Chapter V

DISCUSSION

The pineapple juice composes of many constituents: sugars, organic acids, flavoring compounds, mineral salts, etc. With a little modification, this juice will be more suitable for the alcoholic and acetic acid fermentation. The pH of pineapple juice was adjusted to 4.5 by using NaOH solution. This pH was more suitable for the desired yeast fermentation activity and also inhibited the growth of undesirable organisms. Some kinds of fruit juice, which had high pH, would encourage the growth of undesirable organisms, particularly the anaerobic spore forming bacteria. These kinds of fruit juice should be adjusted to pH 4.5 by using diluted H_3PO_4 solution which was an important mineral for yeast activity in alcoholic fermentation (20).

The experimental results showed that total invert sugars in pineapple juice was about 12.2 gm per 100 ml of juice solution as well as total soluble solid was 16.2° Brix. So this juice would be made up with sucrose to 18° - 20° Brix which was the optimum condition for alcoholic fermentation. On this experiment, the yield of alcohol was 11.0 % which was suitable for further fermentation. If the high concentration of sugar was applied, it might be affecting the yeast activity or it might be inhibiting the activity of the yeast because of the increase in osmotic pressure of the mash. S. ellipsoideus is not an osmophilic yeast

so that water tends to leave the yeast cells by osmosis if there is a higher concentration of solute outside the cells than inside (16).

According to previous investigation (27, 33), many authors recommended that ammonium sulfate, diammonium hydrogen phosphate, and dipotassium hydrogen phosphate were the suitable sources of nitrogen and phosphate for yeast. The first attempt of this work, diammonium hydrogen phosphate was used as a nutrient but the fermented juice had yellowish-brown color, ammonia odor, and the yield was only 3.18 % alcohol. Second attempt, diammonium hydrogen phosphate plus with dipotassium hydrogen phosphate were replaced. The yield of alcohol increased from 3.18 % to 4.15 % alcohol but the ammonia gas was still produced. Third attempt, only dipotassium hydrogen phosphate was used. The color and odor of the juice and the amount of alcohol produced , 3.40 %, were much better than using diammonium hydrogen phosphate. So dipotassium hydrogen phosphate was chosen as the appropriate nutrient for yeast. The last attempt was to find optimum condition of the nutrient and the result showed that the most suitable level which gave the highest yield of alcohol was 0.5 % dipotassium hydrogen phosphate.

Inhibition the growth of the undesirable types of organism in fruit juice by heating was a better method than using chemical substance (43). Cruess et al (10) reported that 125 ppm of sulfur dioxide in the form of potassium metabisulfite completely inhibited

the growth and activity of mold, wild yeast, and acetic acid bacteria, but permitted rapid growth and alcoholic fermentation by S. ellipsoideus. Therefore, in vinegar making, fruit juice should be only pasteurized at 70°C for 10 min without adding any chemical substances because those substances may inhibit the growth of acetic acid bacteria.

Alcoholic fermentation was carried out in 25 litres glass bottle and the fermentation was completed within 3-4 days. The fermented pineapple juice would have 11.0 % by volume of alcohol and it was stored in the same bottle for 2-3 weeks for the settlement of yeast cell and pineapple pulp.

The vinegar making by using generator with bamboo shoot shavings could provide the maximum surface exposure for a volume of fermented juice and enough air for the acetic acid bacteria to efficiently and quickly oxidize the alcohol to acetic acid. The reasons for choosing bamboo shoot as shavings were that they were non-toxic, did not impart odors, flavors or color to the finished product, and did not remove color from the substrate (33).

There were many important factors influenced to the vinegar production in this experiment.

1. The number of recycling of the effluent, percent of acid increased proportionately to the number of recycling from the second through the fourth cycle and reached the maximum level at the fifth cycle. It decreased gradually after the fifth cycle

due to the volatilization and oxidation of acetic acid to carbon dioxide and water by A. aceti culture(6).

2. Zapadinskii (46) showed that the acetic acid fermentation was activated by the addition to the acetic acid mash with inorganic nutrients such as $NH_4H_2PO_4$, $CaH_4(PO_4)_2$, $MgHPO_4$, and K_2SO_4. The result from this experiment showed that 0.5 % of dipotassium hydrogen phosphate in fermented liquor improved the final yield of acetic acid and also improved the efficiency of the fermentation. The maximum yield of acid was attained at the fourth cycle or 48 hours, compared to the fifth cycle in the absence of phosphate. It was so because energy transfer in respiration and fermentation of the cell is accomplished by certain compounds of phosphorus which are designated as energy-rich. The most important compound involved in these energy transfers is adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (32). This experimental results were also well agreed with Shchelkunova (38) who stated that a little addition of phosphate to glucose medium could stimulate the activity and the rate of acetic acid production.

3. Addition of acetic acid, it could inhibit or retard further growth of yeast and possibly decreased the contamination of fermented liquor by undersirable lactic acid bacteria (11). Meanwhile, the present of acidity also improved both yield and efficiency of the acetic acid fermentation for seed purposes (21, 33). Llaguno (24) indicated that the pH of the wine influenced

the rapidity of acetification. Any pH value under 2.5 resulted in a very slow start to acetification and delayed the production of vinegar. The pH values that were closest to 4.0 were the most favourable.

Hromatka (19) also reported that the higher the total concentration of alcohol and acid in submerged fermentation, the better the yield and the less danger of contamination. Fermentations should never be started below 4 % acidity and preferably between 6 and 7 %. Vinegar containing 12 weight % of acetic acid can be produced at full fermentation rate.

In this experiment, the pH of fermented liquor was 2.9 compared to 3.8 prior to addition of acetic acid. The maximum yield of acid was attained at the fourth cycle. A medium should never be acidified before the alcoholic fermentation was complete, because the sugar in the medium would be incompletely converted to alcohol after the addition of acetic acid.

4. In this phase of experiment, it was difficult to control the rate of aeration and to keep the culture in the generator column from being contaminated by mold spores. However, three flow rates of air, 0.035,0.053 , and 0.106 vvm have been done. The first one was the most suitable which gave the highest percent of acid. Using excess air flow rate caused the evaporation of alcohol and acetic acid produced (22).

The way to decrease contamination during the fermentation was that raw material or fruit should be clean, sound, and in the

proper state of maturity; wine or alcoholic liquor should be clear, clean, and free from preservatives. Cleanliness of the plant, equipment, and surroundings were also important.

5. The fermented juice flow rate was other factor taking into account in this experiment. The results indicated that a high flow rate of fermented juice (1500 ml/12 hr) would get longer fermenting time because of its shorter resident time in the generator. This showed that resident time in the column related to the acetic acid production.

According to the reportes by the department of Science, Ministry of Industry, it was found that slow process of acetification took about 45 days to convert alcohol in fermented coconut juice to 4 % acetic acid and about 3 months for grape wine to vinegar. This report also showed that the generator process of coconut toddy took only 1-2 weeks for the acetification (39).

The fermenting time of generator method in this experiment was only 2-3 days to reach more than 4 % of acetic acid. Richardson (35) performed the submerged acetification experiment from waste pineapple juice using a pure culture inoculum. The results showed that vinegars containing up to 7 % by weight of acetic acid could be produced in less that 24 hr. with a conversion efficiency greater than 90 % in both laboratory and pilot plant equipment.

However, the slow fermentation process still plays an important role in Thailand. This process spends more time than

generator process and submerged process but its operation is not complicated and also gave good grade of vinegar without storage and aging step.

Up today, vinegar are eternally appreciated as an important food condiment and preservative. Imitation vinegar was constantly popular in the consumer market because the price was cheaper, large quantity in market, and easy to buy. Fermented vinegar had higher nutritive value and more appreciable flavor and odor than imitation vinegar but the consumer did not attend because of its high price and rarity in the market.

How can the production of fermented vinegar be reduced the price of it? The rapid fermentation process is one of the interested method which will answer the above question. This method could increase the quantity of this kind of vinegar. If most of consumers change their ideas and favor to the fermented vinegar, the production of wine and vinegar would be an attractive means to salvage surplus fruits, overripe fruits, fruits rejected and waste from local fruit processing plants in our country.