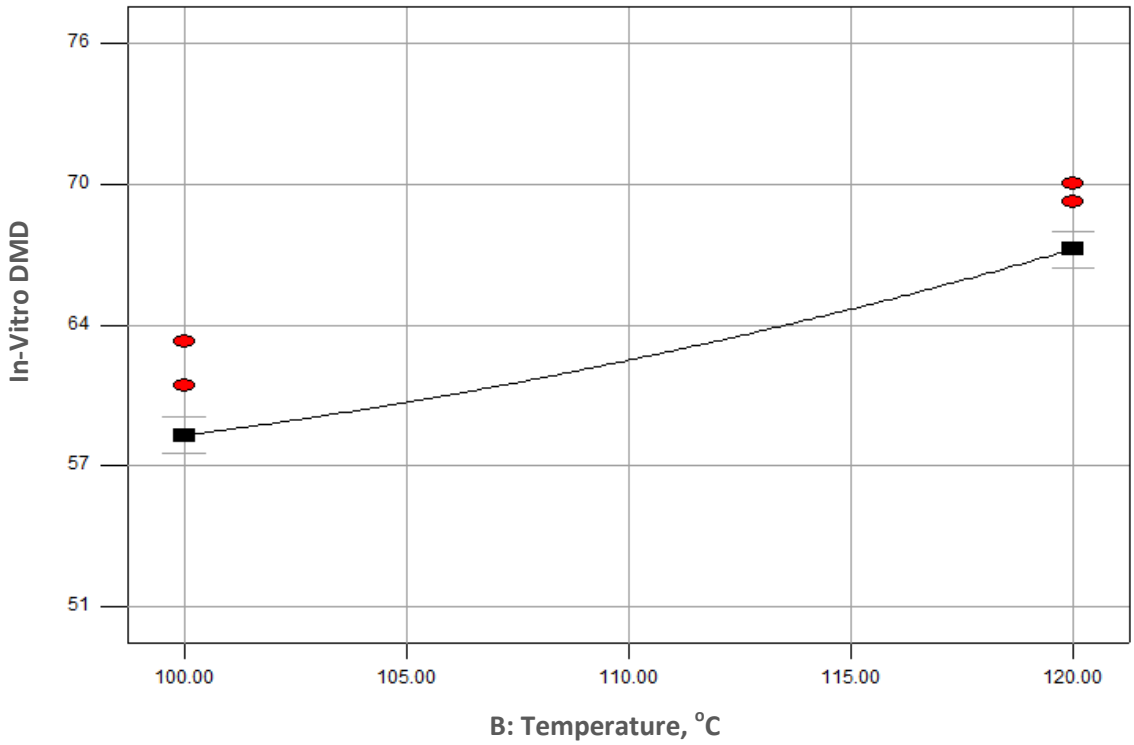


Figure (e) Conc. of SO_2 = 6.00 and Reaction time = 1.50

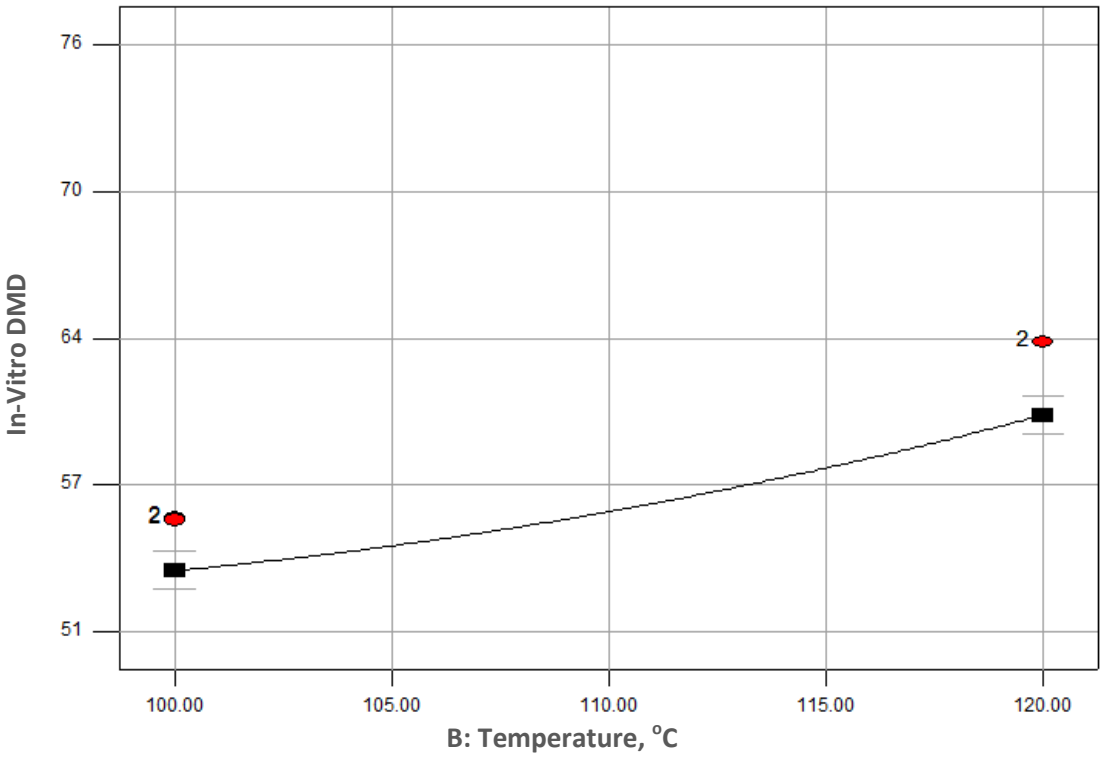


Figure (a) Conc. of SO_2 = 4.00 and Reaction time = 1.50

Figure E-3 (a)-(e) Effect of temperature on In-Vitro DMD at different Conc. of SO_2 and Reaction time.

Table E-1: Experimental Data output of different parameter.

run	Product Sample type	Conc. of SO_2 (%)	Time, hr	Temperature, ^oC	DM%	Ash%	0.5 N Ammonium Hydroxide, ml per batch
1	Prosopis Juliflora Cooked	4	1:30	100	93.88	8.14	18
2	Prosopis Juliflora Cooked *	4	1:30	100	93.61	8.10	20
3	Prosopis Juliflora Cooked	4	2:30	100	94.97	8.24	22
4	Prosopis Juliflora Cooked *	4	2:30	100	94.86	7.94	22
5	Prosopis Juliflora Cooked	4	1:30	120	94.95	6.72	21
6	Prosopis Juliflora Cooked *	4	1:30	120	94.95	6.74	21
7	Prosopis Juliflora Cooked	4	2:30	120	94.87	7.59	25
8	Prosopis Juliflora Cooked *	4	2:30	120	94.81	7.57	24
9	Prosopis Juliflora Cooked	6	1:30	100	95.12	8.01	26
10	Prosopis Juliflora Cooked *	6	1:30	100	94.9	8.01	27
11	Prosopis Juliflora Cooked	6	2:30	100	94.56	6.77	32
12	Prosopis Juliflora Cooked *	6	2:30	100	94.38	6.74	31
13	Prosopis Juliflora Cooked	6	1:30	120	94.22	6.34	27
14	Prosopis Juliflora Cooked *	6	1:30	120	94.17	6.30	27
15	Prosopis Juliflora Cooked	6	2:30	120	94.17	5.33	31
16	Prosopis Juliflora Cooked *	6	2:30	120	94.13	5.24	29
17	Prosopis Juliflora Cooked	5	2:00	110	94.41	6.73	26
18	Prosopis Juliflora Cooked *	5	2:00	110	94.65	6.69	26
19	Prosopis Juliflora Cooked	5	2:00	110	94.27	6.90	27
20	Prosopis Juliflora Cooked *	5	2:00	110	94.27	6.80	28
21	Prosopis Juliflora Cooked	5	2:47	110	94.7	6.64	24
22	Prosopis Juliflora Cooked *	5	2:47	110	94.49	6.75	25
23	Prosopis Juliflora Cooked	5	1:08	110	94.5	8.35	16
24	Prosopis Juliflora Cooked *	5	1:08	110	94.53	8.21	17
25	Prosopis Juliflora Cooked	5	2:00	127.5	94.41	6.88	26
26	Prosopis Juliflora Cooked *	5	2:00	127.5	94.48	6.94	27
27	Prosopis Juliflora Cooked	6.27	2:00	110	93.22	6.93	35
28	Prosopis Juliflora Cooked *	6.27	2:00	110	93.13	6.76	34
29	Prosopis Juliflora Cooked	3.50	2:00	110	94.31	8.27	18
30	Prosopis Juliflora Cooked *	3.50	2:00	110	94.17	7.97	21
31	Prosopis Juliflora Untreated				91.79	7.62	N/A

* indicates the replica

ANNEX – INFORMATION REPORTED IN THE LITERATURE

INFORMATION REPORTED IN THE LITERATURE PERTAINING TO NEUTRAL- AND ACID-DETERGENT FIBER ANALYSES

Fraction	Components	Nutritional Availability for Ruminants
TYPE A - EASILY SOLUBLE		
Cell Contents (soluble in neutral detergent)	Lipids Sugars, organic acids & water soluble matter Starch Non-protein nitrogen Soluble protein	Complete
Cell wall constituents (fiber insoluble in neutral detergent) Soluble in acid Detergent Insoluble in acid (detergent fiber)	Attacked protein Hemicellulose Cellulose Lignin Lignified nitrogen compounds Heat-damaged pectin Silica	Complete Partial Partial Indigestible Indigestible Indigestible

Information Reported in the Literature Pertaining Composition of Some commercial available Feedstuffs [34], (* late cut)

	NDF	ADF	Lignin	DMD
Feedstuffs	%	%	%	%
Alfalfa	40.2	25.1	5.3	62
Alfalfa*	55.2	39.5	8.7	53
Orchardgrass	52.3	27.1	2.7	72
Orchardgrass*	70.4	40.1	4.7	57
Wheat Straw	81.8	53.3	7.6	21

Acid-Detergent Fiber Procedure

Basic Principle:

An acidified detergent solution is used to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash)

Equipment:

* Refluxing apparatus and 600 ml tall form beaker
* Fritted glass (Gooch) crucibles (coarse porosity, 50 mL)
* Analytical electronic balance, accurate to 0.1 mg
* Suction filtering device with trap in line and valve to break vacuum
* Forced-air drying oven set at 100^{o}C

Reagents:

Acid detergent solution:

1 liter of 1.00N Sulfuric acid. Normality must be verified by titration with a primary base standard before adding CTAB (cetyl trimethyl ammonium bromide). A solution approximately 1.0 N sulfuric acid can be made by adding 51.04 g (27.7 mL) of concentrated reagent grade sulfuric acid (95-98% purity) to 972.3 mL water. 20 g Cetyl trimethylammonium bromide (CTAB), technical grade Acetone, reagent grade

Procedure:

1. Samples should be oven dried at 55 °C to 85% dry matter, then ground to pass a 1 mm screen.

2. Dry 50 mL fritted glass crucibles overnight at 100 °C and hot weigh (W1), recording weight to nearest 0.1 mg.

3. Thoroughly mix and weigh sample (W2) (approximately 0.9 to 1.1 g, record weight accurate to 0.1 mg) into Berzelius beaker. Weigh a second subsample for laboratory dry matter determination.

4. Add 100 mL acid-detergent solution at room temperature. Place beaker on heater under the cold water condenser.

5. Heat to boiling in 5-10 min; reduce heat to avoid foaming as boiling begins. Reflux 60 min from onset of boil.

6. After about 30 min, wash down sides of beaker with minimal amount of acid detergent solution. A wash bottle is convenient for dispensing solution.

7. Remove beaker, swirl, and filter through tared (step 2) fritted glass crucible, using minimal vacuum. Police and rinse the Berzelius beaker with boiling water while inverted over the crucible to insure quantitative transfer of all fiber particles into the crucible.

8. Soak twice with boiling (95-100°C) water by breaking up mat and filling crucible each time with vacuum off and allowing to soak a minimum of 15 to 30 sec (2 min recommended) after each wash. While filling the crucible with hot water or acetone, rinse the top edge and sides to remove residual acid detergent.

9. Rinse twice with 30-40 mL acetone by filling crucible each time with vacuum off, allowing a minimum of 15 to 30 sec before vacuuming dry.

10. Dry 3 hr or overnight in forced-air oven (100oC) and weigh hot, recording weight (W3) to nearest 0.1 mg.

Calculation: Percent Acid Detergent Fiber (ADF)

$$\% \text{ ADF (DM basis)} = \frac{W3 - W1}{W2} * 100$$

* W_1 = weight of crucible in grams

* W_2 =oven dried sample weight in grams

* W_3 = dry weight of crucible and dry fiber in grams

Neutral-Detergent Fiber Procedure

Principle:

A neutral detergent solution is used to dissolve cell solubles, Lipids, Sugars, organic acids, water soluble matter, Starch and soluble minerals leaving a residue of cellulose, hemicelluloses, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). The NDF value is the total cell wall, which is comprised of the ADF fraction plus hemicellulose.

Equipment:

* Refluxing apparatus

* 600 ml tall form beaker

* Fritted glass (Gooch) crucibles (coarse porosity, 50 mL)

* Analytical electronic balance, accurate to 0.1 mg

* Suction filtering device with trap in line and valve to break vacuum

* Forced-air drying oven set at 100^{o}C

Reagents:

Neutral detergent solution:

Prepare 1 liter distilled water and add 18.61 gm of ETDA (disodium ethylene diaminetetraacetate) and 6.81gm sodium borate decahydrate and heat until dissolved. Then add to a solution containing 30gm of sodium lauryl sulphate and 10 ml 2-ethoxyethanol. Finally, place 4.56gm of di- sodium hydrogen phosphate in to solution containing the other ingredients.

Procedure:

1. Weigh 0.5-1.0 grams an air-dry sample is then ground to pass 1mm.
2. It is then place into a refluxing beaker and 100 ml neutral detergent solution (room temperature) is added and the mixture is heated to boiling in 5-10 minutes.
4. When the solution begins to boil, the heat is reduced to avoid foaming. The boiling is adjusted to an even level and the reflux is carried out for 60 minutes, timed from the onset of boiling.
5. After the reflux, the beaker is swirled to suspend the solids and poured into the crucible. Vacuum is applied after the crucible is filled; low vacuum is used first, increasing its strength as needed.
6. The sample in the crucible is then rinsed with a minimum of hot (90°-100°C) water. The vacuum is removed, the sample mat broken up, and the crucible filled with hot water. The liquid is again filtered and the washing procedure is repeated once more. Then, in the same manner, the sample is washed twice with acetone and sucked dry.
7. The crucibles are then dried for 8 hours at 100°C and finally, weighed. The yield of recovered neutral detergent fiber is reported as percent of the cell-wall constituents.

$$\%\ \text{NDF (DM basis)} = \frac{W3 - W1}{W2} * 100$$

* W_1 = weight of crucible in grams
* W_2 =oven dried sample weight in grams
* W_3 = dry weight of crucible and dry fiber in grams.