KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

MICROBIOLOGICAL CHALLENGE OF A FUZZY MODELED PINEAPPLE JAM

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY MANGEMENT

BY

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CERTIFICATION PAGE

I hereby declare that this submission is my own work toward the award of MSc. Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Fuzzy logic and reasoning was used to model pineapple jam and to study the trend associated with jam quality change in response to varying imprecise input quantity of three formulation variables: sugar, pectin and citric acid. Sugar/g ranging (100.00-140.00), pectin/g (20.00-30.00) and citric acid/g (0.00-4.00) were combined with 200.00g pineapple pulp at three fuzzy linguistic sets and levels of 'low' sugar /g (50.00-57.50), 'moderate' sugar (55.00g-65.00g), 'high' sugar (62.50g-70.00g); 'low' pectin/g (20.00- 30.00), 'moderate' pectin/g (23.00-27.00), 'high' pectin/g (26.00-30.00); 'low' citric acid/g (0.00-1.50), 'moderate' citric acid/g (1.00-3.00) and 'high' citric acid/g (2.50-4.00). Twenty seven (27) jam formulations with complex nonlinear physicochemical quality outputs ranges of pH (2.8- 3.18), degree brix of (2.5-8.5), percentage moisture content (46.5-59.35) and texture /mJ(0.288- 8.210) were obtained. The Mamdani Fuzzy Inference System (FIS) was used to process input and output data using the "IF–THEN" rules represented in linguistic variables characterized by continuous triangular membership functions. The Maximum conjunctive aggregation and Centre- of- gravity (Centroid) was used as defuzzifier to predict data at 100% accuracy. The jam formulated with 'high' sugar, 'moderate' pectin and 'high' citric acid was deemed best jam formula with good and quality physicochemical property of 'low' pH (2.83), 'high' degree brix (7.85^{o}Bx), 'moderate' texture (4.00mJ) and 'moderate' moisture content of 52.5%. A microbial challenge study conducted on the best jam was done to ascertain the ability of the jam to survive microbial contamination. The result suggested that, storing pineapple jam formulated with resultant 'high' pH, 'high' degree brix, 'moderate' texture and moisture content at refrigerator temperature storage conditions can control the growth of spoilage yeast and pathogenic bacteria that may be present in jam products.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Consumers are particular about food safety and quality and so have high expectations and demands. These demands are rapidly changing, and needs to be met in order to supply food of an expected quality (FAO/ WHO, 2001). It has become necessary to design models that could control and predict food safety and quality and at the same time be able to within the shortest possible time, design food that meets the consumer's ever changing preference (Magkos *et al.*, 2006). According to Singh *et al.*, (2017), models have inherent properties that connect consumer expectation with the product's physical properties thereby reduced production time and expenses as the number of food design experimentations were minimized. Additionally, models enhance production processes by providing predictive capability of performing "what if" scenarios as well as improved process automation and control. Models may be observation or physic based. Observation-based models are inferred from measured data and are useful in providing a practical, useful relationship between input and output parameters of complex processes. Fuzzy logic is a type of observation-based models that gradually assesses membership of elements in a set by use of membership functions. Fuzzy logic models are particularly useful in processes in which human reasoning and perception are involved (Evans, 2004). Fuzzy logic is especially attractive because it focuses on modeling challenges presented in imprecise or ambiguous data and can be applied in food quality evaluation, equipment selection as well as in controlling food processes (Zadeh, 1975; Kasabov and Kozma, 1998).

Consumer choices for healthy and high quality foods have resulted in changes in foods and the development of new production and processing methods. For example, sweet spreads are a household staple in the United Kingdom that offers British consumers an easy and relatively low-cost accompaniment for their breakfast or afternoon tea. However, sweet spread faces a challenge in terms of health and wellness. Mintel (2013) established that about 39% of correspondent consumers were worried about the high sugar content of the sweet spread whereas about 15 % were ready to pay higher price for products formulated with natural sugar-free sweeteners. The consumers preferred sweet spread product manufactured with little amount of sugar for health reasons such as diabetes, low sugar diet, etc. or for the advertisement of final products as an all-fruit or a dietetic preserve.

Jams are a category of sweet spread (Ofosu *et al*., 2011) made by cooking crushed or chopped fruits with sugar that results in a thick sweet spreadable product. The addition of high sugar is essential because it preserves the fruits, and mechanically participates in gel formation. Depending on the gelling properties of the fruit, pectin is added to ensure a good gel. Fruit acids are also added to enhance flavor and to provide the acidic medium required for good gelling particularly since acid concentration differs amongst fruits and higher in under ripped fruits (Fasogbon, 2014). Jam making is technically a food preservation method that utilizes the concept and application of the hurdle principle to ensure microbiological stability and safety of fruits and vegetables. According to Leistner and Gorris (1995) jam's preservative factors such as high temperature during processing (F values), low water activity (a_w), low moisture

content, low acidity (pH), low temperature during storage, preservatives, etc. synergistically restraints the growth of spoilage or pathogenic microorganisms because the microorganisms are limited by unfavourable conditions presented by the preservative factors. The procedure of making jam is complicated (Javanmard and Endan, 2010), because the food industry works with raw materials with varying physicochemical properties that needed to be processed into a product that complies with a fixed acceptable standard as well as the rapidly changing requirements of the consumer.

The standards for quality jams are given by different agencies. For example, according to the specification of the Codex Alimentarius Commission, the finished jam should contain more than 65% total soluble solids, should be of appropriately gel texture, and a desired pH range of 2.5-3.2 (Fasogbon, 2014). Sugar constituents should be more than 40% of total weight and 80% total solids in jam (Codex Alimentarius, 2009).

Pineapple jam production and the prediction of its quality outputs such as pH, total soluble solids or brix, moisture content and jam consistency or texture is very difficult and complex. Mathematically, modeling jam quality output may result in substantial inconsistencies between model results and tentative data (Samhouri *et al.*, 2007). The application of Mamdani Fuzzy Inference System (FIS) in food quality modeling and prediction is an innovative method that quashes challenges existing in the dependency of the manufacturer's or operator's rule of thumb during food processing and production (Shyam *et al*., 2006). The Fuzzy Inference System (FIS) application allows

the exploitation of empirical data and heuristics represented in "if-then" rules and the transference of these rules to a functioning system. The system then converts data sets or linguistic information known as rules to mathematical equivalents resulting in an accurate representation of the way the system performed actually (Ross, 2004; Shyam *et al.*, 2006).

Finished product testing is an important part of food manufacturing control policy. Food products susceptible to microbial contamination, growth or survival, challenge testing is used to assess the product's safety and stability. The test mimics and determines what can happen microbiologically to a product, should there be exposure to microorganism during storage, distribution and subsequent handling. The test involves the inoculation of the food product with appropriate organisms, at suitable levels then stored and tested for their presence or absence during the product's storage life.

1.2 Problem Statement and Justification

Consumers are particular about the quality and safety of their foods. These expectations are imprecise and rapidly changing but needs to be met in order to deliver food of desired quality. It has become imperative to develop and implement models that can serve as a physical connect with these desired food quality and the food products physical properties and to be able to quickly change the food design to suit the customer's expectation. Food products needs to attain a balance between essential properties such as sensory, technology and sanitary because these influence consumer choice and preference as well (Linko and Linko, 1998). But controlling these factors

from start of production is daunting task (Perrot *et al*., 2006; Welti *et al*., 2002). The procedure for making fruit jam is complicated because raw materials with inconsistent physicochemical properties are processed into products that should comply with specifications detailed in standards. In addition, sensory quality perception and assessment of jam such as jam flavor, sweetness, colour, softness, hardness, etc are perceived differently by the human brain which presents with some significant levels of ambiguity. Fuzzy models or systems have been found useful in the food industry especially in modeling processes in which human reasoning and perception are involved (Evans, 2004). The motivation behind this work is to demonstrate how the concept of fuzzy logic reasoning and sets can be used to provide a suitable connect of the physical quality properties of pineapple jam with consumer wishes and at the same time fulfilling technological and safety requirement *(Subuola et al*., 2015).

1.3 Main Objective

The main goal of this study was to demonstrate how the concept of fuzzy logic and reasoning can be applied in the formulation of quality pineapple jam. The specific objectives are; firstly, to obtain the best jam formulation for pineapple jam by means of concept of fuzzy sets and logic using jam formulation variables; sugar, pectin, and citric acid. Secondly, the project sought to challenge jam with the yeast spoilage organism (*Saccharomyces cerevisae*) and pathogenic bacteria (*Escherichia coli*). The microbiological challenge testing was done in order to ascertain the ability of the jam formulation to survive microbial contamination when jam is stored on the shelves at room and refrigerator temperatures.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Pineapple fruit (*Ananas comosus*)

Majority of pineapple is eaten as fresh fruits (Medina, Cruz, and García, 2005). Pineapple (*Ananas comosus*) cultivation and harvest comes second after bananas and it constitutes about 20% of global tropical fruits. Thailand, Philippines, Brazil and China, the main global pineapple producers supplied nearly 50 % of pineapple in 2004 (FAO, 2004), whereas India, Nigeria, Kenya, Indonesia, México and Costa Rica provided most of the remainder 50 % (Medina *et al.*, 2005; Baruwa, 2013). Costa Rica in 2008 led with an annual export of about 300,000 tonnes into the European market, followed by Cote D'Ivoire, with an export of 150,000 tonnes whilst Ghana placed third with 71,000 tonnes in 2008 (Pacific, 2008).

There are about one hundred pineapple cultivars (Medina *et al*., 2005). Pineapple with improved qualities have been cultivated and introduced; For example, *Cayena lisa* has high sugar content of 13 to 19 ˚Bx, has clear yellow colour juice. The Red Spanish cultivar weighs on average between 1.2 kg and 2.0 kg, has medium sugar content of 12 ˚Bx and low acidity content. The Queen variety has an average weight of 0.5 kg to 1.0 kg, has yellow pulp colour, high sugar content of 14 ˚Bx to 18 ˚Bx. Other cultivars which include Perola averagely weighs 0.9 to 1.0 kg has high sugar content of 13 ˚Bx to 16˚Bx. Perolera usually weighing between 1.5kg to 3.0 kg is low in sugar content of 12 ˚Bx. Green selacia and Spanish from Singapur weighs on average 1.0 kg is mostly

used for canning, has golden yellow pulp colour, high quality for juice production, small acid and sugar content of between 10 ˚Bx and 12 ˚Bx (Medina *et al.*, 2005).

2.2 Benefits of pineapple

2.2.1 Nutritional value

Pineapple as food, has both nutritive and anti- nutritive properties. Riped and mature pineapple fruit has high moisture content of up to 86.2 % and total solids of about 19 % which is contributed largely by sucrose, glucose and fructose. Carbohydrate constitutes about 85 % of pineapples total solids and fibre about 2 – 3 %. Citric acid is the most abundant organic acid in pineapple. Pineapple has negligible fat and protein content and very low ash content (Hemalatha and Anbuselvi, 2013). Pineapple's non-nutritive components such as citric acid, malic acids, and bromelin or bromelain are of importance from a dietary and therapeutic stand point. Citric and malic acids which are responsible for the pineapples acidic taste potentiates the action of vitamin C. In spite of its richness in acid, pineapple acts as an alkalizer from a metabolic viewpoint or as an antacid as it occurs with lemon and other fruits. Bromelin, a protein- digesting enzyme commonly used in the food industry as meat tenderizer, acts in the digestive tracts by breaking down proteins and facilitates digestion (Hemalatha and Anbuselvi, 2013).

2.2.2 Medicinal and healing property

Consumption of pineapple is specifically indicated for hypochlorhydria (scanty gastric juice) which is manifested by slow digestion and a sense of heaviness in the stomach; gastric ptosis (prolapsed stomach) caused by the stomach inability to empty itself a condition known as gastric atonia; obesity; and sterility due to its trace manganese content actively involved in the formation of reproductive cells in both males and

females. It has also been shown that, pineapple is a powerful inhibitor of the formation of nitrosamines. Nitrosamine is a carcinogenic substance that forms in the stomach as a chemical reaction between nitrites and certain proteins contained in foods (Roger and George, 2008). Pineapple fruit has antiparasitic, abortive, detoxifier, vermifuge, stomach relief properties. The fruit has also been indicated amongst others to improve digestion, stomach acidity regulation, detoxification, neutralization (Nwaizu *et al.*, 2011).

2.2.3 Other important use

Pineapple fiber bear a resemblance to silk in texture and colour, hence is processed in some Asian countries to make garments and fine flexible sheets of paper. Bromelin in addition to its application in the food industry as meat tenderization is also used in chill proofing of beer, solubilize protein, treat fish waste, colour leather and stabilize latex paints (Hoornstra *et al.*, 2008).

2.3 Pineapple production, Post-harvest loss management and food security

Aworh, (2008) reported that about 50 % commodity grown in developing countries including fruits, vegetables, roots and tubers never make it past the farm gate in West Africa, hence contribute to food insecurity crises. Large amount of food is loss because, developing countries are unable to preserve food as a result, suffer seasonal food shortages, and nutritional deficiency diseases example of which include protein-energy malnutrition (PEM) and the various micronutrient deficiency disorders characterized by vitamin A deficiency (VAD), nutritional anemia due to deficiencies of iron, folic acid and vitamin B12, and iodine deficiency disorders (IDD). PEM and IDD have negative effect on growth and mental development of children whilst VAD, apart from its

destructive consequences on the eye (xerophthalmia and night blindness), is a key cause to the high rates of child and maternal morbidity and mortality. Upgrading traditional food processing and preservation techniques as well as harnessing the potentials of emerging technologies, developing high products for food, health and industrial use, reviewed regulations- quality standards and specifications, that contribute to unintended waste are amongst specific novel postharvest technologies which can improve food security and safety (Linus Opara, 1999; Aworh, 2008)

2.4 Pineapple fruit processing

Pineapples may be processed into products such as pineapple chunks, jams, juice, syrups, and cubed pineapples. The waste from processing the fruit may further be processed into sugar, wines, vinegar, animal feed, etc (Debnath, 2015). Jams differ from each other in the raw materials used, processing methods and additives. Jams produced with a blend of fruits are usually called conserves particularly when citrus fruits, nuts, raisins or coconut are part of the ingredients (Albrecht, 2010; Jayabalan and Karthikeyan, 2013). Acceptable jam quality should not contain less than 60% soluble solids. Sugar constituents should be more than 40 % of total weight and 80 % of total solid. It should have appropriately gelled consistency, normal colour and flavor appropriate to the type and kind of fruits used in its preparation (Codex Alimentarius, 2009).

2.4.1 Jam production technology

Fruits, sugar (mostly sucrose), pectin and edible acids are usually the main ingredients required for jam production (Albrecht, 2010). The ingredients, which are usually combined with 65% sugar, 1% pectin, and an acid concentration of pH 3.10, are

thermally treated at normal or reduced pressure to bring about a sweet jelly textured product of desired pH range of 2.5-3.2 (Fasogbon, 2014).

The fruit gives jam its exclusive taste and colour, and make available the liquid needed to melt the rest of the ingredients. The fruit pulp supplies some or all of the pectin and acid (Albrecht, 2010).

Sugar impacts sensory, physical, microbial and chemical functional properties of the jam. Sugar contributes to flavor or taste that is, the sweet, sour, or bitterness of the jam. Sugar heightens or depresses the perception of other flavours. Sweetness of products depends on the concentration, pH, temperature and the use of other ingredients (Nordic Sugar, 2016). High amount of refined sugar or sucrose, not glucose or pure dextrose, is most preferred due to its low tendency to recrystallize (Javanmard and Endan, 2010). During cooking, the sucrose is partially inverted into glucose and fructose- a splitting process influenced by pH value, temperature and time. Acid and heat catalyzes the inversion process (Cancela *et al.*, 2005). The solubility of pure sucrose is 66 % at 70 °F; hence if the fruit contains enough acid, sufficient inversion will occur during boiling to prevent sucrose crystallization in the finished product. The presence of non-crystallizing or inverted sugar such as glucose or fructose in jam is vital to prevent the growth of sucrose crystals during storage or after opening which gives the jam a grainy and grey colour appearance. The sucrose crystals may increase the water activity of jam as water is “squeezed out” when sugar solids are concentrated in crystals. Inverted glucose and fructose retain moisture and are less prone to crystallization. Jam is preserved by the

osmotic pressure and low water activity properties created by the sugar solution in the liquid phase. Addition of sugar lowers the product water activity value (a_w) and by evaporation down to 0.848 (Bourne, 2013; White, 2014).

All fruit contain some amount of pectin. Apples, grapes and some berries usually contain enough natural pectin. Fruits such as pineapple (Albrecht, 2010), strawberries, etc. have minute amount of pectin and therefore must be mixed with other fruits containing high pectin or with pectin products to achieve gels. Pectin is a gelling agent in powder or liquid form made commercially from apples or citrus fruits. Depending on the degree of esterification on their carboxylic acid moiety, pectin are classified as high methylester or methoxyl if esterification is more than 50% and low methylester pectin if esterification is less than 50%. Dried powdered pectins in water hydrates very rapidly in the process form masses or networks that consist of semidry packets of pectin contained in an envelope of highly hydrated outer coating. Chemically, pectins are composed of D-galacturonic acid molecules, which are linked to each other in alpha-1-4-glycosidic bond to form polygalacturonic acid. The hydrogen bond between pectin molecules free carboxyl group and the hydroxyl groups of neighbouring molecule coupled with hydrophobic interactions aggregate the dispersed pectin molecules into a gel (Oakenfull *et al.*, 1991). In a neutral or slightly acid dispersion of pectin molecules, most of the unesterified carboxyl groups are present as partially ionized salts whereas those ionized produce a negative charge on the molecule, which together with the hydroxyl groups causes it to attract layers of water. However, the repulsive forces between these

negatively charged groups can be strong to prevent the formation of gel or pectin network.

The addition of acid converts the carboxyl ions to mostly the unionized carboxylic; this does not only lower the attraction between pectin and water molecules but also decrease the repulsive forces between the pectin molecules (Sriamornsak, 2003). The presence of sugar molecules decreases the hydration of pectin by competing for water thereby creating a condition of low water activity (Morris *et al.*, 1980; Oakenfull *et al.*, 1991). These two conditions, decreases the ability of pectin to stay in dispersed state. When cooled, the unstable dispersing of less hydrated pectin forms a gel that is, a continuous network of pectin that holds the aqueous solution (Sriamornsak, 2003). The rate at which gel formation takes place is also affected by the degree of esterification. A higher degree of esterification (DE) of above 72% causes more rapid setting. Rapid-set pectins (ie pectin with a DE of above 72%) also gel at lower soluble solids and higher levels than slow-set pectins that is, pectin with a DE of 58-65% (El-Nawawi and Heikel, 1997; Kasapis, 2002).

The weak organic acid, citric acid is naturally found in fruits and vegetable and its responsible for the peculiar sour taste in fruits. The acid is used in the food industry to produce a slightly sour, stimulating taste and balanced sweetness. Citric acid is soluble in water and has no limited acceptable daily intake (FDA, 1991). Organic acids are often added to jam products for tastes adjustment, as a preservative and to set proper level of acidity for gel formation; in fact gel will form in the presence of sufficient acid.

Too much of acid will cause the gel to ooze liquid (weep). In jam manufacture, lemon juice or legal edible acids such as Lactic acid E 270, Citric acid E 330, Tartaric acid E 334, Sodium lactate E 325, Calcium lactate E 327, Sodium citrate E 331, Calcium citrate E 333 and Sodium tartrate E 335 are in cooperated (Codex, 2004; Albrecht, 2010) are added to fruit mixtures that have too little acid to set jam gel.

2.5 Microorganisms and growth response in food

Some bacteria, yeasts and molds can cause food borne diseases and spoilage. Most of these organisms cause food spoilage and food borne diseases due to their ability to grow in foods. Molds and yeast can survive in conditions which are unfavourable to many bacteria such as high osmotic pressures, low pH, low water activity or moisture content. Many strains of yeast and molds contaminate food with mycotoxins and hence, have been connected with food borne intoxication outbreaks. Some mycotoxins are carcinogenic or mutagenic and cause organ specific pathology such as hepatotoxin or nephrotoxin.

Saccharomyces cerevisiae variants constituents a heterogenous group that cause food spoilage. Spoilage is an exponential process that results in change of product quality to one with an unacceptable quality loss of high substantial number of spoilage organisms rendering food unfit for consumption within a relatively small period of time (Verghese, 2012). Osmophilic yeasts and xerophilic molds have been implicated associated with high- sugar products. Zygosaccharomyces and related genera tolerate high- sugar products such as jam, honey etc. The growth of growth white or cream patches on food surfaces is indicative of yeast spoilage. Other signs of microbial yeast spoilage include

bubbles in jams and candy and expansion of flexible packages. There are usually more than one microbial species involved in a single spoiled food but, a specific organism known as specific spoilage organism is usually responsible for the production of off compounds that result in off flavours and odours (Legan and Voysey, 1991).

The pathogenic *Escherichia coli* O157:H7 (*E coli* O157:H7) is a useful enterobacteriaceae, that suppresses the growth of harmful bacteria as well as synthesize some vitamins for the body. However, a minor population of ten Enterohemorrhagic *Escherichia coli* are capable of causing foodborne illness (Adams and Moss, 1995; Frazier and Westhoff, 2003; Pundir and Jain, 2011), hence their presence in food is unacceptable. One may experience severe stomach cramps, diarrhea, and bloody diarrhea few hours after eating food contaminated with *E. coli*. The illness may result in complications such as hemolytic Uremic Syndrome (HUS) which is related to kidney failure and hemolytic anemia (Oladapo *et al.*, 2014). Most *E. coli* O157:H7 disease outbreaks were initially associated the consumption of non-acidic high risk foods such as underdone humburger. Subsequent outbreaks have involved undercooked ground beef, raw milk, yoghurt, water, etc. but, there have been instances where some foods with low pH value have been implicated in *E. coli* O157:H7 disease outbreaks. This was possible because, the causative organism have adapted mechanisms that enable it to tolerate acids. Acidic foods such as mayonnaise, apple cider, or mayonnaise-based dressings have already been implicated.

The growth of a microbial population is controlled by some internal (intrinsic) and external (extrinsic) factors existing in and around the food environment. Microbial growth is expedited through the catabolism and anabolism of some food constituent that provides the required energy and cellular resources and substrates. An increase in the number of vegetative bacteria or yeast indicates growth. To increase in growth, one vegetative Bacteria cell asexually splits into two identical cells. But a yeast cell undergoing asexual reproduction produces a small bud that remains attached to the surface of the original cell. The attached bud continues to increase in size and soon produces a bud which looks like a chain of buds on the surface of the parent yeast cell. It's worth noting that, not all cells in a microbial population asexually reproduce at the same time and rate. The doubling or generation time for the entire microbial population species may differ under different conditions; bacteria have the shortest generation time, followed by yeast and molds when favourable or optimum growth conditions are provided. Factors such as storage temperature, acidity, water activity, oxidation-reduction potential and nutrients influence microbial growth rate.

2.5.1 Hurdle technology and the development of microbiological safe product

Based on the knowledge of the mechanisms of food deterioration, food scientists have developed methods of counteracting losses in food safety and quality (Akua, 2012). In chemically preserved food, the preservative effects of the additives combines synergistically with the foods intrinsic properties such as the food's composition, acidity level, moisture content or water activity and the foods extrinsic conditions such as processing time (F and T values) and storage conditions to prevent or minimize change in the foods microbial and physicochemical quality. The application of the

hurdle technology concept has proven very successful in achieving microbial stability and safety and also stabilizing the sensory, nutritive and economic properties of a food product (Leistner and Gorris, 1995). A food product is microbiologically stable and safe because of the existence of preservative factors or hurdles. The varied nature and strength of these preservative factors synergistically act to control the growth or multiplication of spoilage or pathogenic microorganisms because the organisms are unable to jump over all of the existing hurdles. But if high numbers of microorganism are present due to poor hygienic conditions, the usual set of hurdles may not be adequate to prevent spoilage or poisoning (Juvonen *et al.*, 2011).

2.5.1.1 Moisture content and water activity (aw)

Microorganisms need water to grow. Water activity (a_w) measures how much water is present for biological functions. Water in foods are available in two forms, the free and the bound forms. The bound water hydrates hydrophilic molecules and dissolve solutes hence is not a function of water activity (Barbosa-Cánovas *et al.*, 2008). So a food's water activity is the ratio of the food's water vapor pressure which is usually represented by P and is <1; divided by the vapour pressure of pure water (Po) which is 1. Foods with water activity values of less than 0.91 do not support the growth of bacterial cells. Yeast and molds cells will stop multiplying at a_w levels around 0.81 and 0.85. Addition of solutes such as sugar to foods creates osmotic pressure gradient. Much of the water contained in jams is bound by the added sugar and pectin thereby reduces the amount of free water hence the water activity value is raised. Jams with little sugar content or other solute content, results in increased level of free water available for microbial growth hence the faster the jam deteriorates especially when not refrigerated

after opening. A consumer's wish for jam with less sugar may result in a product with compromised shelf life or lose long term microbial stability at room temperature storage condition (Barbosa-Cánovas *et al.*, 2008).

2.5.1.2 Acidity or pH

The acidic nature or pH value of a food product determines what microorganism can survive and grow in the medium. Products with pH value less than 4.5 are natural acidic foods or are acidic because they are fermented are pickled. Acidic foods are prone to spoilage by yeasts, moulds and some acid tolerant bacteria. Pathogenic bacteria may survive, but few are likely to grow. *Clostridium botulinum* may grow and produce toxin in foods with pH greater than 4.5. In a low pH preservative system, the control of pH values is key to ensuring the safety margins (Prescott *et al.*, 2005; Silva *et al.*, 2011).

2.5.1.3 Pasteurization

Pasteurization or commercial sterility is generally achieved when food is heated below 105°C. The nature of foods, the types and numbers of contaminating microorganism determines the degree of heat for effectiveness pasteurization. Commercially sterilized foods including jams, are not sterile but depends on some other preservative factors such high salt, sugar content or acid and cool ambient storage conditions temperatures to make them shelf stable at room temperature (Prescott *et al*., 2005).

2.6 Food Process Modeling

Perrota *et al.* (2004), observed that food and biomaterials often undergo physical, chemical and biological transformation during production process. Many of these transformation has not been characterized or automated due to the extent of large variation amongst the biomaterials, their biological origin, large quantity of water

contained in the biomaterials coupled with the different physical processes the material undergo. Automation of food processes is a challenge because most on line processing variables are not objective measurable (Singh and Yang, 1997). Measuring quality parameters such as food color, odor, taste, appearance, and consistency, maybe bias and highly uncertain; they are largely evaluated qualitatively and described in linguistic words. For a system such as this, the development and use of symbolic instruments or sensors that has the ability to quantify the subjective measurement, taking into consideration, the vagueness of the problem and then executes a numeric-linguistic conversion may solve the challenge in food process automation (Mauris *et al.*, 1994; Shyam *et al.*, 2006). Fuzzy logic and models have features that transform numeric-linguistic reasoning data that converts linguistic deductions back to numerical depiction. Thus, the fuzzy linguistics shows qualitative parts of linguistic figures/concepts through linguistic factors (Zadeh, 1975).

2.6.1 Concept of fuzzy logic reasoning and modeling

Fuzzy rules enable fuzzy systems processes data or information similar to the way human beings would think in processing information. Since fuzzy systems are constructed with fuzzy rules, the system may be used to simulate the human operator's experience. For instance, Kavdir and Guyer (2003) developed an apple grading method with the assistance of a fuzzy logic model. The model was designed to assist in sorting of apples. The model was 89% consistent with results attained from human operator's grading, so can be confidently used to classify according to expected grading standards.

Fuzzy logic and sets is an extended simplification of the conventional multivalued logic. The former enables the processing of linguistic data (Yager and Filev, 1993). The concept of fuzzy logic and fuzzy sets came about when researchers in 1960 recognized the important role uncertainty played in the optimization of system models (Klir and Yuan, 1994). Researchers had at the time challenges with the development of ways to estimate uncertainties of a modeling problem so as reduce complexity and subsequently increase the reliability of the resultant models. According to the fuzzy set theory, (Zadeh, 1965) the membership of an object in the fuzzy set is a matter of the degree to which they belonged (Yager and Filev, 1993). The different shades of truth and false in the fuzzy set theory is similar to human reasoning process and is what is processed to control a system. In constructing a fuzzy system, "if- then" rules are generated from experimental data, or information from authorities with expertise in the subject area are mapped or classified (Guillaume, 2001) using toolbox provided by the MATLAB computing graphical user interface (GUI) tools (Mathworks, 2002). Five GUI tools exist; these include the fuzzy inference system (FIS) editor, the membership function editor, the rule editor, the rule viewer, and the surface viewer.

2.6.2 Membership functions

Membership functions graphically designate the vagueness of a fuzzy set for formation of fuzzy systems. There is no systematic methodology for developing or designing membership functions (Shahin *et al.,* 2000). Membership function is context dependent and may be depicted in several ways to define the subjectivity of the system. Standard membership functions may be triangular, trapezoid, that are commonly used are shown in (Ross, 2004; Taboada *et al.,* 2006).

2.6.3 Fuzzy Rule base

The fuzzy system is constructed with the "IF –THEN" rules; the rules convenes information that is similar to human thinking. The fuzzy rules also known as fuzzy proposition consist of two parts; the antecedent and consequent. The antecedent carries the "IF" conditions of the rules where is the consequent is convened in the "THEN". The conditions in the antecedent need to be satisfied in order to obtain a conclusion from the consequent. Fuzzy proposition may be atomic fuzzy and compound or fuzzy relation; an atomic fuzzy proposition involves one fuzzy statement whilst, the fuzzy relations involves two or more atomic fuzzy propositions connected with "and" and "or". The connectives are respectively fuzzy intersection and fuzzy union functions of the proposition. The compound fuzzy propositions are used to may be used to characterize values of two or more fuzzy variables (Setnes *et al.*, 1998).

2.6.4 Fuzzy Rules Aggregation

The individual fuzzy antecedents of a fuzzy rule system are combined in order to obtain an overall consequent contributed by each of the consequent rule in the fuzzy rule base. The overall consequent may be calculated on the max-min or the conjunctive and disjunctive functions of the rule based system (Ross, 2004).

2.6.5 Fuzzy Inference

The Fuzzy inference engine processes the fuzzy rules in by mapping information contained in the antecedent or input part of the proposition to the consequent or the output part of the rules. The mapping offers foundation from which conclusions are made. There are two types of inference systems; the Mamdani and Sugeno type. These two differ in the way outputs are represented and determined (Jang *et al.*, 1997); in the Mandani Inference system, both the input and output variables are transformed into

fuzzy proposition whilst the output variable is a crisp function in Sugeno type. The Mamdani-type of inference systems are usually preferred (Shyam *et al.,* 2006).

2.6.6 Fuzzification and defuzzification

Fuzzification converts crisp numerical input variable into defined fuzzy linguistic variable. Defuzzification converts a fuzzy variable into a discrete quantity or crisps value (Shyam *et al.,* 2006). Literature has suggested some method for defuzzification fuzzy variables but the most preferred one is the Center- of- gravity or centroid method (Yager and Filev, 1993; Passino and Yurkovich, 1998; Ross, 2004).

2.7 Microbiological challenge test

Microbiological challenge study remains a very important tool for ascertaining the ability of food product formulation to withstand microbial growth or spoilage and to determine what food storage conditions can control microbial growth or spoilage should there be microbial contamination (Ellin, 2007; Food Safety Authority of Ireland, 2011). Food spoilage according to Ellin, (2007) is a complex process that involves a variety of organisms, and several manipulative physical factors like water activity, pH, food matrices, and temperature (storage and processing) that affect microbial growth. Where the outcome of manipulative physical factors on specific pathogenic or spoilage microorganism is unknown, performing challenge testing becomes may assist in evaluating the safety of the product (Food Safety Authority of Ireland, 2011). The Microbial challenge study focus on how likely the microorganism in question will grow in the medium; and the time it takes to initiate growth or how fast the growth is under certain conditions. Challenge study models have been developed for yeast to find out how temperature, pH and sucrose levels in fruit-based drink or alcohol affects their

growth and or survival Evans *et al.*, 2004). Others have employed challenge study to determine the growth rate of Alicyclobacillus in orange juice under the factors such as pH, temperature, soluble solids concentration (°Bx) and nisin levels.

In doing a challenge study, the food product is inoculated with a sufficient level of pertinent microorganism. The inoculated food product is then stored under certain environmental condition for some time then sampled for analysis to ascertain how favourable the product supports growth under the storage conditions (Food Safety Authority of Ireland, 2011). In the nutshell, the study seeks to assess the growth potential (ie the ability of microorganisms to grow in the food) or to estimate the growth factors that influences their survival. The growth potential (δ) is the difference between the $\log_{10}$ cfu/g at the end of the test and the $\log_{10}$cfu/g at the beginning of the test.

2.7.1 Factors affecting challenge studies

According to Vestergaard, (2001), the appropriateness of selected microorganism, the state and quantity of the inoculum, the preparation methods for the inoculum, the inoculation procedure, period of study, the product formulation, the conditions for storage, and how the samples are analyzed and interpreted are some of the factors considered. The data obtained from the challenge study would indicate if a food product requires time and or temperature control for safety.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

Pineapple fruits (Sugar loaf) used for this work were obtained from the Central Market, Kumasi, Ghana. Granulated sugar and pectin powder were sourced from the Central market, Kumasi, Ghana. Food grade citric acid was obtained from Micrite Ventures, Kumasi.

3.1.2 Inoculum preparation

ATCC standard strains of *Escherichia coli* were used. Whereas, yeast cells *Sacharomyces cerevisae* were isolated from Oye palm wine, a locally fermented product brewed and bottled in Ghana by Oye Winery and Bottling Industry, Kumasi. The isolates were suspended in suitable broth and stored for the studies.

3.1.3 Pineapple pulp preparation

Pineapple where purchased in November 2014 and stored in the department's production room. The fruits, on the day of production were washed under potable running water and subsequently soaked for 10 min in Bleache Clorele sodium hypochlorite solution to ensure disinfection. The fruit where then peeled, cut into pieces using stainless steel knives, weighed and pulverized together with sugar, pectin and citric acid using the food blender Binatone 5080 MP, UK at speed 1 for 60 min.

3.1.4 *Package material preparation*

Bottles for packaging were sourced from the Central market, Kumasi, Ghana. The bottles together with its lids were sanitized in 55% Nitric acid and 32% Hydrochloric acid solution. The sanitized packaging materials were then washed with food grade soap, rinsed with copious potable water and then sterilized by autoclaving at 121°C for 15 min.

3.2 Methods

3.2.1 Fuzzy modeling technique

This phase consisted of allocating degree of membership to each variable, creating fuzzy if-then rule base, performing inference operations using expert knowledge to decide on best jam formulation, and defuzzifying outcome in order to obtain crisps parametric output value. In assigning degree of membership, the triangular functions were applied because of its simplicity, ease of computerization and its ability to estimate most of its non-triangular counterparts (Kandel, 1991; Pedrycz and Card, 1992). Sugar, pectin and citric acids were inputs variables 1, 2, 3 respectively. Each input variable had three membership functions of 'low' (L), 'moderate' (M) and 'high' (H) fuzzy sets. Input variable sugar range of 100.00 g to 140.00 g was used. These quantities represent 50 % to 70 % jam's sugar content. Pectin and citric acid used ranged respectively from 20.00 to 30.00g and 0.00 to 4.00g. The output variables pH, degree Brix, percent moisture content and texture were also given three membership functions namely: 'low', 'moderate' and 'high' fuzzy sets. The output pH variables varied between 2.8 to 3.18. The degree Brix output variable values ranged between 2.5 to 8.5 ^{o} Bx; whereas moisture content and texture ranged respectively 46.50 % to 59.35

% and 0.88 to 8.210mJ in the X-axis. The values in the Y-axis represent the degree of membership or degree to which values on the X-axis actually belonged to the membership function sets. Figuares 3.1 and 3.2 shows the shape and fuzzy sets of ‘low’, ‘moderate’ and ‘high’ membership functions for jam input and out variables.

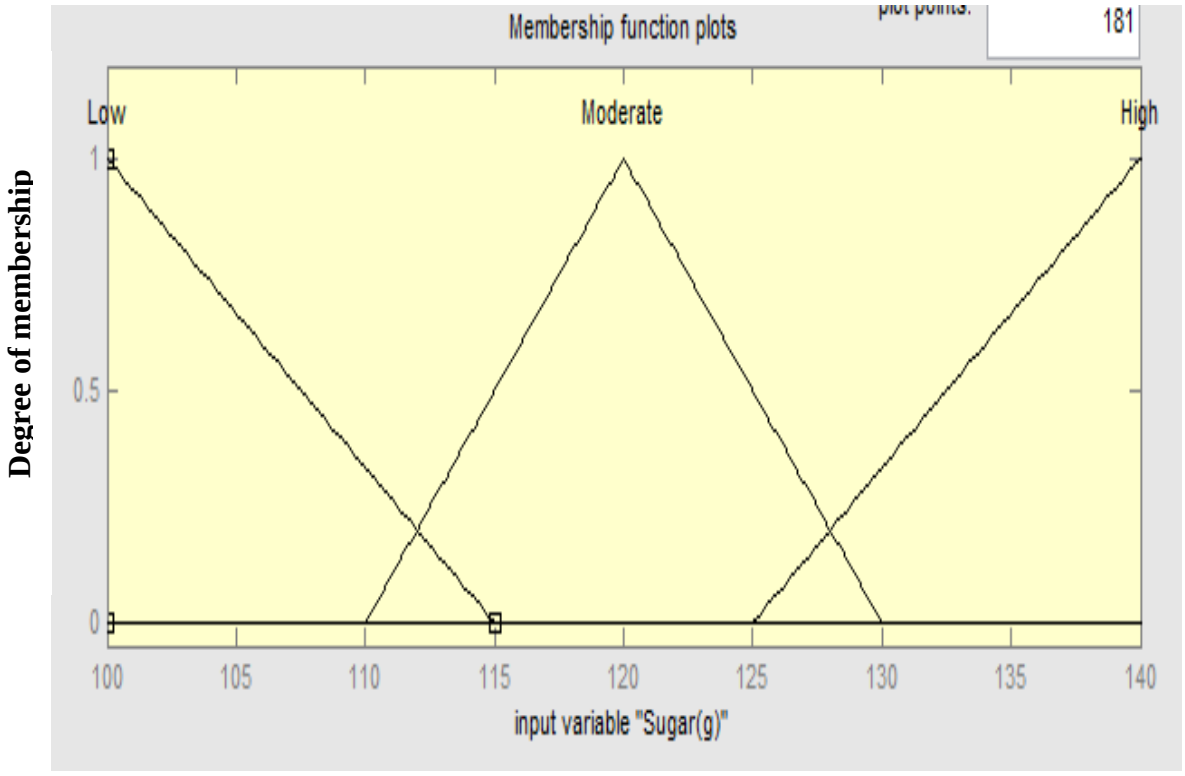

Figure 3.1a: Membership function plots of ‘low’, ‘moderate’ and ‘high’ sugar input per 200.00g pineapple pulp.

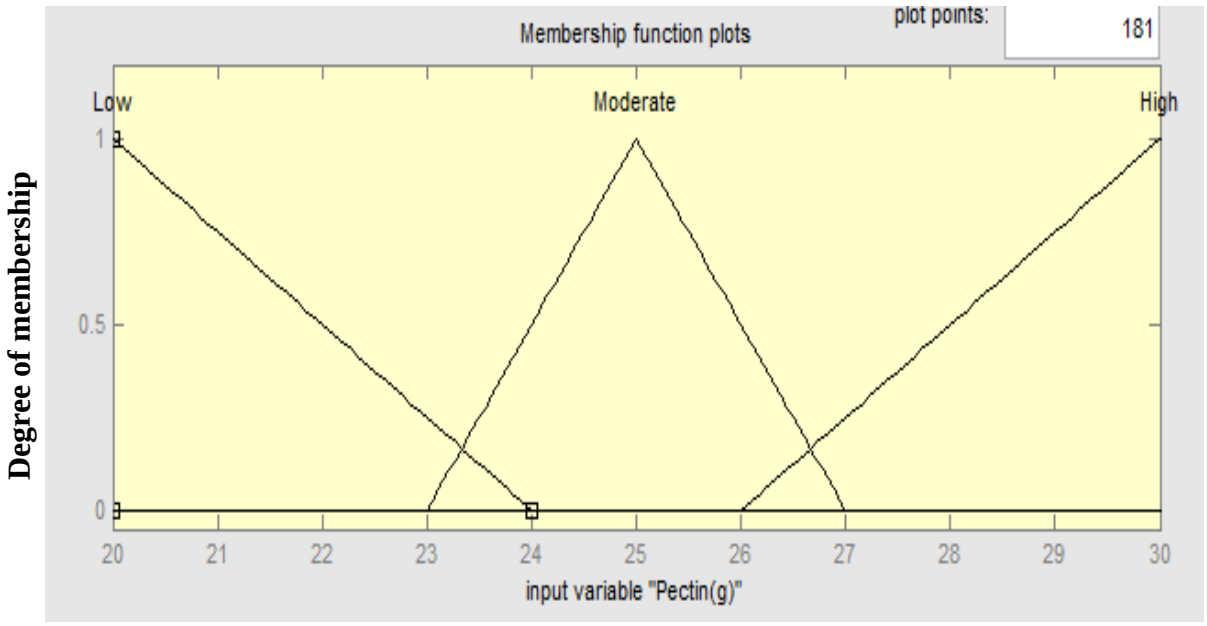

Figure 3.1b: Membership function plots of 'low', 'moderate' and 'high' pectin input

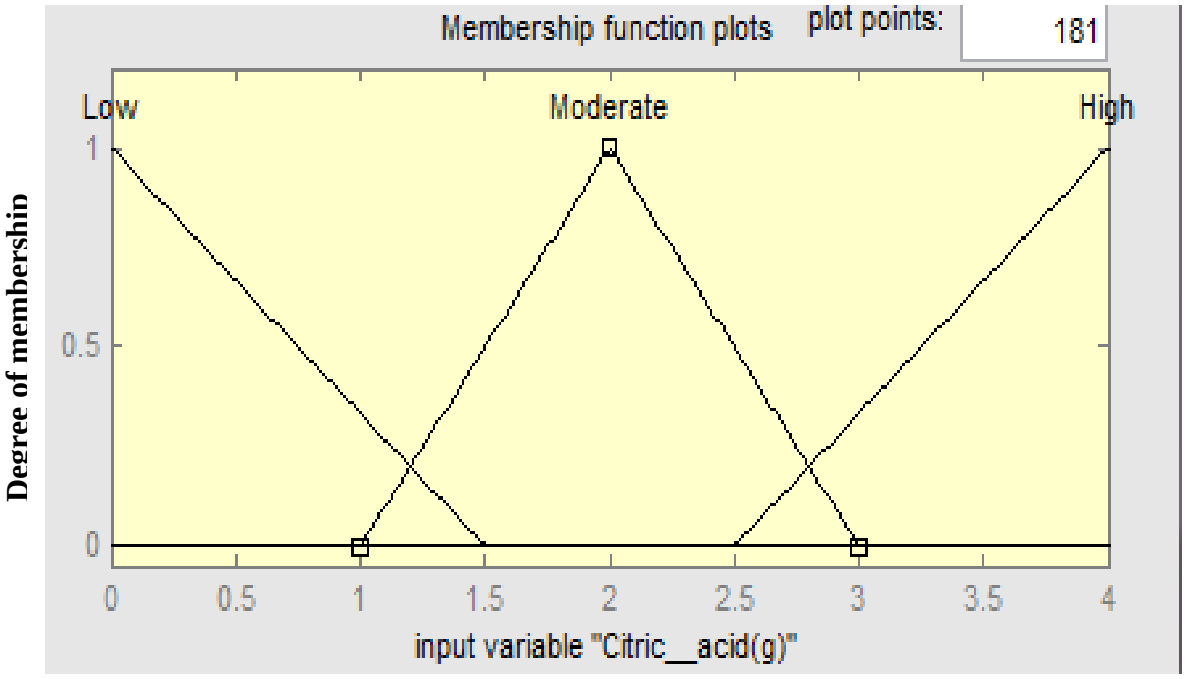

Figure 3.1c: Membership function plots of 'low', 'moderate' and 'high' pectin input per 200.00g pineapple pulp.

3.2.1.1 Formulation Design

In order to set the fuzzy formulation design, low (L), moderate(M), and high(H) linguistics sets of the three input formulation variables; sugar, pectin and citric acid were statistically varied using Design- Expert (2007) to give twenty seven formulations as shown in table 3.1 below. According to the formulation schedule and particular experimental run, a quantity of sugar, pectin and citric acid were weighed and added to 200.00 g of the cut pineapple and then homogenized in a blender beginning at speed 1 and quickening to speed 2 for 60 min till a smooth paste was attained. About 70.00 g of the obtained paste were scoped into the previously sterilized transparent glass jars covered and then cooked in a water bathe at temperature of 95°C for 20 min. The cooked jam was then cooled at ambient room temperature for 24 hours, labeled and then analyzed for its response with respect to physicochemical quality pH, Brix, moisture content and texture.

Table 3.1a: Table indicating precise quantity of sugar, pectin and citric acid used in each pineapple jam formulation.

		Input Formulations variable		
Runs	**Formulation**	**Sugar (g)**	**Pectin(g)**	**Citric acid(g)**
1	LLL	100.70	20.00	0.00
2	LLM	105.00	21.12	1.03
3	LLH	110.23	22.15	4.00
4	LML	115.03	23.28	1.45
5	LMM	112.82	23.50	1.36
6	LMH	102.74	25.15	3.70
7	LHL	106.10	28.60	1.12
8	LHM	104.00	29.00	2.05
9	LHH	108.00	30.00	3.50
10	MLL	130.00	24.00	1.42
11	MLM	104.00	21.84	2.40
12	MLH	124.00	20.60	2.54
13	MML	128.01	26.50	1.20
14	MMM	121.45	25.00	2.00
15	MMH	123.00	26.70	2.82
16	MHL	117.10	24.40	0.92
17	MHM	121.64	29.10	1.62
18	MHH	122.50	27.10	3.26
19	HLL	140.00	20.00	0.00
20	HLM	139.00	20.50	1.75
21	HLH	134.40	21.10	3.92
22	HML	129.03	25.10	1.30
23	HMM	132.02	23.14	1.25
24	HMH	130.42	25.50	3.00
25	HHL	135.02	27.50	0.54
26	HHM	125.00	28.00	2.55
27	HHH	137.04	26.50	2.75

Table 3.2b: A table assigning fuzzy linguistic terms of the crisp quantity of sugar, pectin and citric acid used in each pineapple jam formulation.

		Input Formulations variable		
Runs	**Formulation**	**Sugar (g)**	**Pectin(g)**	**Citric acid (g)**
1	LLL	Low	Low	Low
2	LLM	Low	Low	Moderate
3	LLH	Low	Low	High
4	LML	Low	Moderate	Low
5	LMM	Low	Moderate	Moderate
6	LMH	Low	Moderate	High
7	LHL	Low	High	Low
8	LHM	Low	High	Moderate
9	LHH	Low	High	High
10	MLL	Moderate	Low	Low
11	MLM	Moderate	Low	Moderate
12	MLH	Moderate	Low	High
13	MML	Moderate	Moderate	Low
14	MMM	Moderate	Moderate	Moderate
15	MMH	Moderate	Moderate	High
16	MHL	Moderate	High	Low
17	MHM	Moderate	High	Moderate
18	MHH	Moderate	High	High
19	HLL	High	Low	Low
20	HLM	High	Low	Moderate
21	HLH	High	Low	High
22	HML	High	Moderate	Low
23	HMM	High	Moderate	Moderate
24	HMH	High	Moderate	High
25	HHL	High	High	Low
26	HHM	High	High	Moderate
27	HHH	High	High	High

3.2.2 Evaluation of pineapple jam

Four quality characteristics of the resulted jam were measured. Data of the measured jam output variable were fuzzy hence organized into low, medium and high fuzzy sets for processing as indicated by Table 3.2. The pineapple jam were analyzed for pH, total soluble solids expressed as degree brix, texture (mJ) and moisture content according to the manual for analysis of fruits and vegetable product (ISO 13815: 1993 / ISO 2173: 1978). The pH was measured using Mettler Toledo pH meter (FE20; GB). Total soluble solids expressed as degree Brix was measured using Reichert AR200, digital refractometer at 20°C. Texture analysis was carried out using CT3 Texture analyzer by Brookfield. Texture analysis was done on the basis of Trigger, Deformation and Speed parameters of 0.5 g, 10.0 mm, and 10.0 ms^{-1} respectively yielding responses of peak load, deformation at peak, work and final load based on the Normal Test of the CT3 texture Analyzer. A standard test compresses test sample ones then back to start mode.

Moisture was determined by thermogravimetric method based on the weight loss of mass as described by AOAC, 2005. Figuare 3.2 below shows the shape and fuzzy sets of low, medium and high membership functions for jam output variables pH, Brix, % moisture content and texture.

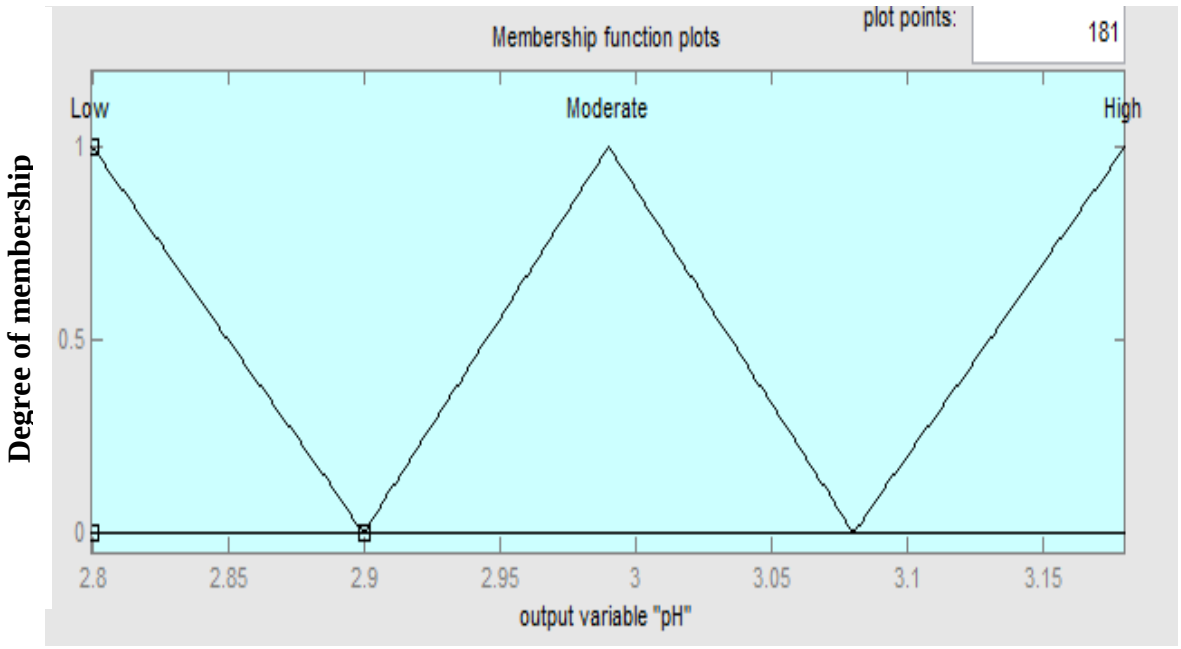

Figure 3.2a: Membership function plots of 'low', 'moderate' and 'high' jam pH output

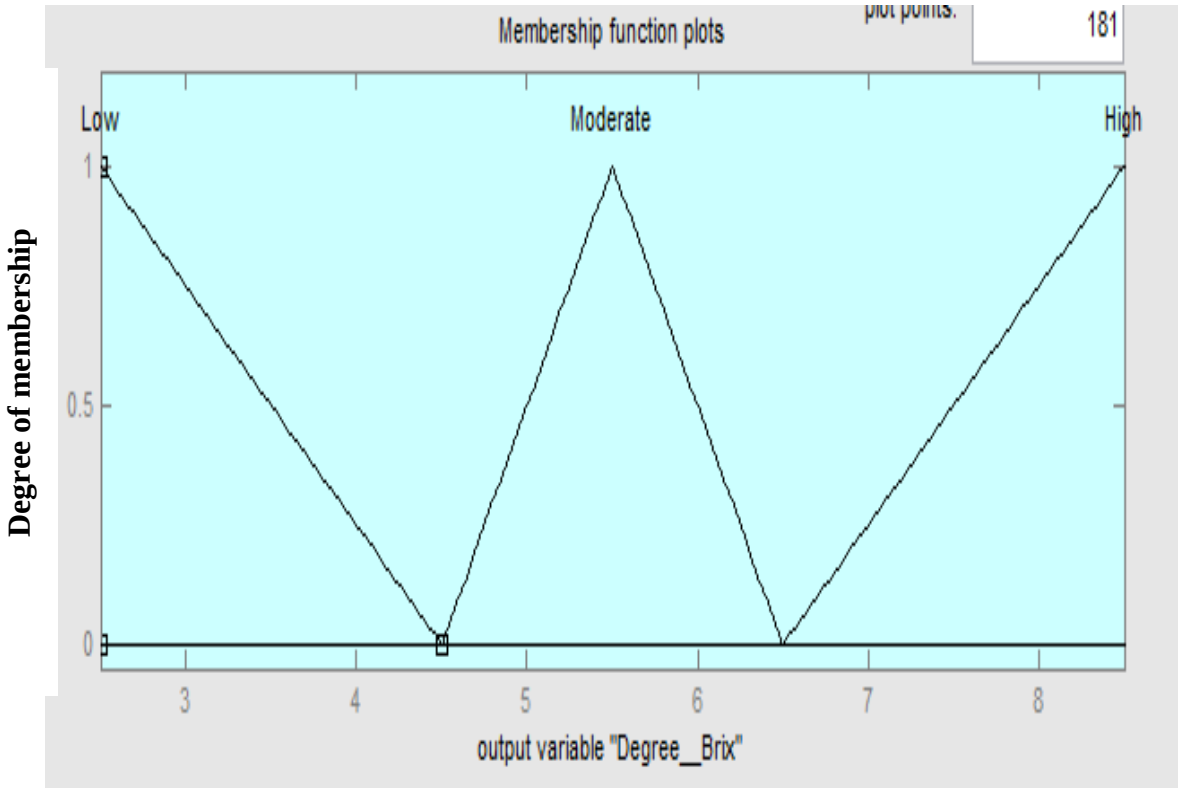

Figure 3.2b: Membership function plots of 'low', 'moderate' and 'high' jam degree Brix output

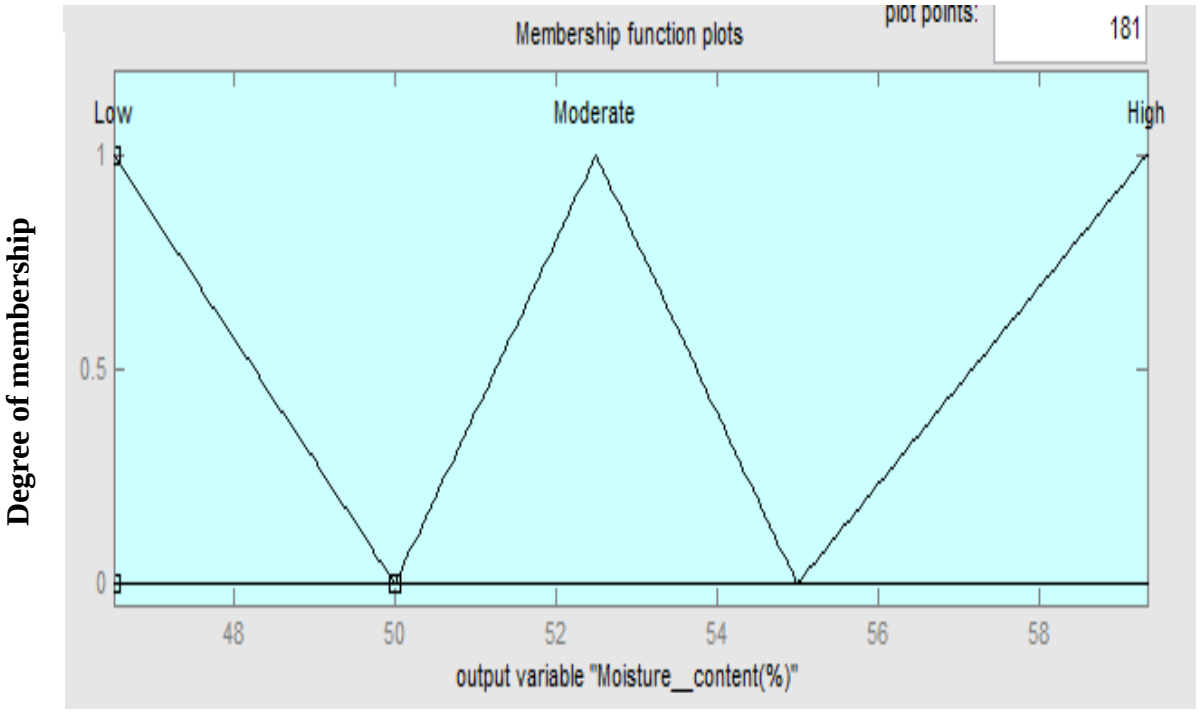

Figure 3.2c: Membership function plots of 'low', 'moderate' and 'high' jam degree Brix output

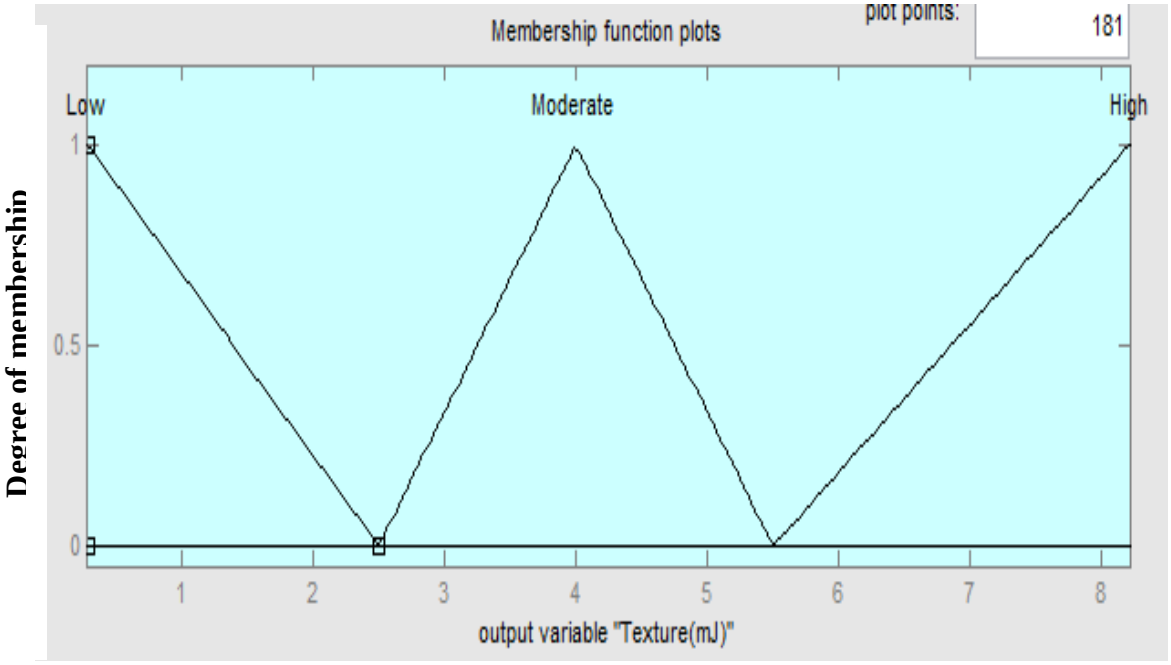

Figure 3.2d: Membership function plots of 'low', 'moderate' and 'high' jam degree Brix output

3.2.3 Formation of If-then rule statements and design of inference engine

Premised on the input and output data obtained from the experiment, a total of twenty seven fuzzy compound propositions were formulated for analysis as indicated in table 3.2 below. The fuzzy conjunction inference or Mamdani inference was used to relate the output part of the rule base in order to obtain a crisp value. The relationship in the Mamdani inference system is symmetric hence can be reversed and was in the form. Inferring for the best jam formulation, experts such as Morris *et al*. (1980) and Borne (2013) established that, jams formulated with high sugar content such as containing 45 parts fruits to 55 parts sugar were graded as standard and with pectin and citric acids as low as 0.5% were found to be the optimum formula for jam containing 45% pineapple. This formulation was found to pass acceptability test with respect to taste, colour, consistency and spreadability. So, performing the inference operations on the fuzzy rules, "If sugar is high, pectin is low and citric acid is low", the resultant physicochemical outputs for jam were high pH with crisps value of 3.15, medium brix of value 5.5^{0}Bx, low moisture content 0f 47.6% and medium texture or firmness of 4.000mJ. But since, pectin content of sugar loaf pineapple was negligible and acid content too low to set gel, the inference engine was then adjusted and simulated on the fuzzy proposition rule, "If sugar is high, pectin is medium and citric acid is high". The resulting quality output for the jam formulation were found to be of a lower pH value of 2.83, higher brix content of 7.84^{o}Bx and unchanged jam texture or firmness of medium quality of 4.000mJ and medium moisture content but a higher moisture content value of 52.5% .

Table 3.2: A table of "if- then" rules generated from the input and output data of experiment to relate the antecedent and consequent of fuzzy proposition

Run	Formulation	Input formulation variables			Jam Output variables			
		Sugar (g)	Pectin (g)	Citric Acid (g)	pH	Brix/ ºBx	% moisture content	Texture/ mJ
1.	LLL	Low	Low	Low	High	Moderate	High	Low
2.	LLM	Low	Low	Moderate	High	Moderate	High	Low
3.	LLH	Low	Low	High	Moderate	Moderate	High	Low
4.	LML	Low	Moderate	Low	High	Low	Moderate	Low
5.	LMM	Low	Moderate	Moderate	Moderate	Low	High	Low
6.	LMH	Low	Moderate	High	Moderate	Low	High	Low
7.	LHL	Low	High	Low	Moderate	Low	High	Moderate
8.	LHM	Low	High	Moderate	Moderate	Moderate	Moderate	High
9.	LHH	Low	High	High	Moderate	Moderate	Moderate	High
10	MLL	Moderate	Low	Low	Moderate	Moderate	Moderate	Low
11	MLM	Moderate	Low	Moderate	Moderate	High	High	Low
12	MLH	Moderate	Low	High	High	Moderate	Moderate	Low
13	MML	Moderate	Moderate	Low	High	Low	Moderate	Moderate
14	MMM	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	High
15	MMH	Moderate	Moderate	High	Moderate	Moderate	Moderate	Moderate
16	MHL	Moderate	High	Low	High	Low	Moderate	High
17	MHM	Moderate	High	Moderate	Moderate	Moderate	Moderate	Moderate
18	MHH	Moderate	High	High	Moderate	Low	Moderate	High
19	HLL	High	Low	Low	High	Moderate	Low	Moderate
20	HLM	High	Low	Moderate	Moderate	Low	Moderate	Moderate
21	HLH	High	Low	High	Moderate	Low	Moderate	Low
22	HML	High	Moderate	Low	High	Low	Moderate	Moderate
23	HMM	High	Moderate	Moderate	High	Low	Low	Moderate
24	HMH	High	Moderate	High	Low	High	Moderate	Moderate
25	HHL	High	High	Low	High	Moderate	Moderate	Low
26	HHM	High	High	Moderate	Moderate	Moderate	Low	High
27	HHH	High	High	High	Moderate	Low	Moderate	High

3.2.4 Microbiological challenge testing

Jam with high sugar content of 130.42g, medium pectin 25.50g and high citric acid of 3.00g were formulated and challenged with $4.7x10^5$ cfu/ml spoilage yeast, *Sacharomyces cerevisae* and $4.03x10^5$ cfu/ml ATCC standard strains of *Escherichia coli*. The challenged samples were then stored for fourteen days at refrigerator temperature storage conditions of 4ºC and at room temperature storage conditions average of 25ºC. A total of 70.00gx64 jam samples were obtained, inoculated and stored for the studies. Two samples (70.00g x2) from each storage conditions and inoculated group were drawn at two days' interval and prepared for microbial load enumeration.

3.2.5 Sacharomyces cerevisae *and* Escherichia coli *load determination*

1.00g of challenged jam samples was mixed with 1ml of distilled water to obtain a uniform mixture. Six test tubes were prepared and labeled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Each of the tubes were filled with 9ml of Ringers solution. 1ml of the uniform mixture was then added to the tube labeled 10^{-1}. To obtain dilution for 10^{-2}, 1ml of mixed solution from test tube 10^{-1} was transferred into the test tube labelled 10^{-2}. The 10^{-3} dilution was successively prepared by taking 1ml of the 10^{-2} dilution solution into 10^{-3} labeled tube and the remainders of the serial dilutions were likewise prepared. By means of sterile or new pipette tips, 1ml each of the six dilutions was carefully added to six test tubes containing molten plate agar at 45^0C and thoroughly mixed by gently revolving between the two palms. The mixed agar was then carefully and gently poured into cleaned petric dishes labeled 10^{-1} to 10^{-6}. The agar was left to harden, and then

incubated at inverted position at 37ºC for 24 h for growth. Labline Electronic Colony counter by Princess Street, Mumbai was used to count the colonies.

3.2.6 Analysis

Data obtained from the microbiological challenge studies were analyzed by plotting the polynomial regression using the statgraphics centurion statistical tool. The data fitted to models were deemed adequate where the largest R-squared statistics interpreted as the percentage of the variability in response variable which has been explained by the model was significant at $P<0.05$. Such models were subsequently chosen to explain trend of microbial colony counts.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.2 Physicochemical quality of pineapple jam

Changing the concentrations of sugar, pectin and citric acid affected the quality features of the formulated pineapple jam. Each jam sample was evaluated for pH, total soluble content as brix, percent moisture content and texture output. The physicochemical attributes of the pineapple jam measured are presented in table 4.1. The data revealed that, the pH of the resultant jam formulations ranged from 2.88 to 3.18. Brix and moisture content ranged from 2.8 ^{o}Bx to 7.2 ^{o}Bx and 46.57% to 59.35% respectively, whereas texture which measures jam firmness, varied between 0.206mJ and 8.206mJ as depicted in Table 4.1 below.

4.1.2.1 Jam moisture content

The moisture content of jam generally reduced as the sugar and pectin input concentrations increased as depicted by figures 4.1a, 4.1b, and 4.1c below but the jam's moisture content fluctuated insignificantly around 52.499% with increasing concentration of citric acid. When sugar concentration of around 130g to 140g where combined with pectin concentrations of 24.0g to 30.0g, the jam moisture content dropped to below 50.0% but fluctuated around 48% to 52% when the same amount of sugar was combined with around 1.5g and 3.0g citric acid.

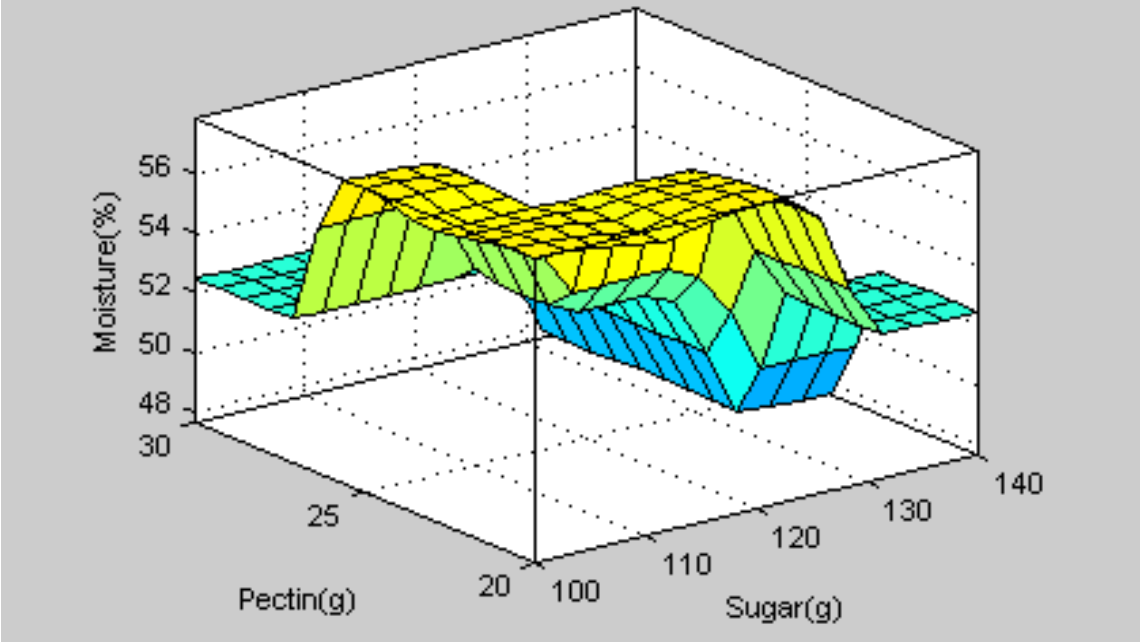

Figure 4.1a: Surface plot showing effect of sugar and pectin on jam output quality moisture content

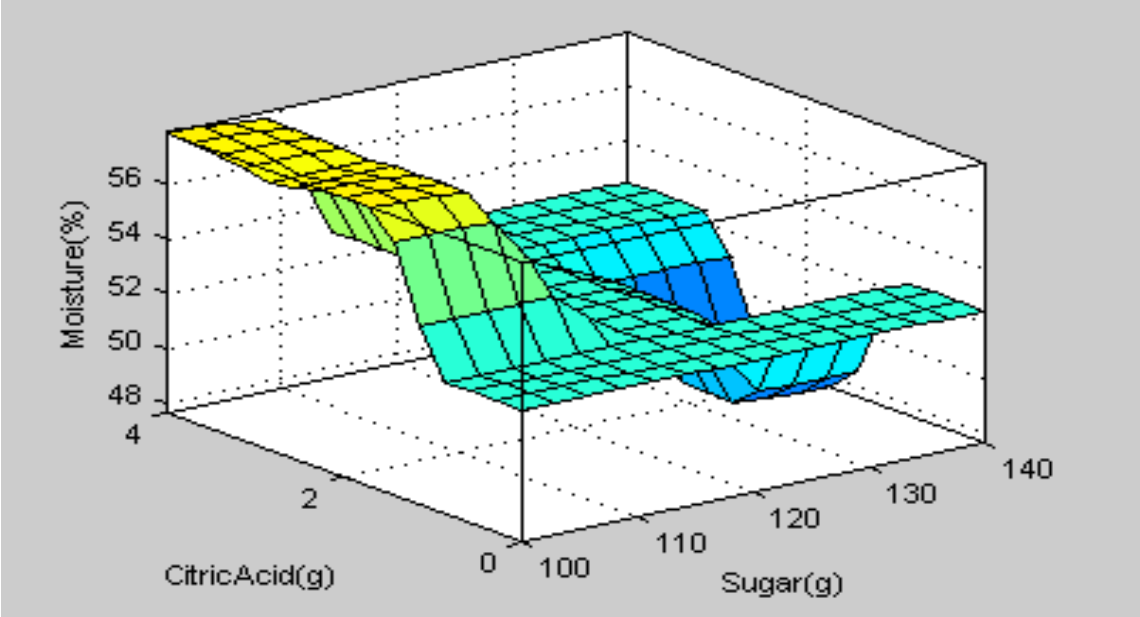

Figure 4.1b: Surface plot showing effect of sugar and citric acid on jam output quality moisture content

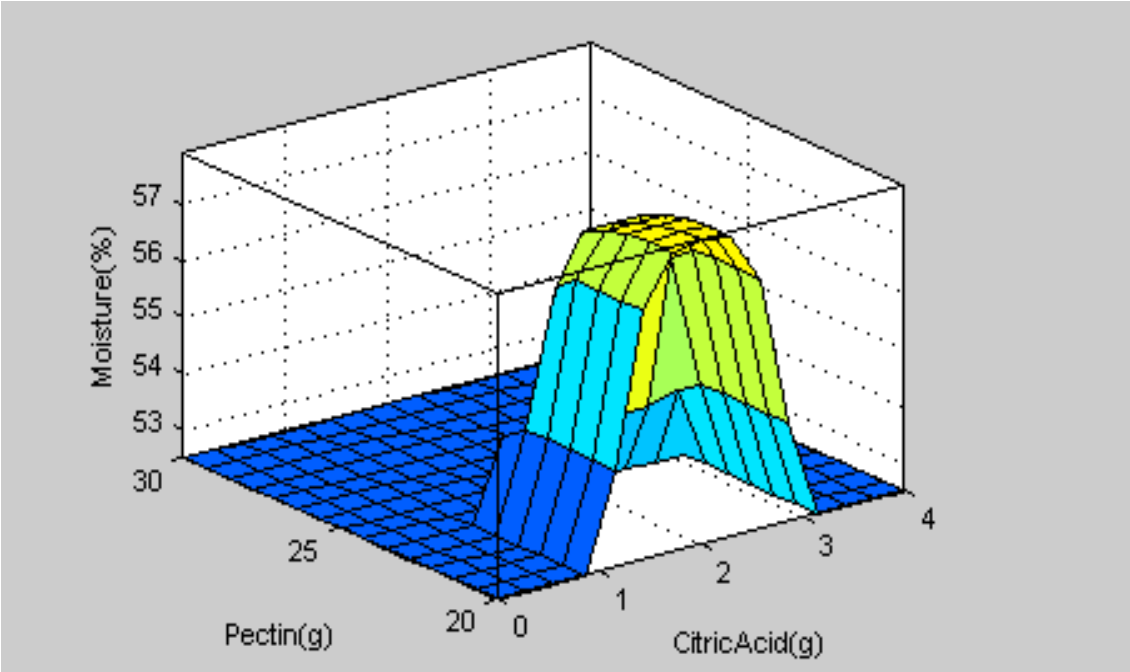

Figure 4.1c: Surface plot showing effect of sugar and citric acid on jam output quality moisture content

4.1.1.2 Jam pH quality

The jam's pH values were raised as jam's input sugar increased but pH decreased with increasing citric acid input. Pectin did not cause significant change in jam's pH; the pH value remained constantly at 2.99 when citric acid input increased. However, the jam's pH values raised and started peaking from 3.02 and 3.14 when sugar and pectin where combined at two levels. These levels are respectively 100.0g to 110.0g sugar verses 20.0g to 23.5g pectin and sugar of 132.5g to 140.0g against pectin of 24.0g to 26.0g pectin. Sugar did not affect jam's pH when combined with citric acid; but jam's pH continues to rise from a pH value of 3.0.

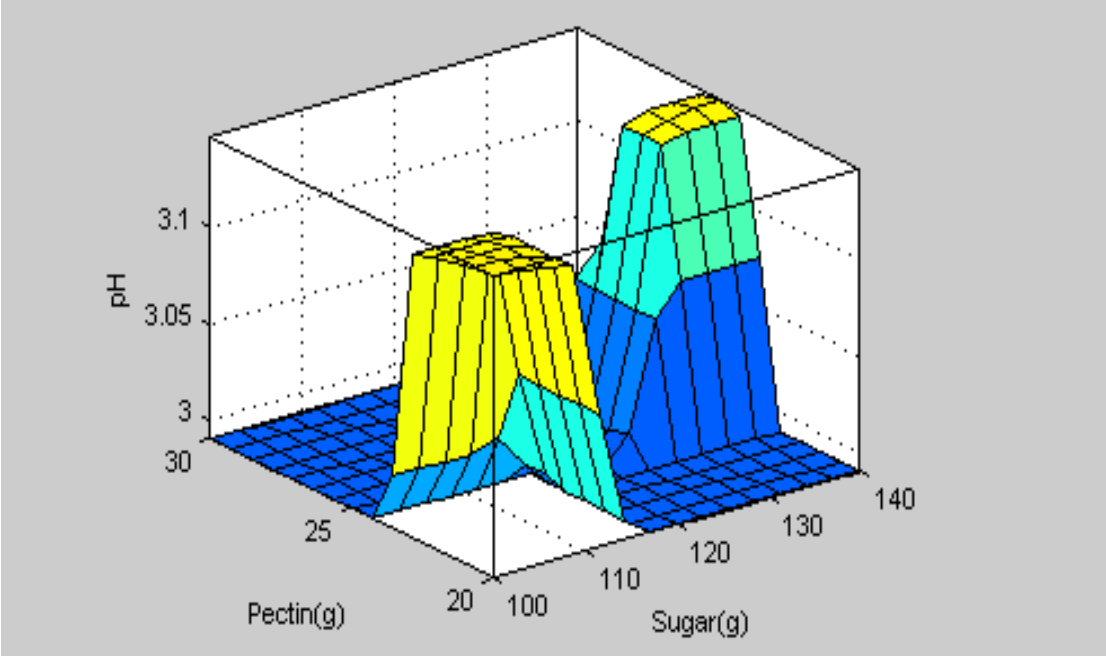

Figure 4.2a: Surface plot showing effect of sugar and pectin on jam's pH quality

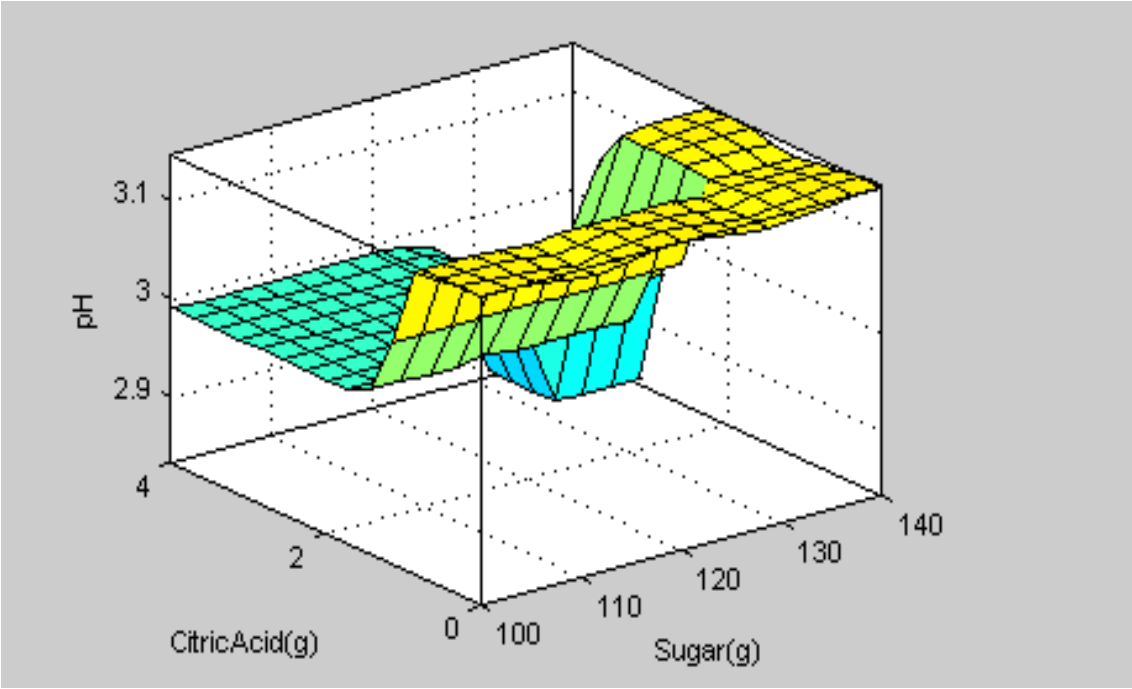

Figure 4.2b: Surface plot showing effect of sugar and citric acid on jam's pH quality

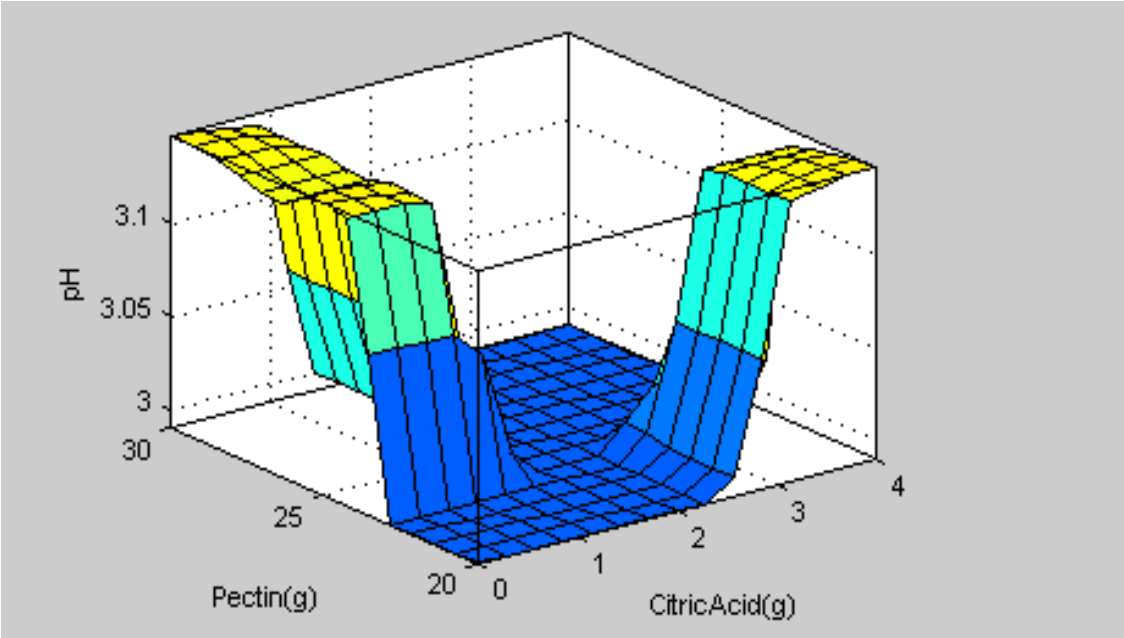

Figure 4.2c: Surface plot showing effect of citric acid and pectin on jam's pH quality

4.1.1.3 Pineapple jams texture quality

Sugar, pectin and citric acid effects on jam's texture quality were similar; the jam texture continued to increase till 50% of the upper range of input sugar, pectin and citric was reached after which they begun to drop. The thickest gel formed at three different combined sugar and pectin levels of 100.00g to 112.5g sugar against 27.0 g to 30.0 g of pectin; 117.5g to125.0 g sugar verses 24.0g to 26.0g pectin and 132.5g to 140.0 g sugar against 27.0g to 30.0 g pectin. Whilst, the thickest jam formulation was at sugar of 117.5g to 125.0g against 1.5g to 2.5g citric acid.

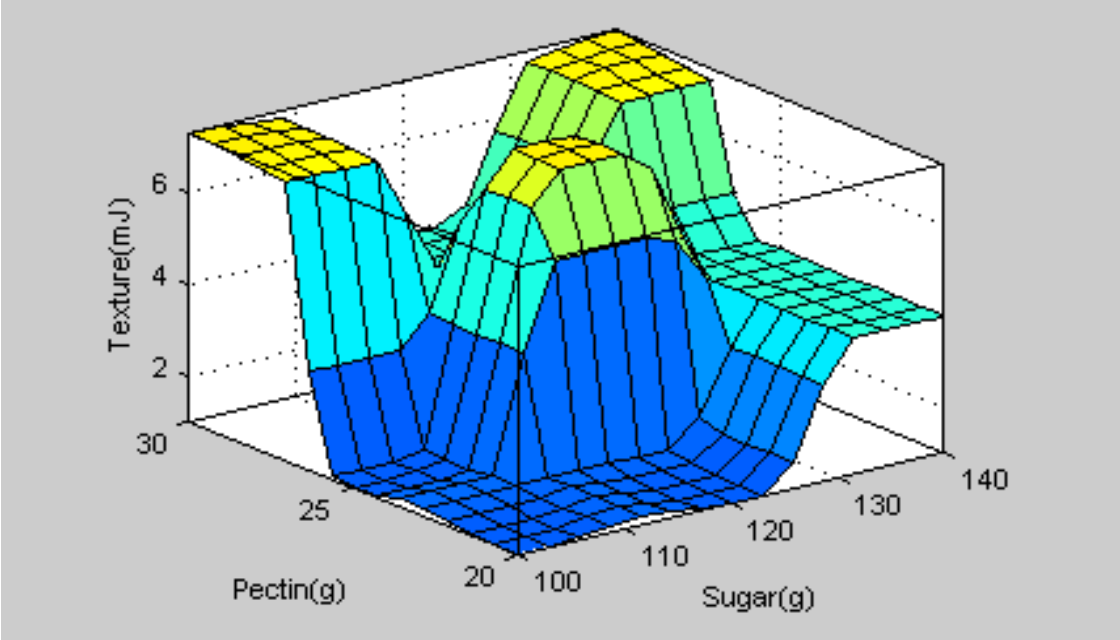

Figure 4.3a: Surface plot showing effect of sugar and pectin on jam's texture quality

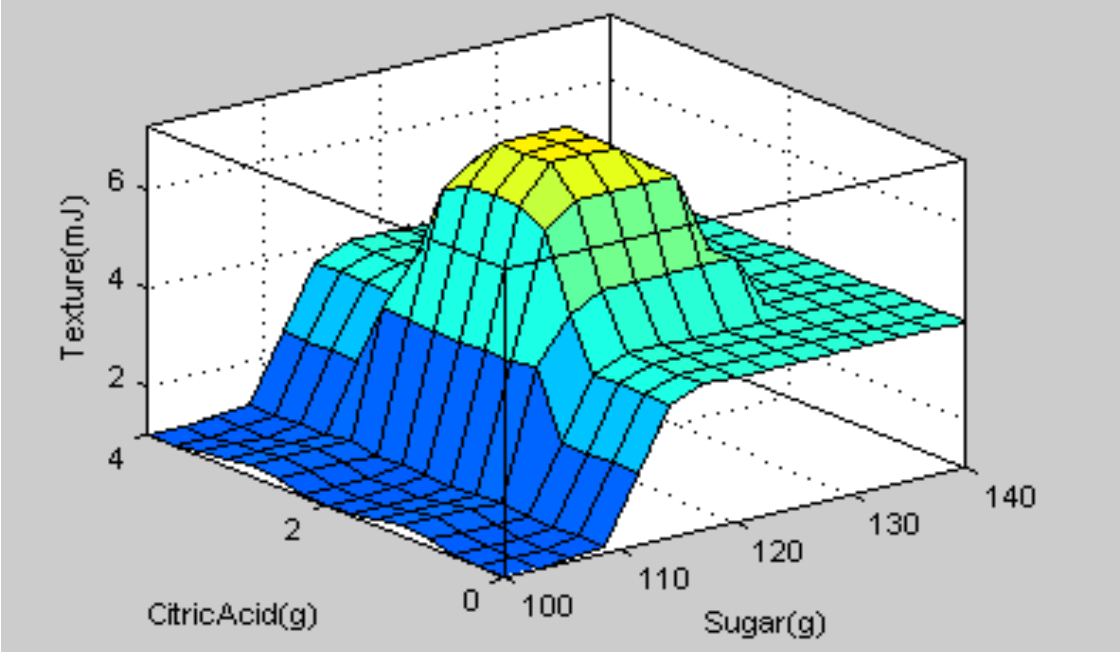

Figure 4.3b: Surface plot showing effect of sugar and citric acid on jam's texture quality

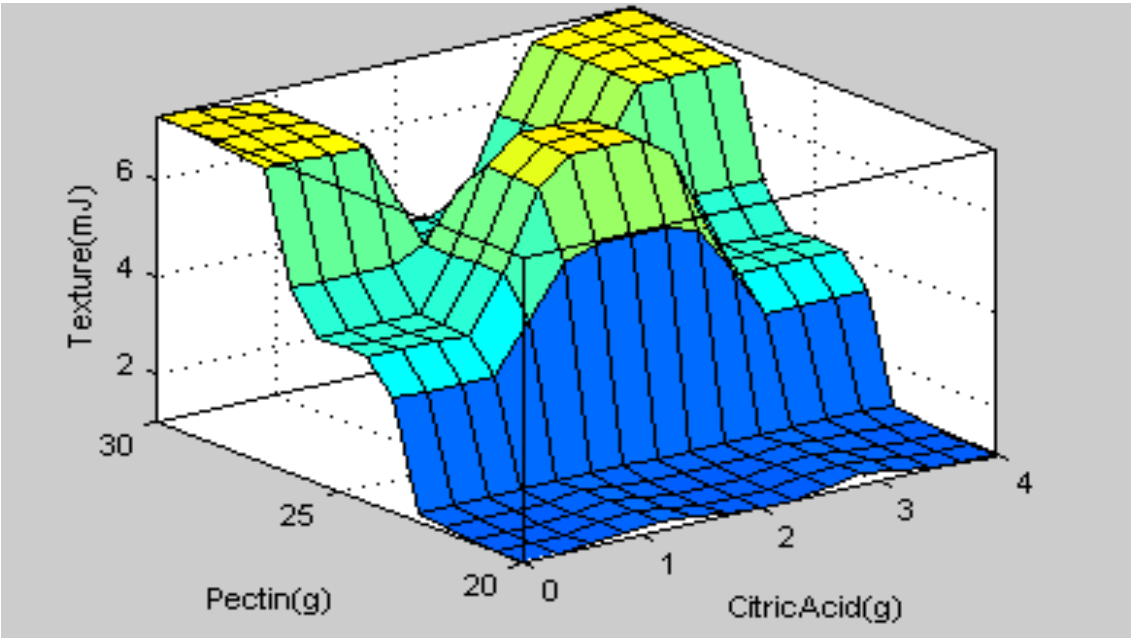

Figure 4.3c: Surface plot showing effect of citric acid and pectin on jam's texture quality

4.1.1.4 Pineapple jams brix quality

Increasing pectin input reduced jam's brix from the highest value of 7.2 ^{o}Bx till it plateau around 5.5 ^{o}Bx. On the other hand, increasing the jam's citric acid generally raised Brix till it plateau around 5.5 ^{o}Bx. The jam's brix value was observed increase with increasing sugar input till it plateau around 5.5 ^{o}Bx when it begun to drop. But the highest brix quality was achieved when sugar of range 117.5g to 125.0g combined with 20.0g to 23.6g of pectin whereas, higher sugar range of 132.5g to 140.0g required high citric acid rang of 3.00g to 4.00 to form jam of high brix quality.

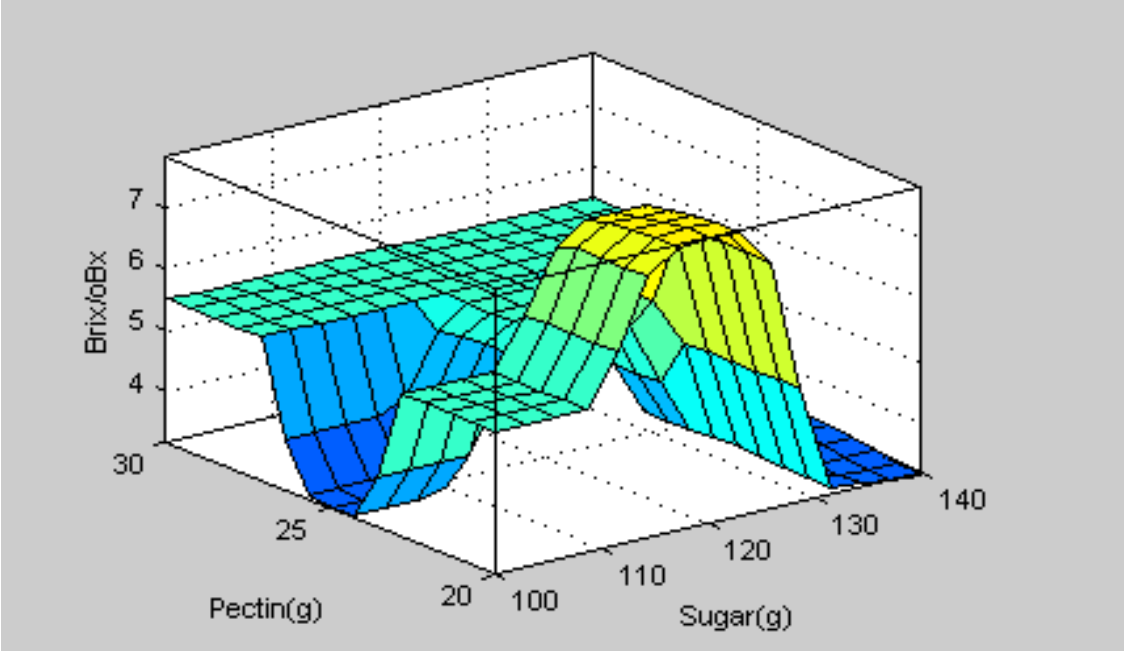

Figure 4.4a: Surface plot showing how of sugar and pectin affects brix quality of jam.

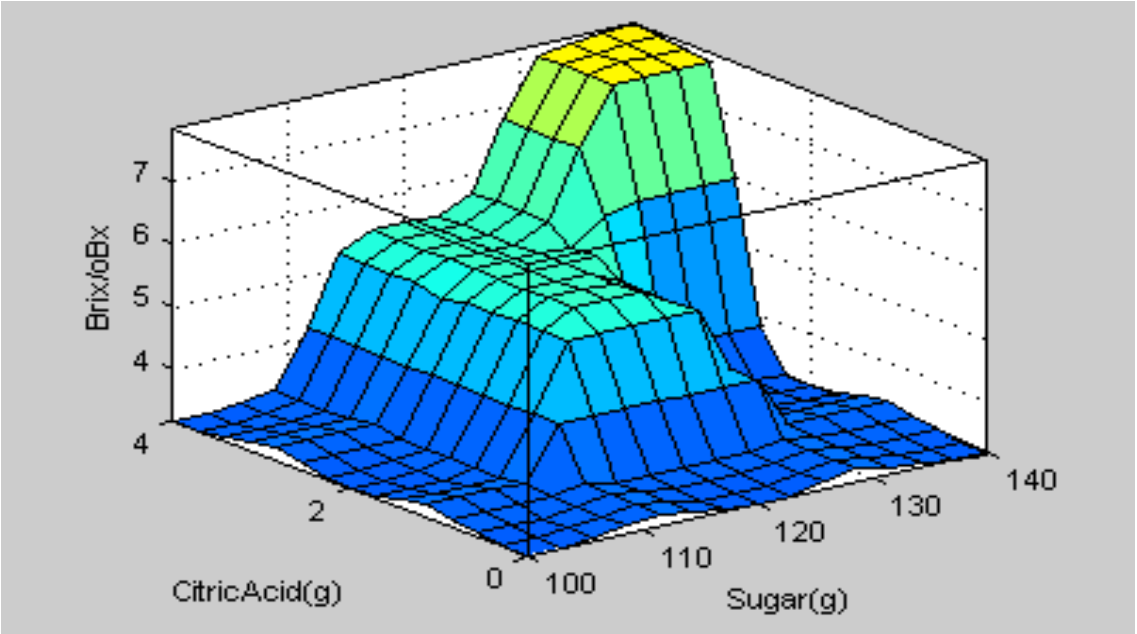

Figure 4.4b: Surface plot showing how sugar and citric acid affects brix quality of jam

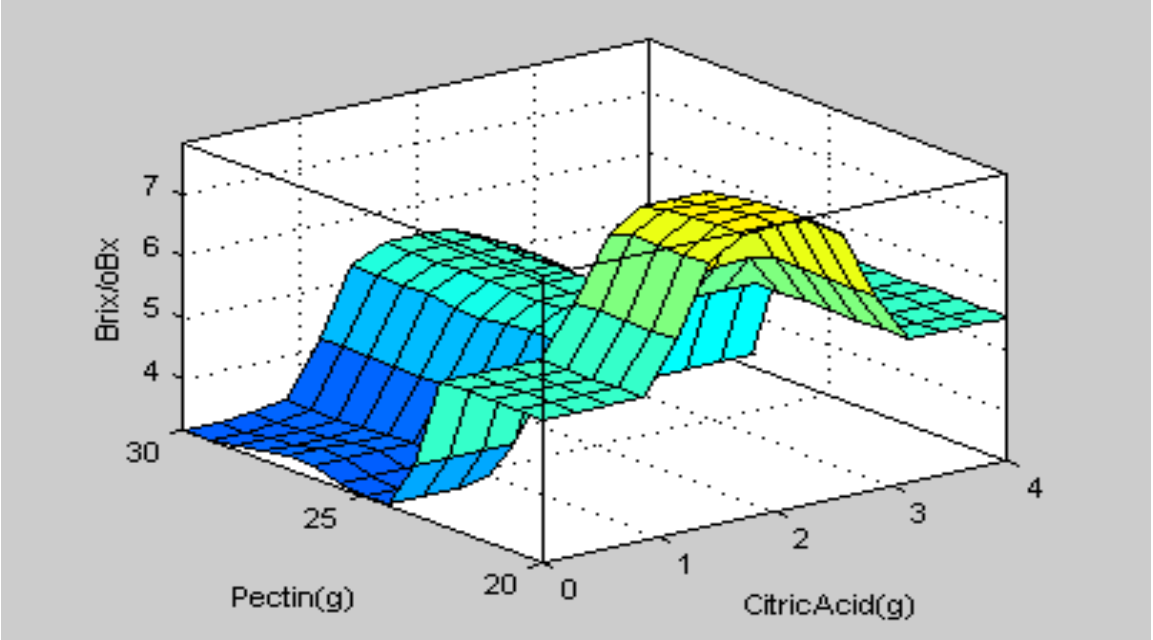

Figure 4.4c: Surface plot showing how sugar and citric acid affects brix quality of jam

4.1.2 Microbiological challenge study test

4.1.2.1 Yeast cells in jam samples stored at room temperature conditions

The number of colony forming units of spoilage yeast cells enumerated in the challenged jam samples stored for fourteen (14) days under room temperature condition at P= 0.021 and with R^2 value of 89.1973% followed a polynomial curve of the order 3: thus, $Y = 5.72227 + 0.268731X - 0.0425511X^2 + 0.00172239X^3$; where X= days in storage and Y= yeast cell colony forming unit per ml enumerated at ambient room storage temperature. The yeast cells number enumerated in jam samples stored at this temperature, proliferated drastically from an initial inoculation level of 4.70×10^5 cfu/ml on the day of inoculation to 1.63×10^6 cfu/ml on day 2.

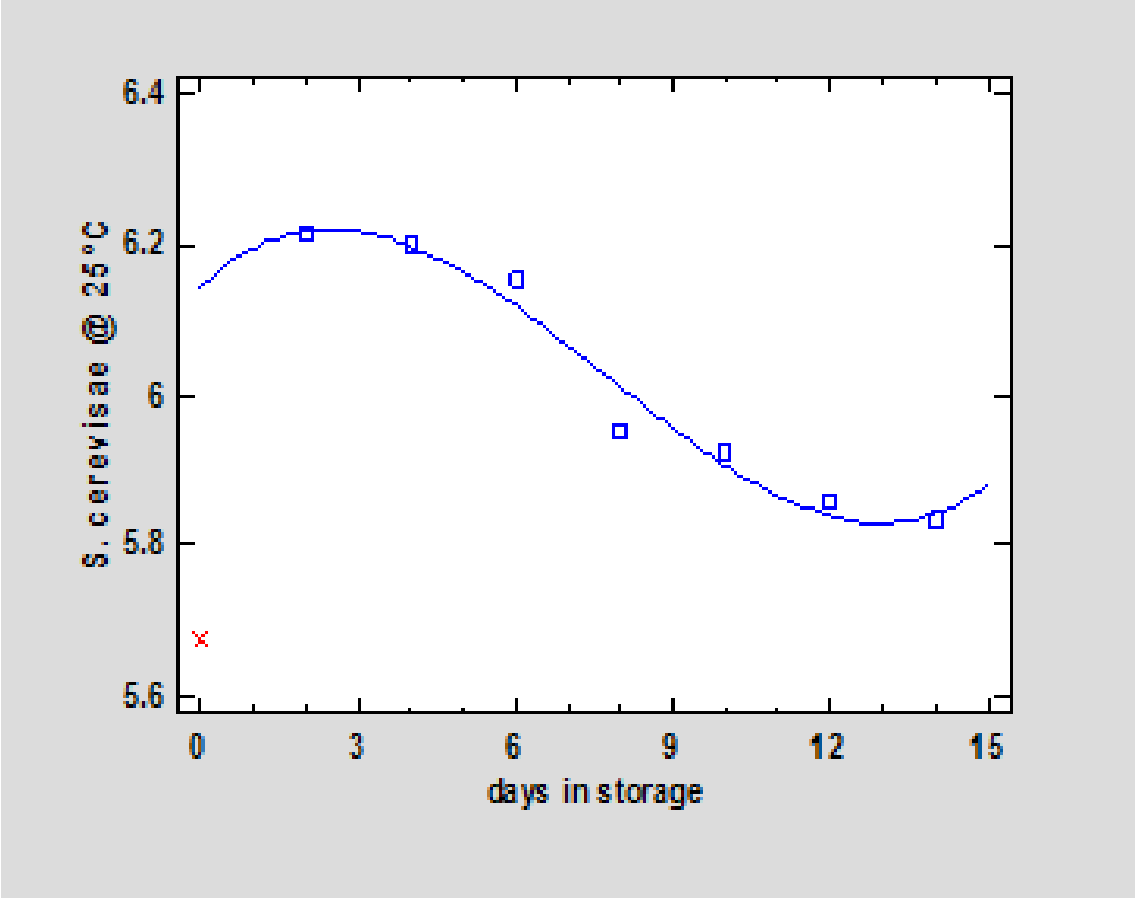

Figure 4.5: Log10 cfu/ml of yeast cells in jam samples stored at room temperature (25°C) storage conditions for 14 days

The increment which was from 470,000 to 1,630,000cfu/ml in two days represents a change of 246.81% change. After day 2, the rate of increase started to drop gently to $7.20x10^5$cfu/ml on day 12 where the log curve began to rise again but at a very slow rate.

4.1.2.2 Yeast cells counted in jam samples stored at refrigerator temperature conditions

The yeast cells enumerated in the challenged jam samples stored under refrigerator condition behaved differently. The yeast cells increased from a count of 4.70 $x10^5$ cfu/ml on day 0 to $1.17x10^6$ cfu/ml on day 2 then dropped to 1.21 $x10^5$ cfu/ml on day 4.

The yeast cells continued to drop tailoring off as indicated by Figure 4.6. The rate of decline followed a Reciprocal-Y Squared-X curve at P= 0.003and R^2 value of 90.71% with the equation ($Y = 1/ (0.176283 + 0.000454516X^2$; where X= days in storage; Y= yeast cells enumerated in jam stored at refrigerator temperature).

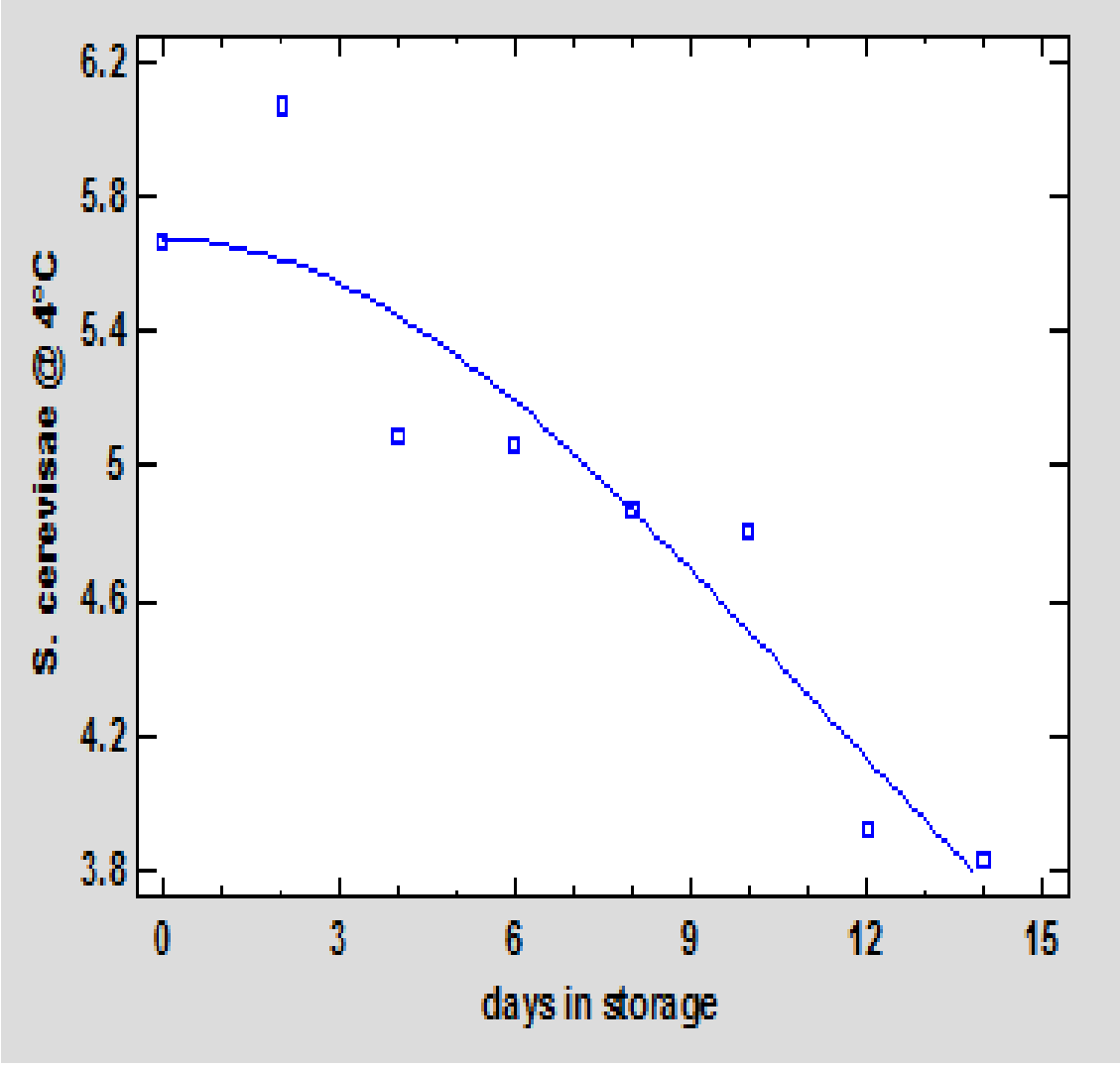

Figure 4. 6 log 10 of yeast cells counts in jam samples stored at refrigerator temperature (4°C) storage condition for 14 days

However, comparing the general rate at which the yeast cells declined under both storage temperatures, it can be deduced that, the yeast cells declined significantly faster in the jam samples stored under the refrigerator (4°C) temperature.

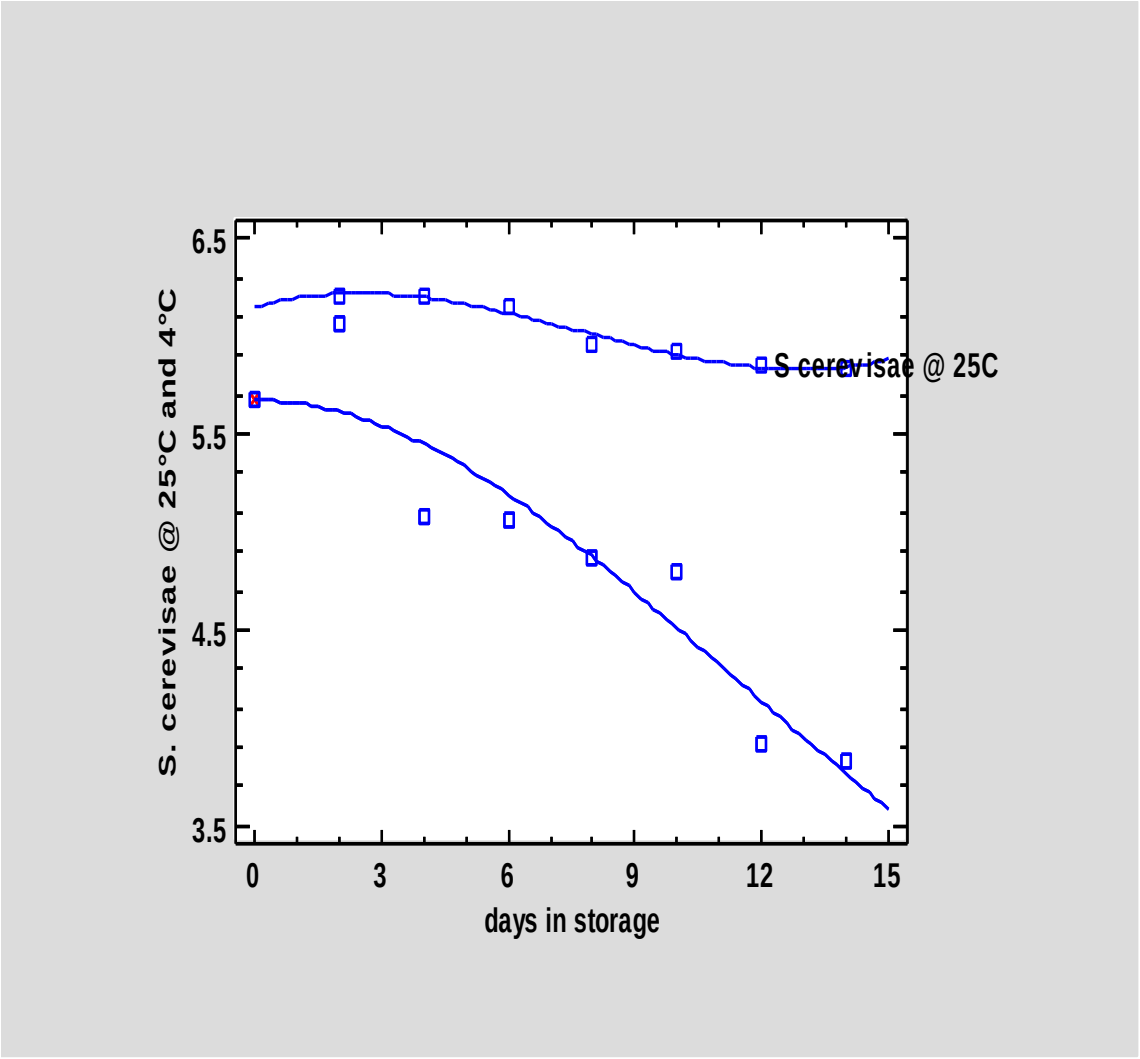

Figure 4.7: A comparism of Log_{10} cfu/ml of yeast cells in jam samples stored at both room and refrigerator temperature conditions for 14 days

4.1.2.3 E. coli in jam samples stored at both room and refrigerator temperature conditions

The pathogenic bacteria cell count generally decreased as storage time increased for both the challenged jam samples stored at room and refrigerator temperature storage

conditions. Bacteria cell count (*E. coli*) decreased from an initial contamination level of 4.03 $x10^5$ cfu/ml, that is a count of 403,000 on day 0 to 3.42 $x10^2$ cfu/ml (342) and 3.20 $x10^2$ cfu/ml (320) on day 4 for jam samples stored at room and refrigerator storage temperatures respectively. No bacteria colony was observed on day 6 and throughout the studies period for both samples under the two storage temperatures. The rate of decline was significantly higher in jam samples stored at refrigerator storage conditions.

Table 4.2: *E. coli* colonies counted in pineapple jam stored under room and refrigerator temperature conditions for 14 days.

Storage days	**Number of *E. coli* colony counted (cfu/ml) in jam samples challenged and stored at conditions**	
	Room temperature (25ºC)	**Refrigerator temperature (4ºC)**
0	$4.03x10^5$	$4.03x10^5$
2	$4.9\ x10^4$	$5.35\ x10^3$
4	$3.42\ x10^2$	$3.2\ x10^2$
6	No colony detected	No colony detected
8	No colony detected	No colony detected
10	No colony detected	No colony detected
12	No colony detected	No colony detected
14	No colony detected	No colony detected

The bacteria count decline rate was fastest from between the day of inoculation or day 0 and day 2 in jam sample stored under refrigerator temperature as against the samples stored under the room storage temperature conditions. That is, the rate of decline was faster in samples stored under refrigerator than in the samples stored at room ambient temperature.

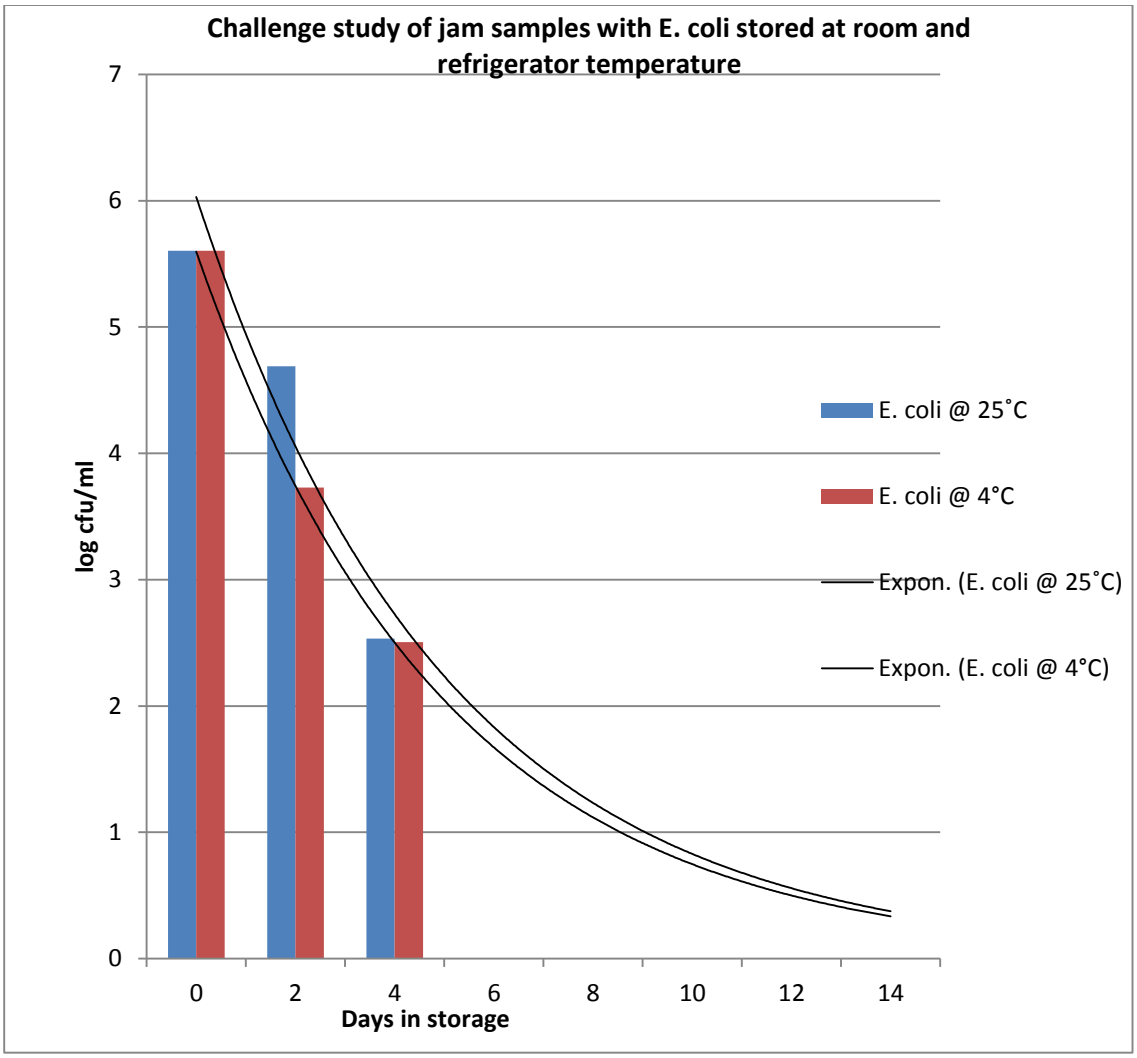

Figure 4.8 A graph comparing decline rate of E. coli in jam samples stored under both ambient room (25°C) and refrigerator (4°C) conditions

4.2 DISCUSSION

4.2.1 Physicochemical properties of pineapple jam

Different concentrations of sugar, pectin and citric acid affected the quality characteristics of the pineapple jam as observed by (Afoakwa *et al*,. 2006). However, balancing these concentrations was necessary to obtain a good jam quality.

The pH values, which is a direct function of free hydrogen ions released by acids present in food was an important jam quality indicator. The pH values of the jam formulations were in the prescribed limits (Codex, 2004; Fasogbon, 2014). The free hydrogen ion gives acid foods their distinct tartness or sour flavor. The more hydrogen ions present, the more acid the food is and the lower the pH value. Addition of citric acid was needed to decrease pH for gel to form and improve jam flavor, especially for the pineapple fruits which are known to be low in acid content. Pectin had no significant effect on jam pH as demonstrated by (Afoakwa *et al.*, 2006). From the study, the jam's pH remained steadily at around 2.99 irrespective of the quantity of pectin added. Decreasing the pH eliminated the negative charges surrounding pectin chain causing more pectin chains to bind which results in the improvement of another quality indicator, texture.

The texture quality output for this work reflected the jam's firmness consistency. Generally, in high sugar concentrations of soluble solids content of more than 60% and a pH value of 2.8 to 3.6, pectin will not dissolve completely (Gigli *et al.,* 2009) because the 3-dimentional molecular network formed immobilizes water in spaces known as junction zones. Addition of sugar removes the water from the junction zones which result in the formation of good gel quality. Thus, mechanical rigidity (Aguilera, 1992) or strength exhibited by the intermediate solid and liquid jam gel were largely affected by sugar and pectin input concentrations. The thickest gel or high texture jam formed at the three different sugar and pectin formulation levels of 100.00g to 112.5g sugar against 27.0 g to 30.0 g of pectin; 117.5g to125.0 g sugar verses 24.0g to 26.0g pectin

and 132.5g to 140.0 g sugar against 27.0g to 30.0 g pectin. These respectively implied that, the thickest gel were formulated with fuzzy linguistics sets of low sugar and high pectin; medium sugar and medium pectin; high sugar and high pectin concentrations. The jam gel texture or firmness increased with increasing pectin and sugar content. The high sugar concentration reduced the available water, increased the jam's texture or firmness and the possible sugar crystallization.

Moisture content in food is critical in determining its shelf life and microbial stability (Fellows, 2000). Sugar in foods usually reduced the amount of moisture or water available for microbial multiplication consequently maintains the shelf life of the food (Afoakwa *et al.*, 2006). The moisture content in this study was found to vary between 46.57% and 59.35%, which is a good indication that each formulation possibly possessed good shelf life potential. But as expected, the moisture content generally decreased as the jam's sugar input increased. The moisture content decrease probably because a lot of the water in the pineapple jam were bounded to the sugar hence reduced its availability. This implies that, the addition of sugar has a dehydrating effect on the jam. The presence of sugar molecules decreases the hydration of pectin by competing for water thereby creating a condition of low water activity (Morris *et al*., 1980; Oakenfull *et al.*, 1991).

Brix measures the content of sucrose or sugar in an aqueous solution. The optimal soluble solid content for jam is usually around 60% to 65%. It is widely acknowledged that, the

higher the brix value, the better the taste or sweetness and the better the resistance to spoilage or the higher the nutrient density of food.

4.2.2 Microbial response in challenged jam samples

When a vegetative yeast culture, like other microorganisms, is inoculated in a fresh growth medium containing sugar and other nutrients and kept at an appropriate temperature and oxygen supply, they go into a lag phase where they are biochemically active but not multiplying. Thus during the lag phase, cells numbers remains fairly constant but actively metabolizing awaiting rapid growth. The initial population size and environmental conditions such as temperature, pH, alcohol, oxygen, nutrient and salt concentration determines how long the lag phase lasts but, once the cells starts actively metabolizing, they soon replicate their DNA and shortly after divide into two identical cells. The cell division marks the start of second phase known as the exponential phase. The time it takes to double is called generation time and is dependent on factors such as, the organism itself, the growth medium, temperature, and all other factors in determining the generation time.

From the result of this study, the yeast cells counted in jam samples stored under room temperature storage and refrigerator temperature storage conditions shot up to $1.63x10^6$cfu/ml and $1.17x10^6$cfu/ml respectively on day 2 after an initial inoculation level of $4.7x10^6$cfu/ml on day 0. The numbers begun to drop to $1.59x10^6$cfu/ml and $1.21x10^5$cfu/ml on day 4. The above observation suggested that, the yeast cells exponential or growth phase were between day 0 and day 2. The cells reproduced by means of budding in which a daughter is initiated as an outgrowth from the mother cell

followed by nuclear division, cell wall formation, and finally cell separation. The growth phase did not prolong because, other environmental conditions such as temperature and probably alcohol, oxygen and pH posed as restraint, resulting the cells moving into the death phase, characterized by the continual decline in yeast cells enumerated from the samples. The yeast cells death phase followed a convex survival curve for samples stored in refrigerator temperature condition but a sigmoid survival curve for samples stored at room temperature conditions.

Temperature difference was the major determining factor that limited microbial growth. Other factors such as nutrient content, pH, moisture content, water activity, brix or total soluble content that could influence microbial activity were kept at constant. High sugar of 130.42g representing 65.21% jam sugar, medium pectin of 25.50g representing (12.75%) and high citric acid of 3.00g (1.5%) was used in formulating the jam for the challenge testing. The challenged jam samples had low pH of 2.83; high brix value of 7.84^{o}Bx; medium moisture content of 52.5% and medium texture of 4.000mJ. pH ranging from 2.75 to 4.25 is considered an important survival and growth requirement of yeast (Fleet and Heard, 1993). *S. cerevisiae* is a facultative anaerobe which is capable of generating energy in both the presence and absence of oxygen using aerobic metabolism and fermentation respectively. *S. cerevisiae* ferments carbohydrate or glucose to form ethanol and carbon dioxide in large quantity. The alcohol released into the environment, eliminates other microbial competitors causing the *S. cerevisiae* become the larger proportion of microbes remaining, a physiological prowess explained by the Cabtree

effect. Yeast cells may switch into the “make, accumulate, consume” ethanol pathway where the waste released is retrieved and used as energy source.

Ambient storage temperature affected the rate at which the number of yeast cells declined. The temperature range for yeast growth is about 0°C - 47°C with 30°C-35°C being the optimum growth temperature. The yeast population decreased fastest in the samples stored in the refrigerator due to the exposure to very low continuous storage temperature of 4°C which was too cold or too low as against the optimum of 32°C. The sinusoidal curve depicted by figure 4.5 shows a phenomenon of majority of distribution or food storage environments where many packaged foods undergo changes in moisture content and temperature as a result of varying temperature and relative humidity conditions in the environment. Again, the rise and fall of the curve depicted by figure 4.5 could also imply, that, the yeast cells experienced a negative feedback reaction, in that, the by- product of respiration, alcohol, may contribute to yeast decline.

The number of *E coli* after inoculation kept reducing till no growth or count was made on day 6. This observation is as result of the unfavorable conditions prevailing within the jam medium.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

The concept of fuzzy logic and reasoning was used to formulate pineapple jam with imprecise ranges of low, moderate and high quantities of sugar, pectin and citric acid. Formulation labeled HMH was found to be the formulation that would result in jam with high taste, good colour and spreadability properties- important qualities that are known to influence consumer preferences. This jam was formulated with high sugar (125.00g to 140.00g), moderate pectin (23.00g to 27.00g) and high citric acid (2.50g to 4.00g) content per 200.00g pineapple. The jam formulation had low pH (2.8 to 2.9), high degree brix (6.5 to 8.5), moderate moisture content and texture quality output of (50% to 55%) and (2.5mJ to 5.5mJ) respectively.

The jam formulation was inoculated with high levels of *Saccharomyces cerevisae* and pathogenic bacteria *Escherichia coli* and stored for fourteen days under two storage conditions; the refrigerator (4^0C) and room temperature (25^0C) to assess growth rate. The microbial load did not increase but dropped exponentially in jam samples stored under refrigerator temperature conditions but gradually in jam samples stored at the room temperature storage conditions. The yeast colony count decreased from a contamination level of $4.7x10^5$cfu/ml on day 0, to $6.83x10^3$cfu/ml and $6.80x10^5$cfu/ml on the fourteenth day in samples stored under refrigerator and room temperature storage conditions respectively. No *Escherichia coli* growth was observed in samples stored under both conditions from day 6 and throughout the rest of the study period.

According to Good Manufacturing Practises microbiological procedure for low acidic foods, foods containing *Escherichia coli* and *Saccharomyces cerevisae* counts of minimum and maximum of 10 to 10^2 and 10^3 to 10^5 respectively are considered microbiologically safe. From the results obtained, the number of *Escherichia coli* and *Saccharomyces cerevisae* counted on the last day of the study were within the acceptable microbial limits.

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APPENDICES

Appendix A 1: Physical and chemical constituents of pineapple pulp

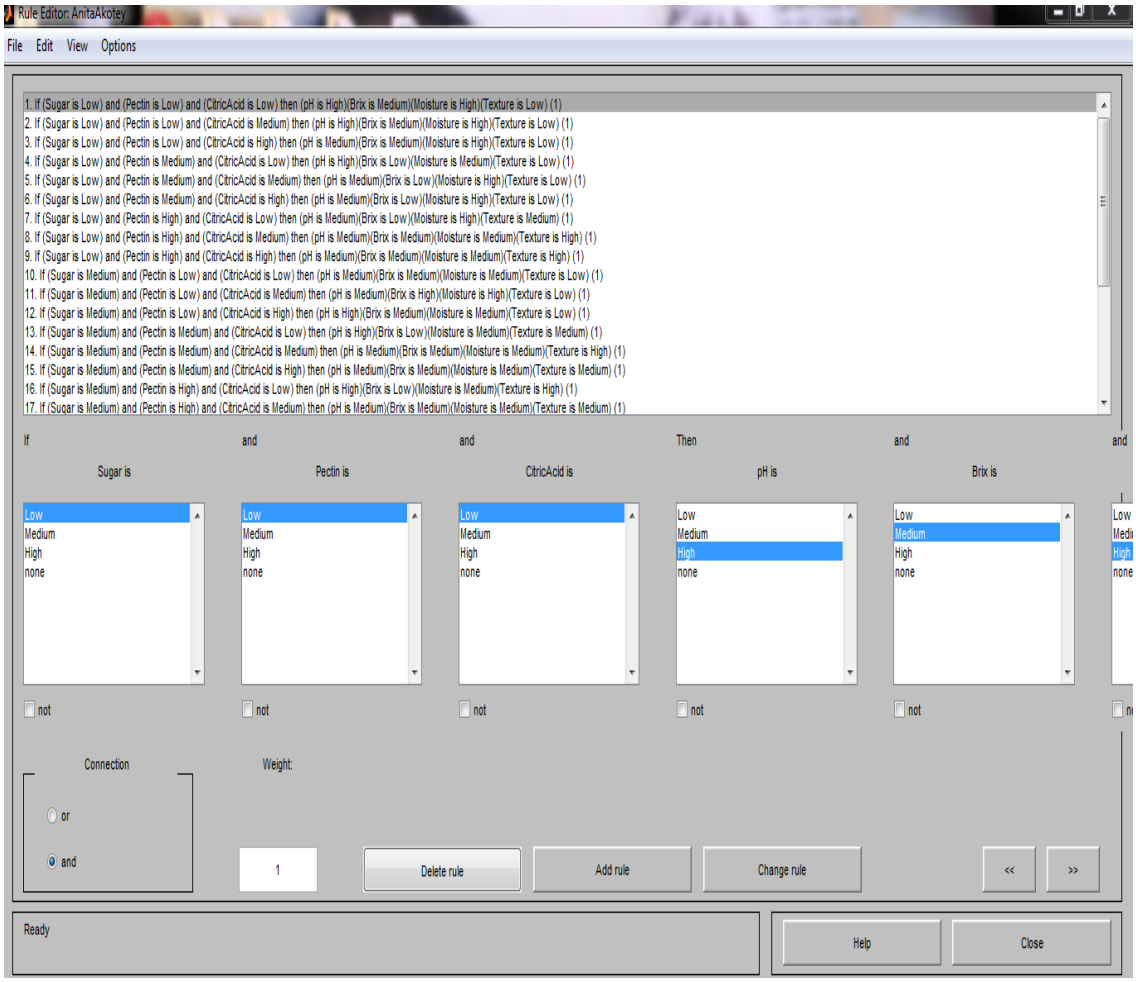
No	Parameters	Pineapple pulp	Pineapple waste
1	Moisture content (%)	87.3	91.35
2	Ash content(mg/100g)	1.8	0.04
3	Titratable acidity (%)	2.03	1.86
4	Ascorbic acid (mg/100g)	21.5	26.5
5	Reducing sugar%	10.5	8.2
6	Non reducing sugars (%)	7.4	8.8
7	Total soluble solids (%)	13.3	10.2
8	Total sugars(%)	8.66	9.75
9	Crude fibre (g/100g-fw)	0.41	0.60
10	Protein (mg/100g)	7.2	10

Appendix B 1: A simplified diagram of a Fuzzy Inference System

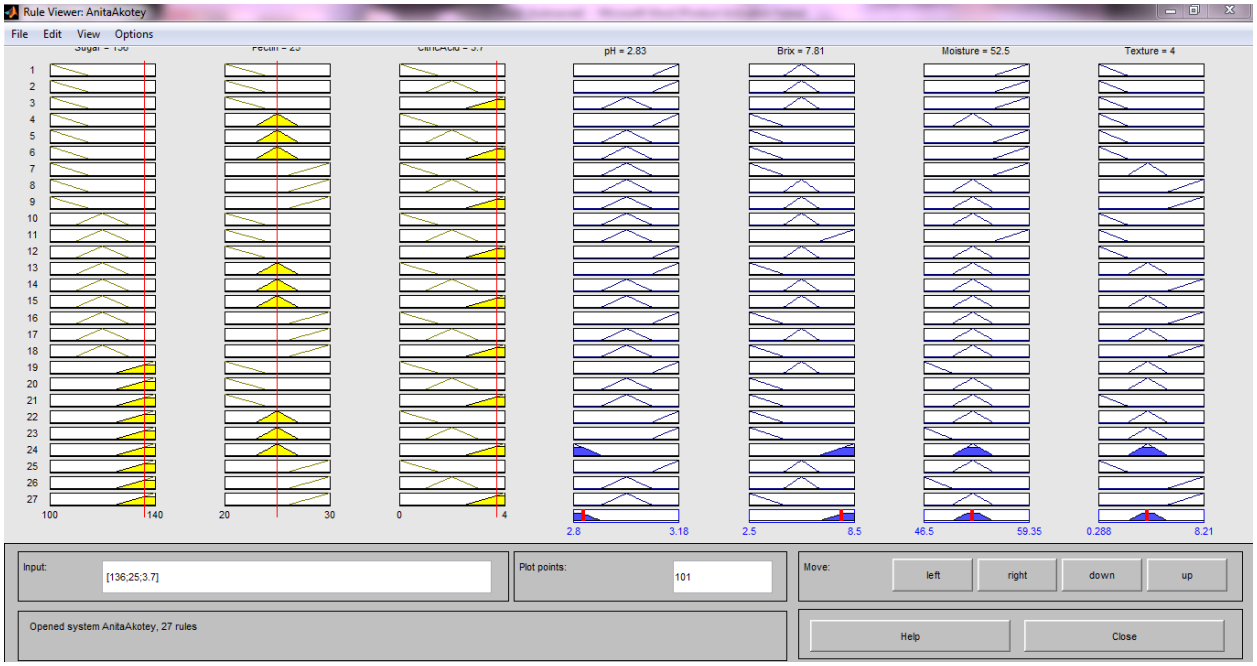
Table 4.1: A table indicating concentration of sugar, pectin and acid variable per formulation run and their resultant pH, Brix, % moisture content and texture outputs

Run	Formulation	Input formulation variables			Jam Output variables			
		Sugar (g)	Pectin (g)	Citric Acid (g)	pH	Brix/º Bx	% moisture content	Texture /mJ
1	LLL	100.70	20.00	0.00	3.15	5.0	57.266	0.594
2	LLM	105.00	21.12	1.03	3.18	4.9	57.400	0.288
3	LLH	110.23	22.15	4.00	2.96	4.8	57.647	1.976
4	LML	115.03	23.28	1.45	3.13	4.1	54.773	0.309
5	LMM	112.82	23.50	1.36	3.05	4.4	55.910	2.171
6	LMH	102.74	25.15	3.70	2.99	3.6	59.351	2.229
7	LHL	106.10	28.60	1.12	3.06	4.0	58.035	5.090
8	LHM	104.00	29.00	2.05	2.98	4.6	53.876	8.206
9	LHH	108.00	30.00	3.50	2.95	4.9	52.589	5.998
10	MLL	130.00	24.00	1.42	3.07	4.6	51.328	2.358
11	MLM	104.00	21.84	2.40	3.04	6.5	55.417	0.206
12	MLH	124.00	20.60	2.54	3.09	5.0	50.330	1.032
13	MML	128.01	26.50	1.20	3.11	4.0	51.824	3.790
14	MMM	121.45	25.00	2.00	2.97	6.2	52.983	6.596
15	MMH	123.00	26.70	2.82	2.91	5.9	52.860	5.228
16	MHL	117.10	24.40	0.92	3.10	4.1	52.251	7.222
17	MHM	121.64	29.10	1.62	3.01	5.0	53.098	4.266
18	MHH	122.50	27.10	3.26	2.99	4.0	54.971	6.187
19	HLL	140.00	20.00	0.00	3.10	5.3	49.136	3.558
20	HLM	139.00	20.50	1.75	3.03	3.2	50.603	3.444
21	HLH	134.40	21.10	3.92	2.91	4.3	51.377	2.045
22	HML	129.03	25.10	1.30	3.08	2.8	51.033	5.147
23	HMM	132.02	23.14	1.25	3.12	3.6	46.573	4.480
24	HMH	130.42	25.50	3.00	2.88	7.2	50.065	2.924
25	HHL	135.02	27.50	0.54	3.08	5.1	50.730	1.877
26	HHM	125.00	28.00	2.55	2.93	5.6	49.643	7.523
27	HHH	137.04	26.50	2.75	3.02	4.2	51.744	5.734

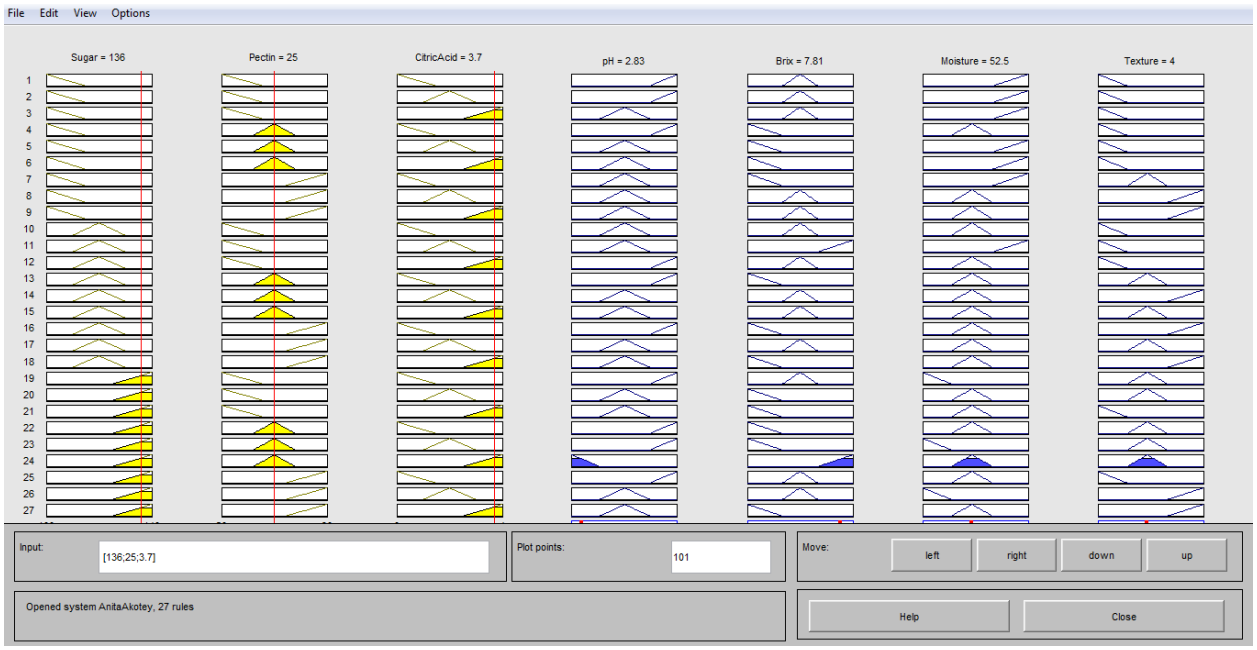
Appendix B 2: Rule Viewer tool showing plots of the 27 "if-then" fired rules constructed with the Rule Editor



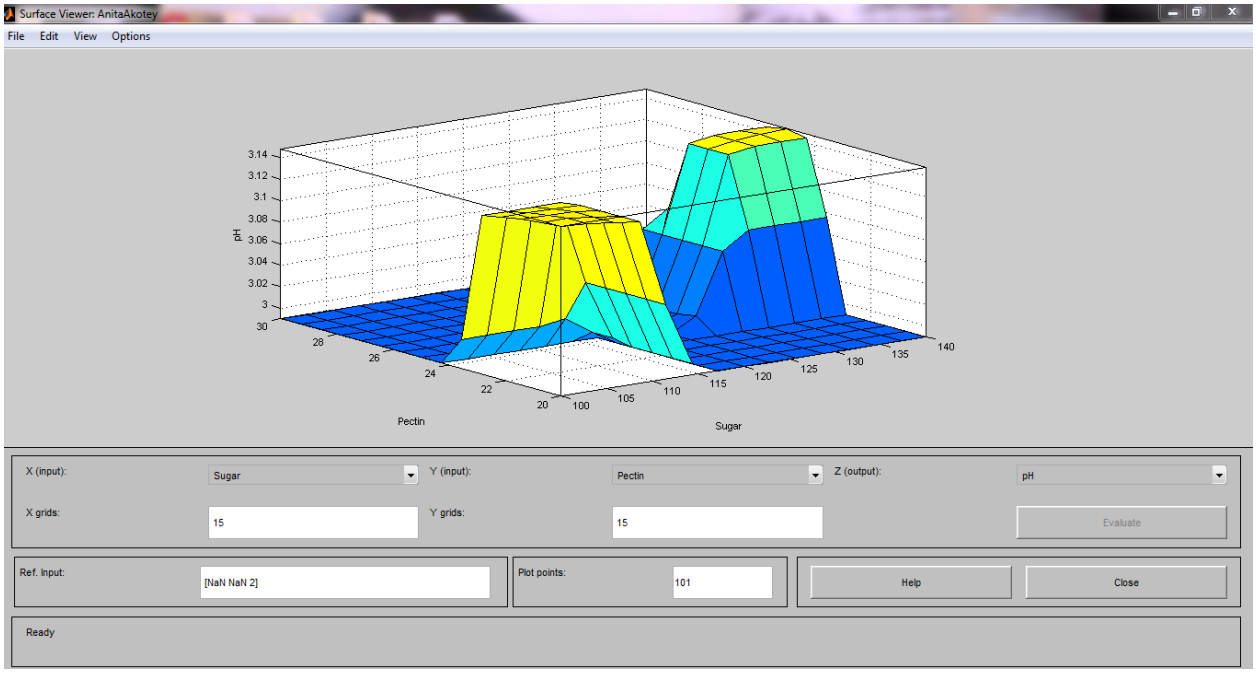
Appendix B 3The Rule Viewer showing the aggregated consequent and the defuzzified output values of fuzzy input values for sugar, pectin and citric acid (predicted pH, brix, moisture content and texture)



Appendix B 4 The Rule Viewer showing the input values for sugar, pectin and citric acid



Appendix B 5 The surface viewer tool displaying the mapping from input sugar and pectin to output pH variable for the pineapple jam.



Appendix C 1 Results for S. cerevisae coliform forming unit of jam samples stored at refrigerator temperature storage conditions

Days in storage	Coliform forming unit per ml of samples stored at refrigerator temperature		
	Sample A	Sample B	Mean
0			$4.7x10^5$
2			1.17×10^6
4			1.21×10^5
6			$1.16 x10^5$
8			$7.35 x10^4$
10			$6.35 x10^4$
12			$8.35 x10^3$
14			$6.83 x10^3$

Appendix C 2 Results for S. cerevisae coliform forming unit of jam samples stored at room temperature storage conditions

Days in storage	Coliform forming unit per ml of samples stored at room temperature		
	Sample A	Sample B	Mean
0			4.70×10^5
2			1.63×10^6
4			1.59×10^6
6			1.42×10^6
8			8.95×10^5
10			8.35×10^5
12			7.20×10^5
14			6.80×10^5

Appendix C 3 Results for E. coli coliform forming unit of jam samples stored at refrigerator temperature storage conditions

Days in storage	Coliform forming unit per ml of samples stored at refrigerator temperature		
	Sample A	Sample B	Mean
0			4.03×10^5
2			5.35×10^3
4			3.20×10^2
6			No growth
8			No growth
10			No growth
12			No growth
14			No growth

Appendix C 4 Results for E. coli coliform forming unit of jam samples stored at room temperature storage conditions

Days in storage	Coliform forming unit per ml of samples stored at room temperature		
	Sample A	Sample B	Mean
0	4.03×10^5		4.03×10^5
2	4.9×10^4		4.9×10^4
4	3.42×10^2		3.42×10^2
6	No growth		No growth
8	No growth		No growth
10	No growth		No growth
12	No growth		No growth
14	No growth		No growth

Appendix D 1 Polynomial Regression - S. cerevisae @ 25°C versus days in storage

Dependent variable: S. cerevisae @ 25°C

Independent variable: days in storage

Order of polynomial = 3

Number of observations: 8

		Standard	*T*	
Parameter	*Estimate*	*Error*	*Statistic*	*P-Value*
CONSTANT	5.72227	0.0804708	71.1099	0.0000
days in storage	0.268731	0.0536371	5.01018	0.0074
days in storage^2	-0.0425511	0.00931269	-4.56916	0.0103
days in storage^3	0.00172239	0.000436517	3.94575	0.0169

Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.239248	3	0.0797492	11.01	0.0211
Residual	0.0289754	4	0.00724384		
Total (Corr.)	0.268223	7			

R-squared = 89.1973 percent

R-squared (adjusted for d.f.) = 81.0953 percent

Standard Error of Est. = 0.0851108

Mean absolute error = 0.0537659

Durbin-Watson statistic = 2.48321 (P=0.1427)

Lag 1 residual autocorrelation = -0.310244

Appendix D 2 Polynomial Regression - S. cerevisae @ 25°C versus days in storage (after excluding outlier)

Dependent variable: S. cerevisae @ 25°C

Independent variable: days in storage

Order of polynomial = 3

Number of observations: 7

		Standard	*T*	
Parameter	*Estimate*	*Error*	*Statistic*	*P-Value*
CONSTANT	6.14519	0.1213	50.6609	0.0000
days in storage	0.0650381	0.0611927	1.06284	0.3658
days in storage^2	-0.0156708	0.00860445	-1.82124	0.1661
days in storage^3	0.000677041	0.00035536	1.90523	0.1528

Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	0.158015	3	0.0526716	30.17	0.0097
Residual	0.00523712	3	0.00174571		
Total (Corr.)	0.163252	6			

R-squared = 96.792 percent

R-squared (adjusted for d.f.) = 93.584 percent

Standard Error of Est. = 0.0417816

Mean absolute error = 0.0207824

Durbin-Watson statistic = 3.00774 (P=0.3734)

Lag 1 residual autocorrelation = -0.515781

Number of excluded rows: 1

Appendix E 1 Calculation for moisture content

$$\% \text{ Moisture content} = \frac{\text{Mass of Water loss (after drying)}}{\text{weight of sample(before drying)}} * 100$$