Pineapple is an herbaceous plant biology essay

Science, Biology

1. INTRODUCTION

Pineapple is an herbaceous plant approximately 1-2 meters tall and wide. The plant has spiral morphology due to the arrangement of the leaves. The stem is a distinct central cylinder, erect and club- shaped approximately 25-50cm long, 2-5cm wide at the base, 5-8cm wide at the top and contains nodes and internodes (Bartholomew et al., 2003). A fully grown pineapple plant has many (68-82) leaves arranged in the form of a dense compact rosette. The older leaves are located at the base of the plant and the younger ones in the center. Leaves are usually sword shaped (except for the ones at the tip) and taper toward the tip (approximately 5-20cm in length). The margins may or may not contain spines. The upper and lower surfaces of the leaf are covered with hairs that are more pronounced on the lower surface (Bartholomew et al., 2003). The leaves enclose the stem up to two thirds of its circumference, are wide at the base and form a sheath around the stem. Due to the tendency to collect water at the base, the leaves are semi rigid and this feature may also provide aerial roots with water and nutrient (Bartholomew et al., 2003). The plant can flower after producing 70-80 leaves (Purseglove, 1972)G: FYPpart of a pineapple. jpgFigure 1. Parts of a pineapple plant(Elfick, 2007)In addition to their delicious taste and intoxicating smell, pineapples have many nutritional benefits. Pineapples provide useful digestive enzymes, several essential minerals, vitamins and fiber. They are also low in calories, rich in carbohydrates, fat free and versatile. Raw, juiced, cooked or canned, pineapples offer tremendous nutritional value. G: FYPPresentationutrition value4. jpgFigure 2. Nutrition information of 100g of fresh pineapple.(Source: USDA National Nutrient data

base)Pineapple contains the bromelain which has the potential anti-inflammatory and digestive benefits. The U. S. National Library of Medicine lists bromelain as a proteolytic digestive enzyme. When taken with meals, bromelain aids in the digestion of proteins, working to break proteins down into amino acids. On an empty stomach, bromelain has anti-inflammatory properties. Certain conditions, such as sinusitis, burns, pancreatic insufficiency and skin rashes seem to benefit from the ingestion of bromelain, according to the National Library of Medicine. Both the fruit and stem of a pineapple contain bromelain. Vinegar may be defined as a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation (Cruess, 1958). Fruit vinegar is a kind of beverage and is becoming more and more popular throughout the world. Fruit vinegars are mainly made of different kinds of fruits and their residues by traditional fermentation and modern food processing techniques (Giudici et al., 2006). Fruit vinegars are rich in organic acids, amino acids, vitamins, mineral substances and so on. The main organic acids in fruit vinegars include acetic acid, tartaric acid, formic acid, lactic acid, citric acid and malic acid (Sáiz-Abajo et al., 2006). The internal qualities of fruit vinegars are different due to different varieties of fruits. In this study, over-ripe pineapple was used in vinegar production because over-ripe fruits have lower market value and are consider as waste because the fruit will soften rapidly during ripening and becomes very mushy and thus, difficult to consume as fresh produce. The objective of my final year project was to review the conversion process of glucose in pineapple to final product, acetic acid during the

pineapple vinegar production. Besides, the determination of the time taken to produce vinegar from the pineapple was also reviewed.

2. LITERATURE REVIEW

2. 1 Introduction of Pineapple

Pineapple with the scientific name of Ananas comosus is sometimes also called as the King of Fruit (D. Arthey, 1995). Ananas comosus is the most economically important plant in the family Bromeliaceae, which is divided into three subfamilies that are Pitcarnioideae, Tillandsioideae and Bromelioideae. Ananas comosus belongs to the subfamily Bromelioideae, order Bromeliales, genus Ananas and species comosus (Bartholomew et al., 2003). Pineapple is grown extensively in Hawaii, Philippines, and Caribbean area, Malaysia, Taiwan, Thailand, Australia, Mexico, Kenya and South Africa. Pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavour and refreshing sugar-acid balance (A. P. Bartolome et al., 1995). The family Bromeliaceae consists of approximately 2794 species and 56 genera that have adapted to a wide range of habitats ranging from terrestrial to epiphytic, shady to full sun and from hot humid tropics to cold dry subtropics. They can grow in moist to extremely dry situations and at varying altitudes from sea level to alpine conditions (Bartholomew et al., 2003). Members of this family are characterized by a short stem, narrow stiff leaves arranged in a circular cluster, terminal inflorescences (racemes or panicles), hermaphroditic and actinomorphic trimerous flowers. Fruits are capsules or berries that contain small naked, winged or plumose seeds, with a reduced endosperm and a small embryo (Bartholomew et al., 2003). The subfamily Bromelioideae is the

most diverse and consists of the largest number of genera but the lowest number of species. The genus Ananas is recognized among Bromeliaceae by the characteristic inflorescence, which is fused into a syncarp, a unique dense rosette of scape-wide leaves and medium to large fruits. Pineapple plants are set apart from other monocots by the characteristic star-shaped, scale-like multicellular hairs and unusual coiling stigmas which fold together lengthwise (Gilmartin & Brown, 1987). Cultivated pineapple was first described and named Karatas and Ananas at the end of 17th century by Charles Plumier on the island of Hispaniola part of Antilles (West Indies) located between Cuba to the west and Puerto Rico to the east. Later all the pineapples were classified in one genus which is the Ananas. Bartholomew et al. (2003) stated that in 1892 Mez recognized in the Flora Brasiliensis only one species A. sativus and five botanical varieties. Pineapple taxonomy underwent further modification several times and it was not until 2003 that the classification developed by Coppens d'Eeckenbrugge and Leal (2003) was internationally adopted. Based on similarity in floral structure, biology and chromosome number (2n= 50), the current classification identifies six botanical varieties of A. comosus that intercross successfully with A. comosus var. comosus to produce fertile offsprings (Coppens d'Eeckenbrugge et al., 1997). The six variesties of A. comosus include the former species given below (Coppens d'Eeckenbrugge & Leal, 2003): A. comosus var. ananassoides (formerly two species: A. ananassoides and A. nanus)A. comosus var. bracteatus (formerly two species: A. bracteatus and A. fritzmuelleri)A. comosus var. comosus (formerly A. comosus)A. comosus var erectifolius (formerly A. lucidus (formely A. erectifolius)A. comosus var.

parguazensis (formerly A. parguazensis)A. macrodontes (formerly Pseudananas sagenarius)Ananas monstrous has been invalidated because the crownless fruit characteristic is not stable (Coppens d'Eeckenbrugge & Leal, 2003). Generally, varieties of pineapple are distributed throughout the tropics and seed production is rare because most varieties of A. comosus possess reduced fertility combined with self-incompatibiluty (Coppens d'Eeckenbrugge et al., 1993).

2. 2 Fruit vinegar

True fruit vinegars such as apple, blueberry, raspberry, blackberry, and black currant are made from fruit wines, which are then fermented into fruit vinegars. This differs from non-fruit vinegars that are flavored with fruit or fruit flavors. The latter is technically called fruit-flavored vinegar. Fruit vinegars have a delicate flavor and aroma with a slightly sweet taste and pair well with fruit and other salads (Cruess, 1958). Table 1 shows that the various type of fruit vinegar and their application. Table 1: Types of fruit vinegar and application

Types of vinegar

Description

Applications

Apple Cider VinegarProduced through the alcoholic fermentation and subsequent apple juice acetification. Made from cider or apple mashIt has a sharp strong flavor at full strength and the better quality ones dilute well to reveal a delicate apple flavor. It has a warm, soft honey color. Used as a condiment and for picklingCan be used for salad dressing. Used as a

powerful astringent and it works very effective in skin toningTreating plants with this vinegar is a solution for almost all plant diseasesUsed as a conditioner. powerful home remedy for wartsworks for a sore throatFruit Vinegar(banana, oranges, pineapples, blueberries)Made of several fruits through alcoholic fermentation and subsequent acetification.- Any fruit or vegetable that contains a lot of sugar could be used for this purpose. (Cruess, 1958)It is very useful in the preparation of fruit saladsUsed as salad dressingUsed as a marinade for meatsGives additional flavor and aroma to salads and cooked vegetablesWine/Grape VinegarProduced through the alcoholic fermentation and subsequent grape juice acetification. Most commonly used in Europe, specifically France and Italy. Its name and characteristics vary depending on the region where it is produced (Tesfaye et al., 2002)Used to bring out the sweetness in strawberries and melonsThe taste of this vinegar reduces the need of salt in recipesIt balances the flavors without adding a fat to the preparationsBalsamicMade from the juice of Trebbiano grapes that has been boiled down to almost syrup. Dark in colorIt is very smooth and mellow with deep complexity and layers of subtle flavors. Used for salad dressings and saucesIt gives additional flavor and aroma when sprinkled on cooked meat(Source: Ning, 2008)Fruit vinegar is obtained after two fermentation stages (Tesfaye et al., 2002). In the first stage, alcoholic fermentation takes place, where sugar is changed into alcohol. This takes place in the absence of oxygen (without air). In the second stage, acetic fermentation takes place and the alcohol is turned into acetic acid. Here oxygen is essential. The alcoholic fermentation is carried out in the temperature range of about 10 to about 30° C. On the other hand, the acetic

acid fermentation may be carried out by in the temperature range of about 25 to about 35° C (Mahanta, 1968). Vinegar for food use must contain between 4% and 5% acetic acid (Food Act 1983 & regulations). Acetic acid is formed by conversion of ethanol to acetaldehyde, and dehydrogenation to acetic acid by aldehyde dehydrogenase (Nichol, 1979; Canning, 1985). The two steps are performed aerobically with the aid of acetic acid-forming bacteria (Mahanta, 1968). Acetic acid yield from fermented sugar is approximately 40%, with the remaining sugar metabolites either lost to volatilization or converted into other compounds. Acid yield improvements can be achieved using high rates aeration of during continuous production (Ghommidh, 1986). untitledFigure 3. Schematic outline of vinegar production(Source: Adams, 2000)Acetic acid bacteria (AAB) are the main microorganisms responsible for the elaboration of vinegar through the oxidation of ethanol into acetic acid by an obligatory aerobic metabolism with oxygen as the terminal electron acceptor (De Ley, 1984). They are also the main spoilage microorganisms in some food products, especially those that may contain ethanol or sugar. Most bacteria strains derived from vinegar factories are able to oxidize acetic acid to CO2 and H2O (over-oxidation) and therefore are classified in the genus Acetobacter (De Ley, 1984). In previous study by Zhang in 2006, at 25 oC of alcohol fermentation, 14 % percentage of sugar, initial pH 4. 4, can get the largest amount of alcohol production after 11 days. And at 30 oC of acetic acid fermentation, pitaya vinegar with brown color and special gentle taste can be obtained after 10 days.

2. 3 Quality Measurements

2. 3. 1 Total soluble solids

The total soluble solids can be referred to refractive index. The refractive index of a sugar solution is a direct measure of its concentration (Aurand et al., 1987). Consequently, the refractometer is widely used for quality inspection in the manufacture of syrup, jams, fruit juice, puree, vinegar and other products. (Aurand et al., 1987). Different foods will indicate different value of oBrix in various ranges, the higher the value the sweeter the product is.

2. 3. 2 pH

pH is an expression for the effective concentration of hydrogen ions in solution (Aurand et al., 1987). In the contemporary food analysis, pH usually determined instrumentally with a pH meter, however, chemical pH indicators also exist. The instrument measures the potential difference developed between the pH electrode and a reference electrode of constant potential. The difference in potential obtained when the electrode pair is removed from the standard solution and placed in the test solution is converted to the pH value (Bennink, 1998). The acidity or basicity of a solution is frequently an important factor in chemical reactions (Aurand et al., 1987)

2. 3. 3 Determination of titratable acidity

Titratable acidity is a measure of the organic acid content of the juice, must or wine sample. Titratable acidity also consider as a measure of titratable hydrogen ions, including free H+ hydrogen ions in solution and those associated with acids and proteins. (Aurand et al., 1987)

2. 3. 4 Alcohol content

Alcohol is produced through alcoholic fermentation of material containing sugar or starch. Starch first has to breakdown into sugar and sugar is then fermented by yeast which converts it into ethyl alcohol and carbon dioxide. Alcohol obtained from fermentation is subject to separation, concentration and distillation. They are several techniques of varying degree of difficulty and analytical accuracy that are used to measure the alcohol content of wine like ebulliometry, distillation, near infrared spectroscopy and gas chromatography (The Australian Wine Research Institute). The analysis of alcohol content can help in indicating the end of alcoholic fermentation and acetic acid fermentation.

2. 3. 5 HPLC analysis for organic acids

The HPLC determination can be carried out to determine organic acids in foods (Cano, 1991). HPLC has simplified the analysis for various food constituents. In fact it allows the fast, sensitive, and nearly specific determination of organic acids in foods and beverages, and involves uncomplicated sample treatment. The quantitative analysis of organic acids is important for the quality control of vinegar, because the classes and content of organic acids give a characteristic taste to vinegar (Cano, 1991). Acetic acid, tartaric acid, formic acid, lactic acid, citric acid and malic acid are main organic acids in vinegar. The analysis of organic acid content can indicate the total acetic acid produced and the end of acetic acid fermentation during production of vinegar

2. 3. 6 HPLC analysis for sugar content

The HPLC method for analyzing fermented products is a composite of methods previously applied to various food products, with modifications (Bugner, 1992). The soluble sugar content indicates the amount of fermentable sugars available for conversion to ethanol at specific process steps (Bugner, 1992). The analysis of sugar content can indicate the alcohol produced and the end of alcoholic fermentation during production of vinegar.

2. 4 Case Study 1: Study of Pineapple Peelings Processing into Vinegar by Biotechnology

In 2009, Yaovi Ameyapoh et al. have published a paper on Pakistan Journal of Biological Sciences with their study of pineapple peelings processing into vinegar by biotechnology. The study is aimed to reduce post-harvest losses of pineapple by the transformation of juice into vinegar through biotechnological process. According to Ameyapoh et al. (2009), developing countries including tropical African countries produce huge quantities of fruits and vegetables. All these are normally consumed fresh but there is a significant amount of the fruits and vegetables have been abandoned at the production sites. The sugars adhering to the fruit processing waste materials are ideal substrate for fermentations (Ameyapoh et al., 2009). Several reports suggested that the possibilities of alcohol and vinegar production from various fruit processing wastes (Ethiraj and Suresh. 1990). But Gullo et al. (2005) reported that the production of vinegar has some sugar in fruit juice and that the concentration of ethanol is not a limiting factor for the growth of acetic bacteria.

2. 4. 1 Review of Materials and methods

2. 4. 1. 1 Plant material

The pineapple Ananas comosus (L.) Merr. (Bromeliaceae) was used in the study. Pineapple peelings were obtained from a local processing factory in Lome town (Togo) during April 2007 to February 2008.

2. 4. 1. 2 Preparation of pineapple juice

A large amount of pineapple peeling about 10 kg was mixed with 5 L of boiling water at 100 oC for 15 minutes. The saccharine extracts were obtained by manual pressing and treated with 50 ppm sulfite. An optimum amount of total dry matter is necessary in the pineapple juice for production of vinegar with high acetic degree. Hence, the obtained juice was concentrated to 20 oBrix by evaporation prior to fermentation.

2. 4. 1. 3 Alcoholic fermentation of pineapple juice

The yeast culture that was used for alcoholic fermentation was obtained by inoculating Sabouraud Chloramphenicol agar (BioRad, France) with natural wort of pineapple juice. The gender and species were identified by seeding on gallery API 20AUX (bioMerieux, France). From the culture of 24 hours to 48 hours in Sabouraud Chloramphenicol agar (BioRad, France), the yeast strain was seeded extensively on 100mL broth culture. After 12 to 24 hours incubation, 25mL broths with 106 cfu mL-1 were collected for use as inoculum for alcoholic fermentation of pineapple juice, by adding to 450mL of juice (oBrix 20%). The mixture was incubated at 30 oC for 72 to 96 hours.

2. 4. 1. 4 Acetic fermentation of pineapple wine

The acetic bacteria culture that was used for acetic fermentation was obtained after exposure at ambient temperature of 28 oC of 250 mL pineapple wine followed by inoculating on Muller Hinton agar (BioRad, France) and of Gram staining. The study of respiration metabolism was performed by growing bacteria on semi-solid beef liver agar (BioRad, France) at 30 oC for 24 hours (Leyral and Vierling, 2007). The production of acetic acid from ethanol was monitored in the mid of yeast extract agar and phenol red (BioRad, France) was added to ethanol. The identification of gender was supplemented by oxidase and catalase tests (Guiraud and Galzy, 1980). From the culture of 24 to 48 hours on Muller Hinton agar (BioRad, France), the acetic bacteria strain seeded extensively on 100mL of broth culture. After 12 to 24 hours incubation, 25mL broth with 106 cfu mL-1 was collected for use as inoculum for acetic fermentation. This was assayed by adding to 475mL of previously fermented juice, 25mL microbial suspension concentration 106 cfu mL-1 acetic bacteria. The mixture incubate at 30oC for 23 to 25 days.

2. 4. 1. 5 Physico-chemical and microbiological parameters during the fermentation

2. 4. 1. 5. 1 Biomass

The development of yeast biomass and bacteria biomass was monitored at different stages of fermentation by direct counting with standard plate count and results were expressed in colony forming unit (cfu mL-1).

2. 4. 1. 5. 2 Total Sugar

The total sugar contents of juice were evaluated during the fermentation of pineapple juice with a refractometer (Euromex, HC type 0-32, Holland). The total sugars in the juice were expressed as Brix degree, indicating the mass in gram of dry matter for 100g juice.

2. 4. 1. 5. 3 The pH

The pH of the wort fermentation was measured with a digital display pH meter (HANNA, France).

2. 4. 1. 5. 4 Acetic acid degree

The production of acetic acid was determined each 48 hours by titration of 1 mL sample with NaOH 0. 1N using phenolphthalein as color indicator. The acidity of vinegar was expressed as degree of acetic acid indicating the mass in gram of pure acid for 100g of vinegar (Lotong et al., 1989).

2. 4. 1. 6 Ethanol and glucose tolerance of isolated acetic bacteria

Acetic bacteria were cultivated on the Muller Hinton agar (BioRad, France) at 30oC for 24 hours and the broth culture seeded was properly diluted. 1mL cell suspension containing 102 microbial germs was inoculated Muller Hinton agar Petri dishes with several concentrations of ethanol (2, 4, 6, 8, 10, 12 and 14%) for ethanol tolerance. Glucose tolerance was assayed with the same Muller Hinton agar with different concentration of glucose (16, 18, 20, 22, 24, 26, 28, and 30%) as indicated by Gullo et al. (2005).

2. 4. 1. 7 Statistical analysis

The results were processed by the software Excel. Statistical significance was set at p <0. 05.

2. 4. 2 Review of the Results

2. 4. 2. 1 Assessment of physic-chemical and microbiological parameters during fermentation of acetic pineapple juice

2. 4. 2. 1. 1 Yeast isolated and identified

The yeast strain isolated was an oval cell, sporulating , apical budding , which fermented and assimilated glucose, galactose, maltose, sucrose, raffinose, and bearing hyphae. It was identified as Saccharomyces cerevesiae. It was coded Saccharomyces cerevisiae (LAS01).

2. 4. 2. 1. 2 Acetic bacteria isolated and identified

The acetic bacteria strain isolated was a Gram-negative bacillus, an aerobic strict, acid producing from ethanol, catalase positive, and oxidase positive. This strain was identified as belonging to Acetobacter genus and was coded Acetobacter sp. (ASV03).

2. 4. 2. 1. 3 Yeast and bacteria biomass

Figure 4. Yeast and acetobacters biomass during the production of vinegar. (Ameyapoh et al., 2009)This is the result of the biomass of yeast and acetobacters during the production of vinegar. From figure 4, we can observe that the growth curve of yeast cells shows three phases. An exponential growth curve in the 1st to 3rd day, a stationary phase in time of 3rd to 4th day and a phase of declination beyond the 4th to 12th days. The maximum biomass is obtained after approximately 4 days at which tell us

that the growth is maximal during the period. This period is the most appropriate time for us to start up the process in continuous culture which is the acetic fermentation. The growth curve for the acetobacter is also showed that the general growth curve indicated three phases. A phase of rapid growth in the period of 1st to 5th days, a stationary phase in the period of 5th to 11th days and a phase of declination after the 11th day until the 23rd day.

2. 4. 2. 1. 4 Acetic acid production and pH

Figure 5. Evolution of pH and acid levels during acetic fermentation(Ameyapoh et al., 2009)These results showed an increase in the production of acetic acid from 1. 1-4. 5 degrees during 1st to 20th day of the acetic fermentation. The change of pH during acetic fermentation show that the pH of vinegar decreased from 4. 4 to 2. 9 during the acetic fermentation. The degree of acidity of vinegar in contrast increased with the length of fermentation. Hence, increase the production of acetic acid will cause the decrease in pH of vinegar.

2. 4. 2. 1. 5 Variation of dry content during of alcohol and acetic fermentation

Figure 6. Variation of Brix during alcohol and acetic fermentation(Ameyapoh et al., 2009)These result showed a variation of dry contents during acetic fermentation due to the use of sugar by yeast and acetic bacteria for growing. It was estimated at 59. 5, 36. 9 and 73. 5% respectively for the alcoholic fermentation, acetic fermentation and alcoholic and acetic fermentation.

2. 4. 2. 2 Ethanol and glucose tolerance of acetic bacteria

Figure 7. Strains of acetic bacteria tolerance to ethanol(Ameyapoh et al., 2009)These results in general showed a slight decrease in the number of strains of acetic bacteria from 86 to 78% between 2 and 6% ethanol and a significant decrease from 78% to 50% between 6 and 14% ethanol. The majority (78%) of acetic bacteria have grown at 6% ethanol and some (58%) at 12% ethanol. Figure 8. Strains of acetic bacteria tolerance to glucose. (Ameyapoh et al., 2009)These results showed a curve of 2 phases which included the first increase in the number of strains of acetic bacteria from 64 to 82% between 16 and 20% glucose and a decrease with increasing glucose and by 24% of sugar, only a small number (28%) of the bacteria strains were able to grow.

2. 4. 3 Discussion

The best known alcohol producing yeast organism is Saccharomyces cerevisiae which is capable of fermenting only hexose sugars into ethanol (Patle and Lal, 2007). According to Gardner et al. (1989), the Saccharomyces genus is effective yeast in the production of alcohol in pineapple juice. According to Ndoye et al. (2006), acetic bacteria are Gram negative, strictly aerobic and normaly found in nature on different plants. Among 17 strains of acetic bacteria that have been isolated from the tropical fruit and sub-Saharan and used in industrial production of vinegar, more than 60% are identified as belonging to the genus Acetobacter. The remaining strains are found to be belonging to the genus Gluconobacter. In order to differentiate these two types, some biochemical features can be distinguished. Acetobacter is positive oxidase while Gluconobacter is negative oxidase

(Guiraud and Galzy, 1980). Both species Acetobacter that isolated from these products are Acetobacter tropicalis and Acetobacter pasteurianus (Ndoye et al., 2006)Alcohol will induce stress in yeast, causing their flocculation, but the stress of yeast is much more related to the acetaldehyde which is the first product of ethanol biological oxidation by Acetobacter. The acetaldehyde disrupts the enzymatic activity of yeast (Claro et al., 2007). Acetic acid content which cause a change in pH extracellular, influencing the intracellular pH of yeast by significantly reducing its production of ethanol as soon as the acid concentration reaches 3% (Graves et al., 2007). According to Valli et al. (2005), gender Acetobacter frequently used in industries of vinegar involves a biological oxidation of ethanol in acetic acid by the combination of two types of enzymes which are the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). At the beginning of acetic fermentation, which constitutes the second stage of the production of vinegar acetic acid production is quick and important because the oxidation of ethanol by dehydrogenases is spontaneous (Frebortova et al., 1997). The stability of acetic acid to 4. 5o indicated the end of the acetic fermentation. The acetic fermentation of pineapple juice requires 23 to 25 days for 4. 5 degree vinegar with pineapple juice of 20 Brix content. The results are compared with those of Ethiraj and Suresh (1990) that the acetic fermentation of the juice of mango peeling gives vinegar at 4. 65o in 12 days. However, the fermentation of vinegar by the simple batch process is generally slow and requires 4 to 5 weeks for a complete fermentation (Ethiraj and Suresh, 1990). According to Ndoye (2007), depending on the strains, acetic bacteria that produce a final acetic acid

concentration of up to 1. 7o are achieved in modern submerged fermentation processes as so called acetators. The study of the evolution of acetic acid and the pH in pineapple juice during the production of vinegar shows a linear relationship between the two settings. The pH decreased with the increasing amount of acetic acid produced. The acidity of the vinegar is due to the presence of acetic acid. But all the volatile organic acids short chain affects the acidity, flavor and quality of vinegar. These volatile acids mainly acetic acid and smaller propionic and butyric acid come from raw materials or are generated by the fermentation. According to Walter(2005), acetic acid and other organic acids (for example: citric acid, tartaric acid, malic acid, succinic acid and lactic acid) determine the acidity of vinegar. Fermentable sugars adhering to residues in fruit processing substrate are ideal for alcoholic fermentation and acetic fermentation of fruit juice (Ethiraj and Suresh, 1990). These results indicated a significant presence of sugar (5. 3%) in pineapple vinegar at the end of its production and are comparable to those found by Ould El Hadj et al. (2001). The preparation and acetic fermentation rarely exceeded 10% alcohol, it would be reasonable not to consider ethanol concentration as a limit to the growth of acetic bacteria. Gullo et al. (2005) argued that the increasing amount of ethanol at acetic fermentation is not significant for the growth of acetic bacteria. High concentration of ethanol was not tested because according to Lotong et al. (1989), higher the total concentration of ethanol and acetic acid in the medium is, the less biomass produced.

2. 5 Case study 2: Production of vinegar from pineapple peel

On June 2012, Mohammed Jibril and co-researchers have published another similar paper on the International Journal of Advanced Scientific Research and Technology with their study of production of vinegar from pineapple peel. The study is aimed at producing vinegar from fermented pineapple peel. The high increase in food deterioration is due to the contamination of food microorganism since the microorganisms colonize the entire environment in which we live. These microorganisms include bacteria, yeast, and mould. Instead of get rid of them, we should actually look into their beneficial aspects that can be noted in the production of vinegar, spirit, wine and some antibiotics. These products enable the utilization of pineapple peels which are usually discarded during the processing of the pineapple (Hang et al., 1986, Chau et al., 2008, Kang et al., 2006 and Kumar et al., 2003). Fermentation microorganisms are capable of degrading all organic substances present in fruits and vegetables. Vinegar can be made from any non-toxic material that has a sugar juice or can be made with the sugar juice. Theoretically, 1 gram of glucose will produce 0. 67 grams of acetic acid. However, this figure can never be achieved as yeasts and bacteria grow during the process and causing at least 2% sugar is required for every 1 % of acetic acid in the final product (Singleton, 1997, Nigam, 1999, Omojasola et al., 2008, Tanaka et al., 1999)

2. 5. 1 Review of Materials and Methods

2. 5. 1. 1 Raw materials

5. 0kg of average sized pineapple were purchased at the fruit stand in Minna Gwari market.

2. 5. 1. 2 Sabouraud and Dextrose Agar (SDA)

65g of commercially powdered SDA and 0. 5g of chlorophenical powder were dissolved into 4L of distilled water in a conical flask. It was shaken and corked with cotton wool. The autoclave was then used to sterilize it at 121oC for 15 minutes and allowed to cool between 45oC to 50oC. Petri dishes were washed and dried using hot dry oven at 150oC for 1 hour and allowed to cool. The liquid SDA was dispersed into three Petri dishes aseptically and allowed to solidify.

2. 5. 1. 3 Inoculums Preparation

The stock yeast was prepared and taking aseptically by using a disposable syringe and needle. 2mL of stock yeast was inoculated in each Petri dish by pouring it on top of the SDA medium with a sterilized wire loop. It was then incubated at 35oC for 48 hours. Growth of the yeast were observed and recorded.

2. 5. 1. 3 Microscopy

2mL of 1% lactopherol was poured in top of a clean slide and the yeast from the agar is picked by using a sterile wire loop and placed on the glass slide cover slip. It was then viewed under the microscope at 40x magnification and the observations were recorded. Same procedure was carried out for another two Petri dishes and the result were also recorded and compared.

2. 5. 1. 4 Raw material preparation and processing

The pineapple was peeled with a well sterilized knife and the peels were cut into thin strips and weighed 179g and put into 1000mL Buckner conical flask. 20. 0 g of sugar and 800 ml of distilled was added. This then followed by

addition of 3. 0 g of viable yeast and yeasts nutrient (ammonium phosphate) of 6. 0 g. Sodium bicarbonate was added to adjust the pH to 4. 0. The fermenter was then corked and adhesive tape was held round it. The fermentation was allowed to take place at 25 - 28 OC for two days. After two days alcohol was first formed by baker yeast, the residue was filtered and the filtrate was then covered with cheese cloth to allow Acetobacter to come in by chanced approach method. This was then allowed to ferment for eleven days with continuous aeration of the filtrate on magnetic stirrer machine. The aeration and agitation in the fermenter would serve to provide oxygen for microbial respiration, to suspend and mix the sludge and other particulates and to strip out volatile non- metallic product such as CO2.

2. 5. 1. 5 Determination of density

This is important in controlling sample purity. It is the measure of the mass (weight of the sample relative to its volume in mL). Certain quantity of sample produced was measured with the aid of hydrometer.

2. 5. 1. 6 Determination of acid value

100 g of sample was weighed into a 250 ml conical flask, 50 ml of previously neutralized mixture of toluene and ethanol was added and the content was titrated against 0. 1 mol/ litre solution of Ethanoic potassium hydroxide solution until the indicator changes pink colour.

2. 5. 1. 7 Determination of viscosity

The viscometer tube was charged with the sample of vinegar produced by inserting the tube’s thinner arm into the liquid sample and by the help of prepared filler, drawn up above the upper timing mark of the viscometer.

The flux timing the flow of the sample as it flowed freely from the upper timing mark to the lower timing mark as noted and recorded.

2. 5. 1. 8 Determination of refractive index

A refractor was used to determine the refractive index of the sample. The surface of the prisms were cleaned with ethanol and allowed to dry before use because of the sensitive nature of the index of refraction to a small of contaminant. The liquid sample was placed on the lower prism and it was ensured that it covered the entire width of the prism plate. A dropper was used for this purpose. The upper prism was brought into contact with the lower prism so that the poly-phenol formed an unbroken layer between the two prisms. The controls were manipulated to bring the light and dark fields into focus with the cross hairs. The readings were then taken and recorded.

2. 5. 2 Review of Results and Discussion

2. 5. 2. 1 Sabouraud dextrose agar (SDA)

Table 2. Colonial morphology of isolates.(Jibril et al., 2012)This is the first stage of the confirmatory test on the viability of the dry beaker yeasts purchased from Bosso market. Saccharomyces cerevisiae is well adapted to surviving in the dextrose agar and it metabolizes this sugar for growth and multiplication.

2. 5. 2. 2 Microscopy

Isolates from the Petri dishes are observed using a microscope and it was observed that the cell oval in shape; like eggs of chicken, which is the normal shape of yeast.

2. 5. 2. 3 Growth observed in yeast isolate

The bottle containing the yeast, after 5 days at 350C in the incubator, when observed amount of yeast cells making the liquid cloudy and this continued when still left in the incubator.

2. 5. 2. 4 Colonial Morphology

Colonial morphology of the isolate in the plate after 48 hours was observed to be as in Table 2.

2. 5. 2. 5 pH measurement

Table 3. pH measurement and corresponding temperature readings. G: FYPable4. jpg(Jibril et al., 2012)The products became increasingly acidic to the desired and optimum value of 2. 80 with temperature varied considerably within the room temperature.

2. 5. 2. 6 Density of vinegar obtained.

The density of the vinegar obtained in this experiment was 1. 08 g/ml. this value compares favorably with the standard value of 1. 049 g /ml.

2. 5. 2. 7 Determination of acid value

Table 4. Sample of vinegar produced. G: FYPable5. jpg(Jibril et al., 2012)The vinegar produced contains at least 4. 77 grains acetic acid per 100 concentrations which is 4. 77%. This however indicates that 0. 0477 of acetic acid molecules were dissociated. And more so the grain strength of vinegar amount to 10 x the acetic acid concentration, so 4. 77% will produce 4. 77 grain vinegar.

2. 5. 2. 8 Summary of measured properties and standard value

Table 5. Measured properties and standard values. G: FYPable6. jpg(Jibril et al., 2012)From the table, the measured properties were found to be in the range of the standard value except for that of pH value. The pH value was measured as 2. 80 whereas the standard value of pH is 2. 40. The results of this study have showed that it is possible to produce vinegar from pineapple peels. This can be done by using the baker yeast which is the Saccharomyces cerevisiae as an aerobic degradation of sugar to ethanol and Acetobacter aceti oxidizes the ethanol produced into the acetic acid which is the final product, vinegar. The various measured parameters are evaluated compared favorably with the standard values.

2. 6 General Discussion

Several parameters have been measured and tested during the conversion process of glucose in pineapple to final product, acetic acid during the pineapple vinegar production. For example, pH, total soluble solids, acidity, alcohol content, and also organic acid composition. From the case studies that have been reviewed, the pH value has found to be decreased to a desired range during the fermentation process. The decrease in pH value during alcoholic fermentation may be due to the presence of carbon dioxide and hydrogen ions (Ning, 2008). Yeast transformed sugar present in pineapple into alcohol and carbon dioxide gas during alcoholic fermentation. The acidity of pineapple vinegar in the second stage of fermentation process (acetic acid fermentation) was most probably due to the presence of organic acids. Alcohol combined with oxygen gas to produce acetic acid and water in

acetic acid fermentation. The increasing concentration of acetic acid caused the lower pH in pineapple vinegar. The pH changes become stable at the end of fermentation was due to the production of acetic acid was reducing. This was the end point of fermentation. For vinegar fermentation, the pH value should fall between pH 2. 0 and pH 3. 5 (Adams, 2000). Yeasts and molds usually grow best between pH 4 and pH 6. The accumulation of acid (H+ ions) decreases the pH (Ning, 2008). An initially high sugar concentration, typically 10 %(w/v) or more, and an acid pH favor the production of ethanol by yeasts. During alcoholic fermentation, anaerobic conditions are created, the pH drops further and the ethanol concentration rises. At the end of yeast fermentation when the sugars have been consumed, aerobic conditions are re-established at the surface of the liquid, permitting the growth of ethanol-utilizing acetic acid bacteria. These produce high levels of acetic acid (between 0. 7 and 2. 0 M in most commercial vinegars), decreasing the pH still further to a value of 3 or below (Adams, 2000). Total soluble solid is a direct measurement of sugar solution, where a reading at 14 oBrix shows that there was 14% of sugar content in 100g of sample. The sugar content was also found to be decreased from the beginning towards the end of fermentation process. The initial rapid decrease observed in the reducing sugar content was due to a rapid multiplication of yeast cells and rapid conversion of the sugars to alcohol via glucose metabolism. After alcoholic fermentation, there were no more changes in sugar content in the acetic acid fermentation. This may due to yeast activity has reached the maximum; no more active yeast could transform sugar to alcohol. The changes observed were due to the metabolism of yeast cells. The sugars served as

substrate for the yeast and thereby using them as energy source and for growth, resulting in the production of alcohol and carbon dioxide as end and by-products respectively. According to Ning (2008), the TSS in fruit vinegar should fall between 4. 5-5. 5. The rapid decrease of reducing sugar content in fermentation process was due to conversion of the sugars to alcohol via glucose metabolism by activated yeast (Adams, 2000). The titration of acid determines the amount of organic acids that contained in the pineapple vinegar. The acidity of pineapple vinegar is found to be increased during the 2-stage fermentation process. The presence of carbon dioxide and increasing concentration of acetic acid affected the acidity of pineapple vinegar. The acidity was constant after 14 days because the vinegar bacterium had exhausted the alcohol by converting it to acetic acid. The process was terminated at this point. Gupta (1985) reported that 1. 56% initial acid concentration was optimum for carrying out acetic fermentation of sugarcane liquor by A. aceti. However, Eapen and Rao (1970) reported 2. 5% initial acidity levels as optimum for acetification. The acidity should be adjusted to 1. 56-2. 5% before acetic acid fermentation. Titratable acidity expressed as percent acetic acid in fruit vinegar. Adams (2000) reported that the range of acidity of vinegar should be 4-5%. Vinegar is regulated by the Food and Drug Administration (FDA) and to be legal must have a minimum of 5% acidity. Alcohol content represents the total alcohol compound produced and consumed in the 2-stage fermentation process. The alcohol content in the pineapple vinegar show a curve where it increase at the beginning until it reach a maximum value and started to decrease after that until the end of fermentation. The maximum alcohol content was achieved at the time that

was also the end of alcoholic fermentation. The decrease in alcohol content during the acetic acid fermentation was due to of the conversion of ethanol with oxygen to produce acetic acid. To obtain a complete conversion of the starch into sugar and alcohol is impracticable (Charles, 1919). Even under the best conditions the yield of alcohol is, from various causes, appreciably lower than the quantity theoretically obtainable if the starch could be all converted into alcohol and carbon dioxide only. This theoretical quantity would be: From the C6H12O6 sugars (dextrose, etc.)51. 1%From C12H12O11 (maltose, sucrose, etc.)53. 8%From starch (C6H10O5)56. 8%(Source: Charles, 1919)According to Prescott et al. (1993), the fermentation product (alcohol) is classified as primary metabolite, since it is formed in the growth phase. This means that, alcohol was formed alongside the increase in the biomass. Ning (2008) reported that the initial alcohol content during acetic acid fermentation should in the range 6-7% because the rate of production acetic acid will be faster and higher production efficiency of acetic acid in this range. High alcohol content in a fermenting medium was reported to slow down or stop the growth of yeast (Prescott, 1993). The minimum acceptable acid content of vinegar is between 4-6% w/v (40 and 60g/L) (Adams, 2000). According to Food Act 1983 & regulations, vinegar shall contain not less than 4 per cent w/v of acetic acid. Acetic acid bacteria gain energy during oxidation of ethanol. Increasing the available oxygen is not only desirable in that it increases the metabolic rate of the bacteria and hence the rate of vinegar production, it is also essential. An interruption in the oxygen supply, even for a short period of time will result in death of the bacteria and failure of process (Adams, 2000). So the acetic acid

fermentation is aerobic process. A. aceti was used in vinegar production (Sievers, 1994). Beppu and Ohmori (1979) reported 30oC as the optimum temperature for acetification by different strains of A. aceti. The temperatures above of 30oC tend to increase damage to bacteria due to the concentration of acetic acid in the cultivation medium (Fregapane, 2001). So it was important to make sure the sample was kept at 30oC through the acetic acid fermentation in this study.

3. CONCLUSION

To conclude, we learned that it is possible to produce vinegar from the pineapple from the study. It can be done by using the Saccharomyces cerevisiae to degrade sugar into ethanol and Acetobacter to oxidize the ethanol to acetic acid. Alcoholic fermentation and acetic acid fermentation of pitaya vinegar were observed to be associated with physical and chemical changes. The process results in decrease pH, reducing sugars, soluble solids, with concomitant increases in the total and volatile acidity over the fermentation period. This is due to metabolism of carbohydrates by yeast and other chemical reactions. The increase in acidity and the stabilization of pH was due to the buffering action caused by unionized weak acids and increases of acetic acid during the fermentation period. This study proved that over-ripe pineapple fruit can be a suitable raw material for the production of fruit vinegar. The production of pineapple vinegar to be at least 4. 5oBrix, 5. 3% acidity, pH 2. 8 and requires 23 to 25 days for alcohol and acetic complete fermentation. All these results can be concluded that post-harvest losses of pineapple fruits may be used to vinegar and have a commercial value. Hence, the waste of fruit or food can be reduced.