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Investigation on Wine Fermentation with Three kinds of Honey

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Abstract: In this research, preparation of starter culture, fermentation of four ratios Honey to Water (1:1, 1:2, 1:3, 1:4) with the fixed starter culture, analysis of product wine, the parameters such as alcohol content, total soluble solid (Brix˚), total (titrable) acidity, pH, ester and aldehyde contents were analyzed. For the microbial safety, the samples were pasteurized at 120 ˚C for ten minutes. The sample containing Honey to Water ratio 1:4 (B Honey) (Pan-Hnun.. Honey) (15% Honey) (Alcohol 9.4%) is the optimum preparation for making wine with Honey.
Keywords: wine; honey; preparation; parameter; microbial safety

I. INTRODUCTION

An ancient use for honey was in medicine as a dressing for wounds and inflammations. Today, medicinal uses of honey are largely confined to folk medicine. Honey has different types of color which depend on the source of nectar. Since Myanmar is a tropical country, Honey is found abundantly. It is the substance that is derived from the tree of the nectar which the bees suck it. Honey is the most complex that contain many biological active compounds. Honey, as explained elsewhere , is readily assimilated, giving athletes a quick source of energy and enabling them to recuperate rapidly from severe exertion with less evidence of fatigue. There are various kinds of honey. Commercial preparations of honey for medicinal and cosmetic used are available in Myanmar.

In nearly all areas of the world, some type of alcoholic beverage native to its region is prepared and consumed. In Africa, fermented alcoholic beverage are consumed in different occasions such as marriage, naming, and rain making ceremonies(Zvauya et al., 1997),at festivals and social gatherings, at burial ceremonies and settling disputes (Steinkraus, 1983). They are also used as medicines for fever and other aliments by adding banks or stems of certain plants (Okafor, 1972). For these reason, these are consumed around the world.

Indigenous fermented alcoholic beverages from different parts of the world are described by Steinkraus (1983) and some of the indigenous African fermented alcoholic beverages include Egyptian bouza, Tanzanian Wanzuki, gon go, tembo- mnazi and gara, Nigerian palm- wines, Kenyan muratinga and uragua, South African kaffir beer.

Fermented beverages produced from cereals are usually referred to as beer while those produced from fruits are classified as wine (Pederson, 1979). Fermentation of a variety of foods or blends of fruit, cereals, milk sap, honey molasses and other foods are also wines that are not crystal clear products. Instead, they are cloudy, effervescent containing residues of substrates and fermenting yeast and other microorganisms (Steinkraus, 1983).

Alcohol in traditional beverages serves as source of calories valuable to the calorie-deficient villager. The primitive beverages provide not only calories but also B vitamin due to residues of the substrate, the fermenting yeasts and other microorganisms. (Steinkraus, 1983).

This study tends to decide the optimum ratio of Honey (*Apis mellifera)* to water, to analyze the wine products and to know the presence of medicinal useful compounds in honey.

The objectives of this study are:

(1) To determine the composition of honey used in the research.

(2) To know how to make honey wine in lab scale.

(3) To search the optimum conditions of honey wine-making based on the ways either yeast starter or only natural present in honey.

(4) To report, if possible, honey wine with statistical point of view.

II. MATERIAL AND METHODS

2.1 Materials

2.1.1. Raw Materials

Honey, yeast, and boiled water were used as raw materials for this research work. Honey is a very versatile tool with which to make wine. Honey is purchased from "Ministry of finishing & livestock, Department of beeking" which is situated southern part of Mandalay Division. "Levuer instance" instant yeast (France) is used for fermentation. They are purchased from local market.

2.1.2Glass wares

Glass wares used for this research are sterilized before using .Sterilization is carried out by autoclaving at 121 ˚C for 30 minutes under 15 psi pressures. After that, they were washed and dried in a hot air oven.

2.1.3. Chemicals

Chemicals and media used for this work are from "British Drug House (BDH) Chemical Ltd, Poole, England," "Australia Medical Diagonostics (AMD) Co.Ltd" and analytical grade chemicals available from local markets.

2.2. Methods

2.2.1. Analytical Methods for Determination of Composition of Honey (*Apis mellifera)*

Before it was used for fermentation process, Honey was sent to Analysis Department of Ministry of Science and Technology to determine the composition of Honey.

The samples were analyzed to know the composition of Honey.

2.2.2. Procedure for making wine with Honey

2.2.2.1. Preparation of Starter Culture

Firstly, the amounts of water were boiled and let it cool down to room temperature and then add the yeast and citric acid. And stir thoroughly and then incubate for 3 hrs or 4 hrs. After that the liquid yeast starter is poured into all 12 samples.

2.2.2.2. Fermentation of Wine with Honey

For this purpose, Honey was mixed the water. In this case, to determine the optimum ratio of Honey to Water , four different ratios of Honey to Water (1:1,1:2,1:3,1:4) were used and the total amount was fixed at 600 ml . So, each of the required amounts of Honey was weighed. The level of starter culture (200 ml) was used to mix with these four ratios. Then, when this starter levels was mixed into four ratios of Honey to Water, 12 samples was obtained. Then, these were fermented for one month and half. Then, they were filtered and transferred into different containers and racked. During racking, total soluble solid (ºBrix) was recorded weekly. When it was constant, the alcohol content, pH, total (titrable) acidity, ester, and aldehyde contents were determined. Then, the samples were stored and aged.

2.2.3. Chemical Analysis of Wine

2.2.3.1. Alcohol Content Determination

Alcohol content was determined by distillation.

2.2.3.2. Total Soluble Solid Content Determination

This content was determined by refractometer.

2.2.3.3. Determination of pH

The pH of the samples was determined by pH meter

2.2.4. Methods For Microbial Safety

2.2.4.1. Pasteurization

The bottles of all wine samples were placed in an oven. The thermometer is used to know the temperature. The temperature is gradually high and when the temperature in the bottle was reached at 120 ºC, they were kept for 10 minutes at this temperature.

2.2.4.2. Gram Staining Method

The method described by Collin (1964) was employed in Gram's stain reaction of the isolated bacteria. After a bacterial smear had been prepared on grease-free slide, it was subjected to gram staining first by staining with ammonium oxalate crystal violet for 1 minute. Next, it is was washed with water, 95 % ethanol was used as decolorizing agent. It was added drop wise for 30 seconds to remove uncontainable stains. Then, it was counterstained with staff run for 1 minute. Finally, the stained bacterial smear washed with water, dried with blotting paper and observed under a microscope using an oil immersion lens.

2.2.4.2. Plate Count Method

In plate count method, 1 ml of sample was diluted in all test tubes having 9 ml of sterile normal saline. 20 µl from diluted test tube were inoculated by dropping on nutrient agar medium. After absorbing the culture suspension inside the medium, the inverted plates were incubated at 37 ºC overnight. The formula for calculating dilution of bacterial count was described as follows.

$$X = m \times d/v$$

Where, X = number of bacterial per 1 ml of sample

m = number of colonies count

d = number of dilution used in series

v = volume of sample

III. RESULT AND DISCUSSION

RESULTS

Honey was used as a "Health Care Product in the fermentation process of wine-making. Before it was used, it was analyzed in Analysis Department of Ministry of Science and Technology. The result of the composition of Honey was shown in Table .

The 12 samples with each of the fixed level of starter culture combined with 4 ratios of Honey to Water (1:1,1:2,1:3,1:4) were prepared and fermented for one month and half . Photographic appearance of the 12 samples preparation was shown in plate . After fermentation, wine products obtained from fermentation process were analyzed. The parameters measured were alcohol content (%), total soluble solid (°Brix) , pH ,total (titrable) acidity, ester and aldehyde content . The results of 12 samples were shown in Appendix .

The graphical presentations of alcohol content (%) and total soluble solid (°Brix) of four ratios of Honey to Water for the three types of Honey were shown in figure respectively.

The graphical presentation of pH, and total (titrable) acidity of four ratios of Honey to Water for three types of Honey were shown in fig. respectively.

Table (1) Analytical Composition of the Determination of Honey

Composition	Honey A	Honey B	Honey C
Moisture (%)	19.87	19.13	19.47
Ash (%)	0.45	0.34	0.47
Glucose (%)	30.2	33.6	33.2
Sucorose (%)	9.78	1.14	9.97
Fructose (%)	33.9	38.2	30.5
Chloride (%)	0.02	0.025	0.03
Nitrogen (%)	N.D	N.D	N.D
Protein (%)	N.D	N.D	N.D
Acid(Total Acid)(mg Na OH/g of sample	2.5	8.9	3.7

Table (2). Analytical Determination of Composition of Mineral Contents in Honey

Mneral	Honey A	Honey B	Honey C
Silica (Si) (%)	N.D	N.D	N.D
Iron (Fe) (%)	N.D	N.D	N.D
Manganese (Mn) (%)	N.D	N.D	N.D
Phosphorus (P)(%)	0.005	0.015	N.D
Magnesium (Mg) (%)	0.008	0.003	0.004
Copper (Cu) (%)	0.001	0.0005	0.0015
Sulphur(S) (%)	N.D	N.D	N.D

Plate1. “The Sources of Honey” Used in the Research

Plate2. Preparation of Wine with Honey

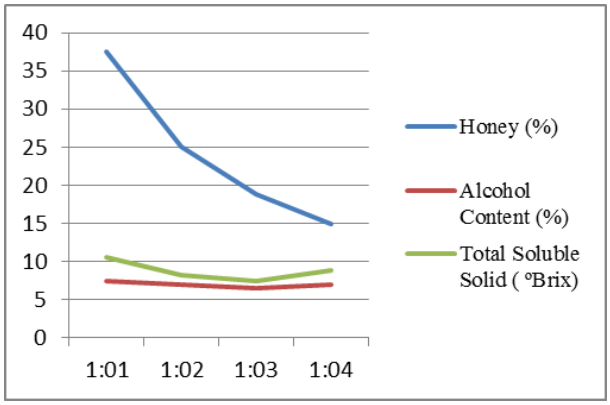

Figure 1. Alcohol content and total soluble solid (˚Brix) for four ratios of Honey to Water for Honey A(Zee)

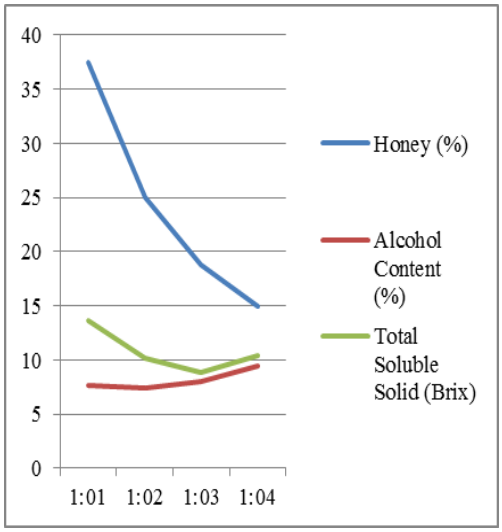

Figure 2. Alcohol content and total soluble soli (˚Brix) for four ratios of Honey to Water for Honey B (Pan-hnun)

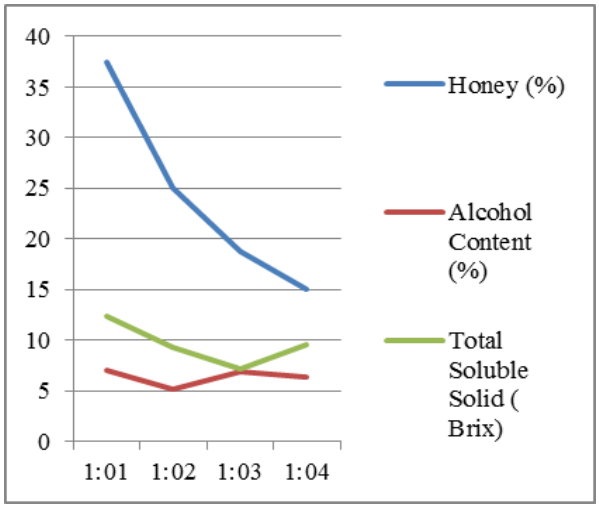

Figure 3. Alcohol content and total soluble solid (˚Brix) for four ratios of Honey to Water for Honey C(Mixture)

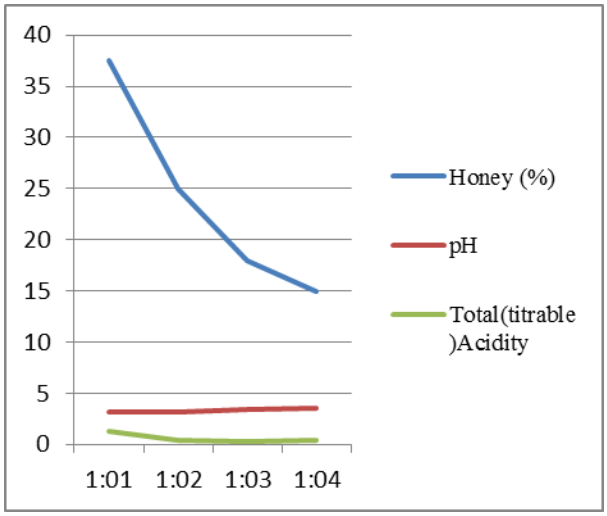

Figure 4. pH and total (titrable) acidity of four ratios of Honey to Water for Honey A(Zee)

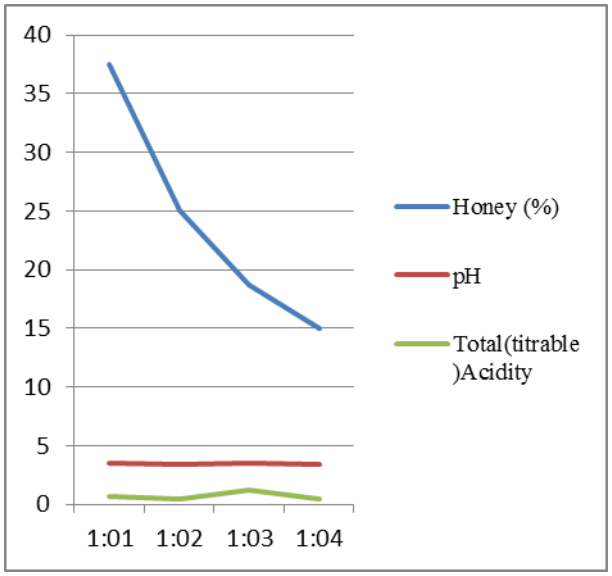

Figure 5. pH. and total (titrable) acidity of four ratios of Honey to Water for Honey B (Pan-hnun)

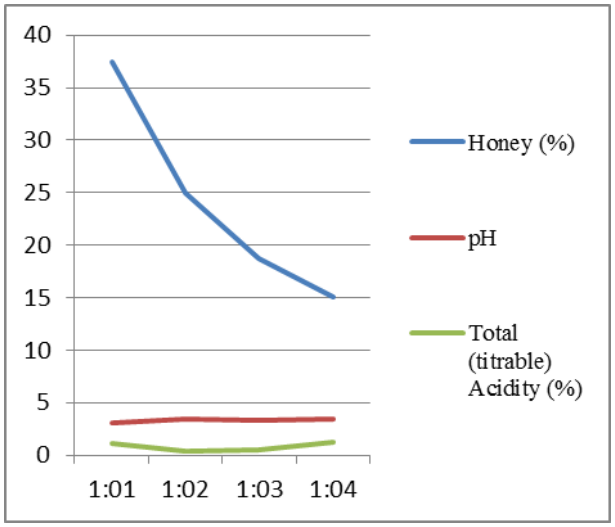

Figure 6. pH and total (tirable) acidity of four ratios of Honey to Water for Honey C (Mixture)

DISCUSSIONS

Wine is a kind of alcoholic beverage made from fermentation of fruit juice. It is becoming increasingly popular and is well accepted in Myanmar. And, Honey is used as folk medicine. Honey is found in every parts of the country around the world. So, Honey is used in wine-making as a fortifying agent. Starter culture preparation was necessary done to ferment the mixtures of Honey to Water.

To determine the composition of Honey, I sent the samples of Honey to Analysis Departments, Ministry of Science and Technology (MOST) and measured the parameters. Due to the results of Table , Honey was suitable to use in experiment because it includes much minerals and proteins.

To get the optimum ratio of Honey to Water in wine-making, 12 samples preparations were made on three sources of Honey. Four different ratios of Honey to Water (1:1,1:2,1:3,1:4) were fermented with fixed levels of starter culture .In this way, 12 samples preparations were obtained .

For wine analysis, pH of all samples was measured in which all pH values existed within the specification range. Wines are diluted acid solutions. Without acid, wine would spoil easily and unpalatably flat in taste.

Total (titrable) acidity was also measured in wine products. Wine producers need to know the titrable acidity of

musts in order to determine the proper amount of sulfur dioxide to add and also to decide on whether correction of the acidity needs to be made.

The titrable acidity is used during processing and finishing operations to standardize the wine and to follow undersirable changes due to bacteria, yeasts, etc. Commercial standards dictate total (titrable) acidity of wine is about 0.4-0.65%. According to figures, titrable acidity values of 1:2 ratio and 1:4 ratio of (A) Honey (zee honey) to water and 1:4 ratio of (B) Honey (pan-hnun honey) to water and 1:2 ratio and 1:4 ratio of (C)Honey (mixture honey) to water were 0.45,0.42,0.45,0.40, and 0.49 respectively . They were within the specification range. This could be due to the lesser formation of citric acid in wine products.

The alcohol content of wine products was determined and that of 1:1 ratio and 1:4 ratio of (A) Honey (zee honey) to water and 1:3 and 1:4 ratio of (B) Honey (pan-hnun honey) to water were highest alcohol content (%) . Other ratios had lower alcohol content. This could be due to incomplete fermentation or unequal presence of yeast cell in starter culture. Alcohol content and total soluble solid (°Brix) for four ratios of Honey to Water could be seen in fig respectively.

The ester and aldehyde content were important to measure the parameters of wine products in the research .Below about 200 mg/L ester content may give a desirable odour while above this content appear to give a spoiled character of wine . At a concentration of which it gives adverse effect. Content for wine specification should be below 750 mg/L. In this research, all 12 samples had ester and aldehyde contents within the specification range. These conditions pointed out all wine products not only have desirable odour but also are safe for drinking.

The essential research work was to detect microbial safety for wine products in which pasteurization at 120 ºC for 10 minutes was enough to sterilize all contamination microbes. The low pH value in wine products also inhibited the growth of contaminants. These facts pointed out all wine products had microbial safety for consumption.

IV. CONCLUSION

In the research, 12 samples with starter culture mixed four ratios and three sources of honey to water (1:1, 1:2, 1:3, and 1:4) are prepared. Then these wine samples are analyzed for the parameters such as pH, alcohol percentage, ester, ºBrix, total (titrable) acidity, are determined. Aledhyde content also determined in these samples for chemical safety. For the microbial safety, the samples are pasteurized at 120 ºC for 10 minutes. It can be concluded that sample preparation containing (B) Honey (pan-hnun honey) of Honey to Water 1:4 is the optimum preparation for making wine with honey. Because this sample had the higher alcohol percent.tage and the parameter of this such as aldehyde, ester, pH, and total (titrable) acidity are within the wine specification range.

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REFERENCES

[1] Haydak, Mykola H, ABC and XYZ of Bee Culture, 1951 Edition; Page 420.

[2] Maxwell, W, W, M.D., The Uses of Honey in Diets and Medicine.

[3] Chaloner, Reginald, Reverend, -Bible Bees Bee Craft (England) Volume XL III, No.3, March 1961, Pages 23-24.

[4] Townsend, G.F. 1939. Time and Temperature in relation to the destruction of sugar tolerant yeast in honey. J. Eco-Entomol 32:650-654.

[5] The sugar content of nectars. Biochm. J. 53:294-296.

[6] Smith, M.R., W.F. Mc Caughey, and A.R. Kemmerer-1969. Biological effects of honey. J. Apec.Res. 8:99-110.

[7] Seymour, F.I., and K.S.West. 1951, Honey- its role in medicine. Med. Times 79:104-107.

[8] Sacket, W.G.1919. Honey as a carrier of intestinal disease Col.Arg. Expt.Sta Bull. 252.

[9] Rubin, N, A.R.Gennaro, C.N.Sideri, and A.Osol 1959. Honey in medicinal preparations. Am.J.Pharm. 131:246-254.

[10] Nelson, E.K., and H.H.Motten. 1931. Some organic acids in honey. Ind.Eng. Chem: 23:335-336.

[11] Morse, R.G. 1954. Honey-Wine G1-Bee Cult. 82:716-717.

[12] Morse, R.G. 1958. Annotated bibliography on honey wine (Mead). Dept. Entomol. Cornell Univ.

[13] Lochhead, A.G.1933. Factors concerned with the fermentation of honey. Zent.Bakt.Parasotent. 2 abt. 88:296-302.

[14] Hatdak, M.H., L.S.Palmer, and M.C.Tanquary. 1942. The role of honey in the prevention of cure of nutritional anemia in rats. J.Pediatrics 26(6): 763-768.

[15] Hatdak, M.H., L.S.Palmer, M.C.Tanquary, and A.E.Vivino. 1942. Vitamin contents of Honeys. J.Nutrition 23:581-587.

[16] Church. J. 1954. Honey as a source of the antistiffness factor. Fed. Proc, 13:26.

[17] Dennis, C.B. 1957. A background to mead making. 3rd Ed.Central Assoc.Beekeepers.Ilford, Essex. England.

[18] Bulman, M.W. 1955.Honey as a surgical dressing. Bnt. Bee J. 83:664-665.

[19] Amrine, M.A. and Curess, W.V.1960. The technololgy of Wine Making, AVI Pub.Inc, Westport.

[20] Amrine, M.A. and Ough, C.S. 1974. Wine and Must Analysis.

[21] Aye Aye Mar. 1999.M.S Thesis. Product Development of Rice Wine. Department of Chemical Engineering, Yangon Technological University.

[22] Bell. R.C 2000. Updates on Wine and Health, American Journal of Clinical.