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Optimization of Fermentation Parameters for Grape

Aye Aye Mar[*], Nwe Nwe Aung[**]

Abstract

This research was emphasized on the fermentation of grape, grown in Meikhtila Township, Mandalay Region, Myanmar. The main objective of this research was to stimulate agricultural production by obtaining marketable products and to generate both rural and urban employment, etc. The fermentation of grape was carried out with cultivated yeast. The optimum parameters such as the effect of sugar syrup concentration, the effect of yeast amount, the effect of sugar, the effect of diammonium phosphate, the effect of pectinase enzyme, etc, were determined. The characteristics of fermented grape juice such as pH, soluble solids, acidity, specific gravity, alcohol content, sugar content, turbidity and organoleptic properties were also investigated. It was observed that sugar concentration (1.15) sp.gr, yeast (0.25)g, sugar(5)g,diammonium phosphate(0.01)g, pectinase enzyme(0.20)g were the most suitable conditions for the fermentation of grape.

Key words: Grape, Fermentation, Sugar syrup concentration, Yeast, Sugar, Diammonium phosphate, Pectinase enzyme

Introduction

Grape is one of the finest fruits and healthiest food. Grapes are a rich source of vitamins and minerals that can contribute to a balanced healthy life. Among the fruits, grape is a delicious, refreshing and nourishing fruit. Grape can be eaten fresh as table grapes or they can be used for making wine, jam, juice, jelly, raisins, vinegar and grapes seed oil. The grape vines are cultivated mainly in all temperature regions of the world. (http://www.shodhganga.inflibnet.ac.in/jspui/pdf)

Wine is an alcoholic beverage typically made of fermented grape juice. The natural chemical balance of grapes is such that they can ferment without the addition of sugars, acids, enzymes or other nutrients. Wine is produced by fermenting crushed grapes using various types of yeast. The yeast is responsible for the production of ethanol in alcoholic drink. *Saccharomyces cerevisiae* has adapted in several important ways and be able to break down their foods through both aerobic respiration and anaerobic fermentation. Different varieties of grapes and strains of yeasts are used depending on the type of wine being produced. (http://www. wine dining net)

Many varieties of wine are made throughout the world. The general classification of wines are red wines (made from grapes without removing the skins), white wines (made from grape juice) and sparkling wines (carbonated white wine). Wine naturally contains about 80-85 % water, 10-14 % alcohol, less than 1 % fruit acids and hundred of aroma and flavor components in very small amounts. The specific gravity of wine should have between 0.985-1.16. (http://www.apitco.org/profiles/wine%20 project. pdf)

To replace imported products like squash, sauce, wines, pickles, etc., the fruit and vegetable processing activities should be set up in Myanmar, one of the developing countries. In addition, Myanmar is an agro-based country and grape cultivation is also extended in the Shan State besides the Divisions of Central Myanmar such as Meikhtila, Pyawbew, Yamethin, Kyaukpadaung townships.

[*] Assistant Lecturer, Department of Industrial Chemistry, Yadanabon University.
[**] Professor, Dr., Department of Industrial Chemistry, Yadanabon University.

Materials and Methods

Materials

Grapes, grown in Meikhtila Township, Mandalay Region were used. Yeast, sugar, diammonium phosphate and pectinase enzyme were obtained from local markets.

Preparation of Sugar Syrup

Sugar syrup of desired Brix was prepared by adding sufficient sugar to the water to get the desired specific gravity of syrup.

Method of Preparation of Fermented Grape

Good sound and fresh mature grapes were destemmed and washed thoroughly with water to remove all adhering dirt. The grapes were weighed and then crushed by blender.

Fermentation of Grape

About 100 g of grape must was mixed with 100ml of sugar syrup (specific gravity 1.15), 0.1 g of yeast, 5 g of sugar, 0.02 g of diammonium phosphate and 10 ml of distilled water were added into the beaker and stirred thoroughly for 15 minutes. In this manner, the yeast culture solution and 0.1 g of pectinase enzyme were added to the above mixture and stirred thoroughly. The mixture was poured into the pre-sterilized bottle and stored to ferment at room temperature for 10 days. Later, CO_2 gas was evolved from fermentation bottle and allowed to release the evolved CO_2 gas. When fermentation was slow down, the mixture was racked out of the pre-sterilized bottle and aged for one month. After that, the sediment was filtered from the wine and sterilized. Finally, grape wine was stored in cool and dark place.

Effect of Sugar Syrup Concentration on the Characteristics of Grape Wine

The effect of sugar syrup concentration on the characteristics of grape wine was determined by varying sugar syrup concentration [specific gravity (1.01, 1.05, 1.1,1.15, 1.2)] using the same procedure mentioned in above. The value of pH, soluble solids, alcohol content and organoleptic properties were determined and the results are shown in Table (1).

Effect of Yeast on the Characteristics of Grape Wine Based on Constant Amount of Diammonium Phosphate (DAP) and Sugar in Yeast Culture

To investigate the effect of various amount of yeast on the characteristics of grape wine, the amount of yeast in the range of (0.05,0.1, 0.15, 0.2, 0.25, 0.3) g was used respectively, while the other variables were fixed using the same procedure mentioned in above. The value of pH, soluble solids, alcohol content and organoleptic properties were determined and the results are shown in Table (2).

Effect of Sugar in Yeast Culture on the Characteristics of Grape Wine Based on Constant Amount of Diammonium Phosphate (DAP) and Yeast

The effect of sugar in yeast culture on the characteristics of grape wine was determined by varying amount of sugar (1, 5, 10, 15, 20,) g while the other variables were fixed using the same procedure mentioned in above. The value of pH, soluble solids, alcohol content and organoleptic properties were determined and the results are shown in Table (3).

Effect of Diammonium Phosphate (DAP) on the Characteristics of Grape Wine Based on Optimum Amount of Yeast and Sugar

The effect of DAP was determined by varying amount of DAP in grape wine in the range of (0.005, 0.01, 0.02, 0.03, 0.04, 0.05) g while the other variable were fixed in the

fermentation of grape. To optimize the amount of DAP, pH, soluble solids, alcohol content and organoleptic properties were investigated and the results are shown in Table (4).

Effect of Pectinase Enzyme on the Characteristics of Grape Wine

The effect of pectinase enzyme on the characteristics of grape wine was determined by varying amount of pectinase enzyme (0.05, 0.1, 0.15, 0.20, 0.25) g while the other variables were fixed using the same procedure mentioned in above. The value of pH, soluble solids, alcohol content and organoleptic properties were determined and the results are shown in Table (5).

Analysis of Grape Wine

Determination of pH

The sample solution was tested with a glass electrode of pH, which was standard standardized with buffer solution at pH 6.99. pH of the solution was obtained by reading from pH meter. (KL-009(1), CE, made in China). Then, the pH value of grape wine were determined using the method P4 in Food Analysis; Analytical and Quality Control Methods for Food Manufacture and Buyer, Lees, 1975. The determination of pH was carried out at Industrial Chemistry Department, Yadanabon University. The data of different samples are shown in Tables (1) to (6).

Determination of Soluble Solids

The soluble solids content of grape wine was determined by refractometer (CHENG DU TAI HUA GUANG XUE Co. LTD. SHOU CHI ZHE GUANG YI, made in China) using the method S8b in Food Analysis: Analysis and Quality Control Methods for Food Manufacturer and Buyer, Lees, 1975. The data of different samples are shown in Tables (1) to (6).

Determination of Specific Gravity

Specific gravity of grape wine were determined by Sike's hydrometer (made in UK) at Mandalay Rum Factory. The data of different samples are shown in Tables (1) to (6).

Determination of Alcohol Content

About 200 ml of sample and 200 ml of the H_2O were added into the distillation flask. 250 ml of measuring cylinder was used to receive distillate. The strength of the distillate was determined by using Sike's hydrometer and thermometer. After having the strength, the corresponding percentage of alcohol by volume was obtained from any Standard Alcohol Tables. The data of different samples are shown in Tables (1) to (6).

Determination of Sugar Content

The sample was prepared in above procedure by distillation. From the residue in the distillation flask, final sugar content by weight was determined by using refractometer (MASTER-M) and the data are shown in Table (6) .

Determination of Turbidity

The amount of turbidity present in wine sample was determined by HACH meter (model no. DR 2000) at Myanmar Pharmaceutical Factory (Sagaing), Ministry of Industry. The data of sample are shown in Table (6) .

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Determination of Acidity

About (5)ml of grape wine was added in the conical flask and mixed with (50) ml of distilled water. Then, titrated with 0.1 M standard sodium hydroxide solution from the burette and phenolphthalein indicators was used using the method A4a in Food Analysis: Analytical and Quality Control Methods for Food Manufactures and Buyer, Lees, 1975. The titrant volume was noted as soon as it turned pink (end point) and its total acidity was calculated using tartaric acid present and data are shown in Table (6) .

$$\text{Acidity} = \frac{\text{volume of NaOH x (molarity of base) x (M.eqwt acid)}}{\text{volume of sample}} \text{ x } 100$$

Acid factor, for 0.1 M solution, tartaric acid = 0.0075 gms

Results and Discussion

Good, sound and fresh mature fruit must be selected to obtain good quality of wine. The grape must was fermented with yeast which converted the sugar from the fruit to alcohol and carbon dioxide. Fruits can be fermented with wild yeast but this wine have not good flavors, good taste and to go to oxidation. Therefore, in this experiment, grape was fermented by using cultivated yeast. And then, the properties of prepared wine was determined and compared with literature values.

In this fermentation, the various amount of [sugar syrup, specific gravity (1.01,1.05,1.10,1.15,1.20)] were used, respectively . Among them, the sugar syrup (specific gravity 1.15) was chosen as optimum condition according to alcohol content and organoleptic properties. The alcohol content of sample was within the accepted range of 6-14% but alcohol content of grape wine with [sugar syrup specific gravity (1.10,1.2)] was more than literature value. The pH of all samples were nearly the same.Table (1) represents the effect of sugar syrup concentration on the characteristics of fermented grape.

The effect of yeast on the characteristics of fermented grape based on the constant amount of diammonium phosphate (DAP) and sugar in yeast culture was shown in Table (2). The alcohol content of grape wine using yeast amount (0.05,0.10)g were closely to the literature value. However, the alcohol content of amount of yeast (0.25)g was more than literature value. (0.25) g of yeast was chosen as optimum condition for the preparation of wine because of organoleptic properties which was better than that of the other samples. It was noted that the amount of yeast (0.25) g gave the bright red color and pleasant odor of wine.

From the results of Table (3),the sugar amount in yeast culture (5)g was chosen as the optimum condition because of its organoleptic properties. The value of alcohol content was found to be nearly the same for all samples. Although the amount of sugar (5,10,15,20,25)g gave bright red color and pleasant odor, (5)g was chosen as the optimum condition because of the organoleptic properties and economic point of view.

The effect of yeast nutrient diammonium phosphate (DAP) on the characteristics of fermented grape based on the optimum amount of yeast and sugar was also studied and shown in Table (4). The values of pH and alcohol content were within the acceptable range of 2.9-4.2 and 6-14% respectively. Moreover, from the organoleptic properties , the amount of DAP (0.01)g was fit for the fermented grape.

Table (5)show that the effect of the amount of pectinase enzyme in wine. When the more amount of pectinase enzyme was used, the more clarification of wine was obtained. (0.20)g of pectinase enzyme was chosen as the optimum amount because of the appearance of product which was more clear than that of the other samples and economic aspect.

For wine making by using cultivated yeast as shown in Table (5), the optimum condition [sugar syrup (specific gravity1.15)] and yeast culture including the amount of (0.25)g of pectinase enzyme gave good result due to its organoleptic properties.

According to Table (6), the characteristics of sample such as pH, acidity, specific gravity, alcohol content were within the acceptable range of literature values. The values of soluble solids and sugar content of sample were close to the value of May Rose from market. However, the data for the turbidity of sample was more than that of May Rose. The more value of turbidity indicated that aging time and fining agent were also the important parameters for making wine. So, aging time was still needed to improve the clarification of wine and fining agent such as gelatin should be used.

Table (1) Effect of Sugar Syrup Concentration on the Characteristics of Grape Wine

Grape = 100g Yeast = 0.1g Pectinase Enzyme = 0.1 g
DAP = 0.02g Water = 10ml Sugar Syrup = 100ml Sugar = 5 g

Sr. No	Sugar Syrup (sp.gr)	pH	Soluble Solids (°Brix)	Specific Gravity	Alcohol Content (%)(v/v)	Organoleptic properties
1	1.01	4.3	3	0.9870	9.7	pink color, fermented odor, slightly bitter taste, slightly clear
2	1.05	4.5	5	0.9853	11.1	pale red color, fermented odor, slightly bitter taste, slightly clear
3	1.10	4.3	7	0.9810	14.8	pale red color, fermented odor, slightly bitter taste, slightly clear
4	1.15*	4.3	12	0.9819	14	red color, pleasant wine color, sharp and sweet taste, slightly clear
5	1.20	4.1	13	0.9784	17.1	red color, pleasant wine color, sharp and sweet taste, slightly clear

*Optimum Sugar Syrup Concentration = sp.gr1.15

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Table(2) Effect of Yeast on the Characteristics of Grape Wine Based on Constant Amount of Diammonium Phosphate (DAP) and Sugar in Yeast Culture

Grape = 100g Sugar = 5 g Pectinase Enzyme = 0.1 g
Water = 10ml DAP = 0.02g Sugar Syrup = 100ml

Sr. No	Yeast (g)	pH	Soluble Solids (°Brix)	Specific Gravity	Alcohol Content (%)(v/v)	Organoleptic properties
1	0.05	3.8	12.0	0.9851	11.3	red color, fermented odor, slightly bitter taste, slightly clear
2	0.10	4.3	12.0	0.9814	14.5	red color, fermented odor, bitter taste, slightly clear
3	0.15	3.9	11.0	0.9761	19.2	red color, fermented odor, bitter taste, slightly clear
4	0.20	3.6	10.5	0.9761	19.2	red color, pleasant wine color, sharp and sweet taste, slightly clear
5	0.25*	3.8	11.0	0.9804	15.3	bright red color, pleasant wine color, sharp and sweet taste, slightly clear
6	0.30	3.8	11.0	0.9765	18.9	bright red color, pleasant wine color, sharp and sweet taste, slightly clear

*Optimum Amount of Yeast = 0.25 g

Table (3) Effect of Sugar in Yeast Culture on the Characteristics of Grape Wine Based on Constant Amount of Diammonium Phosphate (DAP) and Yeast

Grape = 100g Yeast = 0.25 g Pectinase Enzyme = 0.1 g
Water = 10ml DAP = 0.02g Sugar Syrup = 100ml

Sr. No.	Sugar (g)	pH	Soluble Solids (°Brix)	Specific Gravity	Alcohol Content (%)(v/v)	Organoleptic properties
1	1	3.5	8	0.9802	15.5	pale red color, fermented odor, slightly bitter taste, slightly clear
2	5*	3.8	11	0.9804	15.3	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
3	10	3.7	14	0.9804	15.3	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
4	15	4.0	14	0.9826	13.4	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
5	20	4.0	17	0.9815	14.4	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear

*Optimum Amount of Sugar = 5 g

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Table (4) Effect of Diammonium Phosphate (DAP) on the Characteristics of Grape Wine Based on Optimum Amount of Yeast and Sugar

Grape = 100g Yeast = 0.25 g Sugar Syrup = 100ml
Sugar = 5 g Water = 10ml Pectinase Enzyme = 0.1 g

Sr. No	DAP (g)	pH	Soluble Solids (°Brix)	Specific Gravity	Alcohol Content (%) (v/v)	Organoleptic properties
1	0.005	3.6	11	0.9794	16.2	pale red color, fermented odor, bitter taste, slightly clear
2	0.01*	3.6	11	0.9819	14	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
3	0.02	3.8	11	0.9804	15.3	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
4	0.03	3.3	9	0.9801	15.6	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
5	0.04	3.4	7	0.9814	14.5	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear
6	0.05	3.7	9	0.9787	16.8	bright red color, pleasant wine odor, sharp and sweet taste, slightly clear

*Optimum Amount of DAP = 0.01g

Table (5) Effect of Pectinase Enzyme on the Characteristics of Grape Wine

Grape = 100g Yeast = 0.25 g Sugar Syrup = 100ml
Sugar = 5 g Water = 10ml DAP = 0.01g

Sr. No.	Pectinase Enzyme (g)	pH	Soluble Solids (°Brix)	Specific Gravity	Alcohol Content (%)(v/v)	Organoleptic properties
1	0.05	4.1	8.0	0.9795	16.1	pale red color, fermented odor, bitter taste, hazy
2	0.10	3.6	11	0.9810	14.8	red color, pleasant wine odor, bitter taste, slightly clear
3	0.15	3.9	10	0.9816	14.3	red color, pleasant wine odor, bitter taste, slightly clear
4	0.20*	3.9	9.0	0.9819	14	red color, pleasant wine odor, sharp and sweet taste, sparkling clear
5	0.25	3.9	9.5	0.9807	15.0	red color, pleasant wine odor, sharp and sweet taste, sparkling clear

*Optimum Amount of Pectinase Enzyme = 0.20 g

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Table (6) Comparison of Physico-Chemical Properties of Experimental Values, Literature Values and Commercial Values of Grape Wine

Sr No	Properties	Experimental	Literature	May Rose
1	*pH	3.9	2.9-4.2	3.5
2	*Soluble Solids(°Brix)	9	-	11
3	*Acidity(%) (v/v)	0.315	0.3-0.55	0.319
4	**Specific Gravity	0.9819	0.985-1.16	0.9819
5	**Alcohol (%) (v/v)	14	6-14	14
6	**Sugar Content (%) (w/w)	9	-	11.2
7	***Turbidity (FTU)	912	-	664.50

* Determination of acidity, pH and soluble solid were carried out at Department of Industrial Chemistry, Yadanabon University.

** These analysis were carried out at Mandalay Rum Factory.

***Turbidity was determined at Myanmar Pharmaceutical Factory (Sagaing), Ministry of Industry.

Note: The literature values were obtained from the Chemical Analysis of Food, seven Edition, Pearson, D. (1976), New York.

Conclusion

Grape is one of the favorite fruit in Myanmar. Grape consists of proteins,lipids,carbohydrates,energy,water,calcium,iron,magnesium,phosphorus,potassium,sodium,vitamin C, vitamin B_1,vitamin B_2,vitamin B_3 and vitamin B_6.Good quality products were made from good quality raw materials. The quality of wine was dependent on the fermentation time, storage temperature and aging time. Excessive alcohol consumption has adverse health effect but small quality of wine should only be consumed for medicinal and health gain purpose.In this research, yeast culture including sp.gr (1.15) sugar syrup concentration, 0.25 g of yeast , 0.01 g of DAP,0.20 g of pectinase enzyme and 5g of sugar gave the best result due to its alcohol content, organoleptic properties, pH and soluble solids.

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References

Dauthy Enahesu, M. (1995), Fruit and Vegetable Processing, FAO Agricultural Service, Rome.
Myat Su Su Khin (2007) ,Preliminary Study on the Preparation of Pineapple Wine, MSc Project Term Paper, Department of Industrial Chemistry, Yadanabon University.
Pearson, D. (1976), The Chemical Analysis of Food, Seven Edition, New York.
The American Wine Society, (2003), ninth Edition, The Complete Handbook of Winemaking, Published by G.W. Kent, Inc.
Woodman, A.G, (1941), Food Analysis, McGraw-Hill Book Company, Inc., NewYork, London.

Website

http://www. wine dining net
http://www.apitco.org/profiles/wine%20 project. pdf
http://www.invista.com/health/herbs/grape.html
http://www.fao.org/docrep/003/×6897e/×6897e09.html
http://www. how to things. com/ home---/a 3060.how.to.harvest.grapes.html
http://www. fresh central.com
http://www.wed md.com/vitamin-supplements
http://www.wikipedia.org
http://www.extension.isostate.edu/NR/radonlyrers/.../composition of grape.pdf
http://www. healing daily.com
http://www.eckraus.com
http://www.wikipedea.org/wiki/Diammonium phosphate
http://www.homewine making.com/wine making-2a.html
http://www.eckraus.com/wine-recipes