Chapter II

LITERATURE REVIEW

2.1 Pineapple juice

The pineapple is a monocotyledonous plant closely resembling in its growth character and morphological structure such plants as corn and bananas(41). In Thailand, it is grown mostly in Chonburi, Prachubkerekhun, and Lampang Province. The wellknown products from pineapple are canned pineapples and canned pineapple juice which are important exports. Pineapple juice from pineapple fresh meat, small fruits, cores, peels, and juice drained during processing can be used as raw material for vinegar production.

A typical proximate andlysis of pineapple juice was shown in Table 1. Important ash constituents (41) in pineapple juice are potassium 0.148 %, sodium 0.0012 %, calcium 0.0132 %, magnesium 0.0122%, and phosphorus 0.0075%. The most prevalent of the non - volatile organic acid occurring in pineapple juice is citric acid. Pineapple juice is well buffered (41). The pH of various lots range from 3.5 to 3.7. The average total sugar is 12.55 gm per 100 ml of juice. The mean Brix level is 14.44°. The flavor of pineapple is owing to a variable mixture of non volatile and volatile constitutents such as ethyl methyl esters.

2.2 Vinegar

Table 1 Proximate analysis of pineapple juice (30).

	Percent
Moisture (by drying)	84.7
Protein (Nx6.25)	0.3
Crude fiber	0.1
Titratable acidity (as anhydrous citric)	0.6
Ash	0.3
Total carbohydrates other than crude fiber and acid by difference	14.0
Total sugar as invert	13.9
pH	3.5-3.7

Vinegar may be defined as the condiment made from sugary or starchy materials by alcoholic and subsequent acetous fermentations. The term "vinegar" derived from the French vinaigre, means literally sour or sharp wine (vin=wine : aigre = sour or sharp)(45). The composition of a vinegar will depend somewhat on the nature of the raw material that has undergone alcoholic and acetic acid fermentations. The conditions of manufacture, aging, and storage will also influence the composition of the product. In a cider vinegar, acetified product made from fermented apple juice, for example, one might find at least 4 grams of acetic acid(CH_3COOH) per 100 ml of vinegar at 20° C, trace or small amounts of alcohol, glycerol, esters, reducing sugar (as invert sugar), pentosans, salts and other substance (43).

Kinds of vinegar has been adopted by The Food and Drug Administration of the United States (42) as follows :

Vinegar, cider vinegar, apple vinegar. The product made by the alcoholic and subsequent acetous fermentations of the juice of apples.

Wine vinegar, grape vinegar. The product made by the alcoholic and subsequent acetous fermentations of the juice of grapes.

Malt vinegar. The product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt.

Sugar Vinegar. The product made by the alcoholic and subsequent acetous fermentations of sugar sirup, molasses, or refiners sirup.

Glucose vinegar. The product made by the alcoholic and subsequent acetous fermentations of a solution of glucose.

Spirit vinegar, distilled vinegar, grain vinegar. The product made by the acetous fermentation of dilute distilled alcohol.

All of these kinds will contain in 100 cubic centrimeters (20), not less than 4 grams of acetic acid.

In Thailand there are 3 types of vinegar (44) :

1. Imitation vinegar which is prepared by diluting acetic acid with water. The concentration of acetic acid is between 4 gm and 7 gm in 100 ml of vinegar.

2. Distilled vinegar is a product obtained by acetic acid fermentation of diluted spirit or of diluted alcohol, and should contain not less than 4 gm of acetic acid in 100 ml of vinegar.

3. Fermented vinegar which obtained by alcoholic and subsequent acetic acid fermentation of sugary and starchy fruits and vegetables, and should not contain less than 4 gm of acetic acid in 100 ml. Fermented vinegar is a better kind of vinegar because of its good original flavor and odor.

The works of Levine and Fellers (23) showed that acetic acid adjusted to a certain hydrogen ion concentration was more toxic to bacteria, molds, and yeasts than was lactic acid or hydrochloric acid of the same pH. Vinegar added to foods prior to heat treatment was very effective in destroying the organisms expecially heat resistant bacteria. These investigations found that acetic acid inhibited bacterial growth in direct proportion to the amount present.

2.3 General Requirements in Alcoholic Fermentation

As has been indicated, the manufacture of vinegar from sugary materials involves two steps (16) :

1. the fermentation of sugar to ethyl alcohol and
2. the oxidation of alcohol to acetic acid.

In the first step the hexose sugars of the raw material, whether there at the beginning or formed by enzymic or chemical hydrolysis, are converted by the hign-alcohol-producing strains of *Saccharomyces ellipsoideus* into alcohol through anaerobic fermentation. This biochemical conversion may be represented by the simple reaction,

$$\underset{\text{Hexose}}{C_6H_{12}O_6} \xrightarrow{\text{Yeast Enzymes}} \underset{\text{Alcohol}}{2CH_3CH_2OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

This reaction is only an approximation. It does not take into consideration that some residual hexose is left unfermented or that small amounts of other products are formed in the fermentation. Thus, the solution to be acetified may contain, in addition to alcohol, trace of sugar (hexose), glycerol, formic, acetic, lactic,and succinic acids, acetylmethylcarbinol, and other constituents characteristic for the raw material used. These natural ingredients include malic and tartaric acids, esters, pigments, pentosans, proteins and minerals (43).

In each of the processes, success depends on the selection of type of yeast, the efficiency of preliminary treatment, such as the use of an optimum concentration of sugar, an optimum pH, and an optimum temperature and the addition of nutrient substances to the mash. Certain types of yeasts are desirable, namely, those which are able to produce a high concentrations of alcohol and which posses uniform and stable characteristics. Strains of *Saccharomyces ellipsoideus* are commonly used in this step(33). Khattak (20) also suggested that the *S.ellipsoideus* culture produced larger amounts of alcohol as compared to *S.cerevisiae* or *S.fragilis* cultures. Having selected the yeast for the fermentation and having isolated it in pure culture, a starter is then prepared by means of aseptic technique with using sterile pineapple juice as medium. This starter was then used for the fermentation of the same media and under the same conditions, whose volume was 2-10% that of the final fermentor charge.

Maldonato et al (27) found that the highest yields in alcoholic fermentation (alcohol produced, % vol, per sugars consumed, % wt,) would process with blackberry, orange, banana and cashew fruit while pineapple and tamarind gave lower values. Pineapple presented the shortest time necessary to reach maximum alcohol production as well as the highest specific growth rate. The authors also found that all of these fruits can be used sucessfully as raw material for the production of alcohol with possible use in the manufacture of wines and vinegars.

Treatment of fruit juices is necessary for the alcoholic fermentation. Attempts were made to sterilize the fruit juice by heating, or by inhibiting the growth of the undesirable type of organisms by the use of SO_2 (43). Cruess et al (10) reported that 125 ppm SO_2 in the form of potassium metabisulfite, in apple juice fermentation, completely inhibited the growth and activity of mold, wild yeast, and acetic and lactic acid bacteria, but permitted rapid growth and alcoholic fermentation by S.ellipsoideus. So if it is used, care must be taken to eliminate all but traces of free SO_2 in the vinegar stock before acetification, because it is very toxic to the acetic acid bacteria.

A sugar concentration in fruit juice of 10 to 18 per cent is usually satisfactory, although other concentrations are used (33). Khattak (20) proposed that in alcoholic fermentation, the evaporation and oxidation losses were higher, when the sugar content was below 9-12 %, and if above this range the amount of alcohol produced might be affecting the yeast activity, or the concentration of the sugar might be inhibiting the activities of the yeast. Charley (8) fortified raspberry juice with different types of sugar (sucrose, invert sugar, and glucose). His results revealed that raspberry juice fortified with glucose remained very sweet (indication of incomplete fermentation) and had an acidic taste, whereas the juices fortified with sucrose or invert sugar produced more complete and better fermentation with similar end products. Bilford et al (5) reported that the preparation of blackstrap molasses for fermentation required adjustment of the sugar concentration to 12% and the pH to 4.5. At this pH, Khattak (20) stated that it was suitable from the point of yeast activity and inhibiting the undesirable bacteria but also improved the efficiency of fermentation from 66.46 % at pH 5.9 to 78.74 % at pH 4.5 with S.elliposideus.

Yeasts, like bacteria and other forms of life, require certain food materials and environmental conditions for proper growth and reproduction (33). S.elliposoideus, one strain of yeast, can utilize fermentable sugars such as glucose and sucrose as carbon sources. Ammonia and ammonium salts, particularly ammonium sulfate and diammonium hydrogen phosphate, appear to be most suitable sources of nitrogen on account of their availability, low cost, and ready assimilation (33). Other suitable conditions are oxygen tension, temperature, and time required for fermentation. Oxygen in large amounts is necessary in the early stages for the optimum reproduction of yeast cells but is not required for the production of alcohol. Initial aeration of the media shortened the lag period and increased the pronounced consumption of carbohydrates and consequently,elevated production of alcohol during this portion of the run. Suitable temperature for alcoholic fermentation is at 70^{o} to 80^{o} F., depending somewhat on the external temperature. At temperatures much above 80^{o} F, alcohol evaporates rather rapidly and bacterial growth is also favored. A fermentation is usually complete in 50 hr. or less, depending on the temperature, sugar concentration, types of yeast, and kinds and amount of nutrients or supplements (33).

2.4 General Requirements in Acetic Acid Fermentation.

In the second step of vinegar manufacture, the alcohol contained in the vinegar stock is converted to acetic acid by oxidative enzymes produced by the acetic acid bacteria through aerobic fermentation. This second stage in the production of vinegar may be represented by the reaction (16),

$$2CH_3CH_2OH + 2O_2 \xrightarrow{\text{Acetobacter enzyme system}} 2CH_3COOH + 2H_2O$$

The acetification of vinegar stock never goes to completion, so that alcohol, together with other minor constituents, and products of fermentation of the raw material are present in the finished product. These constituents include, among others, aldehydes, esters, acetylmethylcarbinol, glycerol, and lactic, malic and tartaric acids, the last being found only in wine vinegar. Vinegar may be made by slow acetification of vinegar stock in barrels, by rapid oxidation in generators, or by submerged oxidation process.

In the classification presented in Bergey's Manual (6) there was 2 genus of bacteria which could oxidize ethanol to acetic acid. Firstly, genus Gluconobacter could oxidize ethanol to acetic acid, sometimes weakly, at neutral and acid reactions (pH 4.5) Did not oxidize acetate or lactate to CO_2. Temperature optimum 25 - 30^{o} C ; range tolerated 7 - 41^{o} C . The species of this genus were G.oxydan, G.dustrius, G.suboxydans, and G. melanogenes. Secondly, genus Acetobacter which could oxidize ethanol to acetic acid with strong rate. Oxidized acetate and lactate to CO_2 and H_2O (overoxidizers) The species of this genus were A.aceti, A.xylinum, A.orleanensis, A.pasteurianus, and A.peroxydans.

Lopez et al. (25) conducted experiments to determine conditions under which submerged acetic fermentation could be performed under laboratory conditions and found that A.acetigenum was the most suitable bacteria among six acetobacter cultures used. Khattak (21) reported that A.orleanense produced higher yield of acetic acid from fermented watermelon liquor than A.aceti and A.xylinum. The author also noted that recycling the effuent was an important factor affecting the yield of acetic acid.

Rao et al. (34) suggested that species other than A.aceti might be able to grow with ammonium nitrogen if supplied with essential growth factors and with an appropiate source of carbon and energy. Yeast extracts and peptones were necessary for the growth of many species of the genus Gluconobacter including G. suboxydans and G. melanogenum. Yeast extracts supplied not only factors essential for the growth of the exacting strains but also contained factor necessary for the growth of many of the relatively less-exacting strains with $(NH_4)_2SO_4$ or hydrolyzed casein when ethanol, lactate, or pyruvate as the source of carbon. Ruttkay (36) recommended the Acetopep (phosphate-containing food for Acetobacter) which could be used as bacterial food in vinegar generators. Shchelkunova (38) reported that 0.4 - 0.6 % KH_2PO_4 or Na_2HPO_4 added to glucose medium stimulated reproduction and increased the rate of glucose oxidation by G. suboxydans and G. melanogenum. This stimulative reaction was due to an increase in the buffer capacity of the medium. Maceda and Palo (26) reported that optimal conditions for growth and production of acetic acid for acetic acid bacteria were pH 5; temperature, 28° to 32° C ; nitrogen requirements preferably organic in nature and phosphatic form of ammonium salt; cool temperatures and sunlight, inhibitory to growth.

Prescott and Dunn (33) stated that the adjustment of alcohol content of the medium might be necessary to ensure a sucessful fermentation. The solution with alcohol content of 10 to 13% was an excellent condition of fermentation. When the alcohol was up to 14% the zoogloeal mat formed with difficulty as well as the alcohol was not completely oxidized to acetic acid. On the other hand, too low concentration of alcohol (1-2%) was oxidized to carbon dioxide and water.

Cruess (11) reported that S. ellipsoideus ceased to grow when the concentration of acetic acid exceeded 0.5% in the fermented liquor. It was an accepted practice in the vinegar industry to supplement the fermented liquor with vinegar to inhibit the development of undesirable types of bacteria and to supply desirable acetic-acid-producing bacteria for seeding purposes (33). The amount of vinegar added to the alcoholic medium would depend upon the nature of the process. Usually 10 to 25 per cent by volume of strong vinegar was considered to be sufficient. Cruess also reported that the addition of apple cider vinegar had a desirable effect on both yield and efficiency of the acetic acid fermentation. The increase in acidity inhibited or retarded further growth of the yeast and decreased the possibility of contaminating the fermented liquor by undersirable lactic acid bacteria.

Recycling the effluent was an important factor affecting the yield of acetic acid. Hausen (17) reported that recirculation of acid-alcohol mixture through the 14-by-15 ft Frings generator under controlled conditions increased production of white vinegar 3--fold over that of the standart 4 - by - 8 ft noncirculating generator. The sealed construction of the generator prevented losses by evaporation and excaping vapors and resulted in increased yields. Because of a much larger volume of mixture passing through the shavings continuously, there was very little tendency toward sliming.

Since the conversion of ethanol to acetic acid is primarily an oxidation process, or a dehydrogenation in which atmospheric oxygen acts as the hydrogen acceptor, the success of the fermentation will depend on the availability of large quantities of oxygen. The following equations will illustrate the requirement for oxygen (43) :

$$CH_3CH_2OH + \frac{1}{2} C_2 \longrightarrow CH_3CHO + H_2O$$

Ethanol — Acetaldehyde

$$CH_3CHO + \frac{1}{2} O_2 \longrightarrow CH_3COOH$$

Acetaldehyde — Acetic acid

Generally, the generators were packed rather loosely with zoogloeal supporting materials to permit sufficient ventilation in all parts of them. The generators thus act as a stack or chimney in which a strong upward current of air takes place, bringing the oxygen in contact with the bacterial film resident on the surfaces of the supporting material for the film. Maldonato et al (27) suggested that high rates of aeration and agitation favored evaporation of alcohol from the culture medium with a resulting decrease in yield. Using too high temperature also favored the loss through evaporation of alcohol, acetic acid, and the volatile substances. The exact temperature to be used would depend on the organism and prodess being employed. The temperature between 80 and 85^{o} F (26.7 to 29.4^{o} C) were usually favorable for the acetic acid fermentation

Kreipe (22) indicated that the rate of access of air considerably affects the evaporation in the Schiizenbach generator. Since the acetic acid bacteria could function satisfactorily at low O_2 concentration, the air supply should be reduced to a minimum sufficient to give good ventilation of the waste gas.

2.5 Methods of Manufacture.

The commercial methods of manufacture included slow, rapid, and submerged oxidation process :

2.5.1 Slow acetification

The slow acetification process commonly used for production of commercial quantities of vinegar is known as the French or Orleans process. Barrels of approximately 200 liters capacity were use. Each container of about 200 litres or barrel was filled about one-third full with a good grade of vinegar, which constitutes the starter or culture, and 10 to 15 liters of wine were added. At weekly intervals for 4 weeks, the same amount of wine was added into barrel. When 5 weeks has passed, 10 to 15 liters of vinegar were withdrawn from the barrel, which was now about one-half filled, and the same amount of wine was introduced. The operation might be repeated, thus becoming a slowly continucus process.

Air was introduced into the barrels through holes, one usually at each end of the barrel, which was placed on its side above the level of the vinegar medium. The acetic acid bacteria formed a thin film on the surface of the solution, and this film later became quite thick and gelatinous. This gelatinous zoogloeal mat, which contained very large numbers of bacteria, was known as the "mother of vinegar" (33).

Adriano (3) reported that banana and coconut milk could be used as raw materials in fermented vinegar production. The banana vinegar was clear, pale brown in color, and retained the aroma of the original substance with containing over 4% acetic acid. Cohee (9) stated that a concentration of 15.2% dextrose with 2% of barley sprouts gave alcohol yields of best appearance and largest amount (6.61%). Saccharomyces cervisiae, Frehberg bottom type yeast, was found to be the best type to use. Studied on a plant scale of slow acetification process showed that a vinegar of 6.5% acidity was obtained.

2.5.2 Rapid acetification

Rapid acetification of vinegar was a process variously known as the generator process, quick vinegar process, or the German process. The generator consisted of a large, cylindrical, straight-sided to slightly conicial wooden tank divided into three compartments. The upper compartment contained the distribution apparatus to insure even application of the vinegar stock over the generator medium. The central compartment might be filled with shavings which offered large surface areas. The lower chamber serve as a sump for the collection of the vinegar and contained ports for the admission of air (33).

The oxygen necessary for the bacteria was supplied by the air which entered the generator through the ports or vents in the sides of the generator at the bottom compartment. The air passed up through the packing and out through the losefitting top, its passage upward being assured by the heat generated by acetification of the vinegar stock. Oxidation of the alcohol was accomplished by droplet diapersion of the vinegar stock which was applied to the upper surface of a mass of packing medium of some depth. The vinegar stock trickled through the generator packing. The acetic acid bacteria presented on the generator packing surface find conditions satisfactory for rapid oxidation of the alcohol.

The temperature of the generator had to be carefully regulated. In the simple generator, the temperature was regulated by adjustment of the rate of flow of the vinegar stock down through the generator and the volume of fresh air passing up through the packing to balance the temperature at 80 to 85^{o} F. In the large, closed, recirculating-type generator, the air might be pumped through the generator at a more or less constant rate and the temperature controlled by cooling the vinegar stock.

Fabian (15) used eight-in glazed tile for constructing the generator. The joints of the tile should be cemented together but no concrete should come into contact with the vinegar. Corncobs or wood shavings were practical packing materials. The generator should be well inoculated with *Acetobacter aceti* before use. This generator was suitable for converting 5 - 50 bbls. of hard cider into vinegar at the rate of about 1 bbl. a day.

2.5.3 *Submerged production of vinegar*

The oxidation of ethyl alcohol during the fermentation was performed by air bubbles, flowing from bottom to top through the fermentation vessel. To ensure equal distribution of the air bubbles throughout the mash, the air was introduced and finely distributed by rotating air jets. The mash was foamed up and fresh liquid was led into the mixture through the foam layer, thereby enlarging the active surface for quicker oxidation (28).