Global Scientific JOURNALS

GSJ: Volume 9, Issue 10, October 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Mixed fruits wine production from *Saccharomyces cerevisiae*

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ABSTRACT

Juices of carrot, orange and, papaya were mixed to produce and investigate, fermented wine by using *Saccharomyces cerevisiae* (a starter culture). The mixture of these fruits juice has been subjected to anaerobic fermentation. During this period, aliquot samples were used to analyses the various parameters that were pH, specific gravity, temperature, sugar, titrable acidity, alcohol. Analysis of these parameters was done by using the standard protocol. During the investigation of the above mentions parameters changes were recorded as follows gradual decreases in specific gravity [1.070 to 0.953], change in pH [5.5 to 4.99] increases in alcohol content (0 to 5%) was noted on the final day of fermentation [after 24 days]. The consequence of this study's reflecting those juices of fruits Papaya, carrot and orange can acceptable for the mixed fruits juice wine with a starter culture of *Saccharomyces cerevisiae*.

INTRODUCTION

Wine is one of the alcoholic products of fruit juices prepared by fermentation which is also the most consumable drink in the world. (1). In consonance of the European Union, the fermented juice of grapes is juridically considered as a wine (2). It has been stated by Antsier in the year 1987, consumption of three to four glasses of fortified wine in a day was the ample amount that can be routinely taken because it does not stimulate any chronic symptoms (3).

In the process of fermentation, yeast acts as a starter culture means it initiates the process of conversion of glucose into alcohol and other secondary product which can be carbon dioxide, organic acids, that further can form many chemical compounds such as ester aldehyde (2,4).

Intake of mixed fruits juices wine in an appropriate amount having positive impact on body because of the presence of nutritional components such as vitamins, amino acids, sugar ethyl alcohol and other flavoring constituents (5). If it is consumed in an adequate amount, it shows good health related-positive impacts such as helps in reducing heart-related diseases especially prevent coronary heart diseases also act as an antiaging supplement. In Monica project (Monitoring Of trends and determinants in cardiovascular disease), which was a major international collaboration study which had the objective to study the trends and determinants in cardiovascular disease, they conclude that the intake of red wine helps in paradoxical protection from cardiovascular diseases in the European population of French (6). Red wine one of the wine contains procyanidins which provide protection against heart diseases and lung cancer (7).

Pawpaw belongs to the family of *caricaceas*. Papaya comes under herbaceous fleshy plants means a pulpy fruit (8). Leaf bark and twig tissues of pawpaw plants having annonaceous acetogenin (natural compound) that shows reactivity towards anti-tumor and pesticides. Milk constituent in papaya fruit has an active component, which is papain. It has been used for beer clarification (9). Mixed fruit juice wine can be an effective carrier for many nutrients either natural or synthetic such as minerals, vitamins, and much functional foods (10) Carotenoid which having Beta- Carotene also associated with more lycopene's is also present in fresh papaya fruits (11).

Domestic carrot is a seasonal biennial plant of spring and summer with a rosette of leaves while having tap root and these roots can store a large number of carbohydrates (12). Also used in skin and hair care products example of which is Theo derma skincare lotion having carrot extract which is mainly for dry skin type. Lutein is a chemical compound present in carrot that helps in reducing health-related problems like inflammation in the brain and memory deficits (13).

Orange is a citrus fruit which has been processed into wine. Kazan is one of the citrus sinensis

variety of orange reproduce in very large amount in the Kazan area in Southern Turk (14). Grapefruit wine is differ in flavor when compared with orange wine because of the metabolism of the fruits volatile and secretion of yeast volatile during fermentation (15).

Flavoring compounds are the key contributor in determining the final product of fermentation as they having much medicinal value (16). Phytochemicals are present in fruit plants and any other part of the plant, which can be considered nutritive and non- nutritive biological compound. Phytochemicals also having health-related beneficial effects (17,18). Various types of Phytochemicals such as Anthraquinone, flavonoid, protein, carbohydrate, phenolic compound etc. Phytoalexins is one of the unique and delicious class of phytochemicals. Its uniqueness is, its good response towards external stress i.e. draught, sunburn, etc. A stipend type of constituent is having effectiveness against heart diseases that is a cardiovascular disease also having a positive impact against the tumor, and they are generally used in juices of red grapes wine (19). Physiochemical tests are commonly used to determine wine certification. These test include determination of specific gravity, alcohol content, etc.(20,21,22)

2. MATERIAL AND METHOD :

2.1 Procurement of raw material and must Fermentation:

Three different fresh fruits were used which include Carrot *(Daucuscarrota)*, Papaya *(Carica papaya)*, and Orange *(Citrus sinesis)* for making wine, which has been purchased from the local market of Panchkula.

2.1.1. The exaction of fruit juice (Fermentation media)

The fruits were washed thoroughly with sterile water and rinsed to remove unwanted material. Pulp from papaya was extracted and filtered with a muslin cloth. Fresh juice of orange and carrot was also extracted by filtration.

2.1.2. Must Preparation:

Fruit juices from papaya, orange, and carrot has been taken and mixed in 10:17:9 ratios respectively. The fruits were cut, manually deseeded, blended and filtered to obtain the must. The must was then transferred to a sterile 500 ml flask, yeast has been inoculated (as a starter culture) and incubated at 42^{o}C for 24 days with the analysis of parameters such as pH, temperature, Specific gravity, Titrable acidity, sugar content and ethanol content.

2.2 PHYSIOCHEMICAL ANAYLYSIS OF WINE

Physiochemical parameters were routinely analyzed at a regular interval of 3 days. These parameters included the monitoring of pH, sugar, temperature, specific gravity, Titrable acidity and ethanol content. These tests usually help in fixing the wine quality (for wine consumer as per health concern).

2.2.1 pH determination

The pH scale is used to determine the acidic and basic strength of any solution. pH meter has been used to check the pH. During wine fermentation decrease in pH has been observed because of the consumption of sugar in used fruits.

2.2.2 Sugar Content

The content of sugar was measured with the help of refractometer routinely. Change in sugar content was recorded Sugar is utilized by the yeast for the production of ethanol.

2.2.3 Specific gravity

It is measure as the ratio of the density of a substance to the density of a reference substance. For which special specific gravity bottle has been used. 25 ml specific gravity bottle was thoroughly cleaned with distilled water then put in a hot air oven at 50^{O}C for drying. After that weight of the bottle was recorded as (W_1) and filled it with distilled water. Surface of the bottle was cleaned with a wall of cotton wool and weighed as (W_2). Thereafter the bottle was filled with the “must” and weighed as (W_3). With the help of recorded values, the specific gravity was calculated (23).

$$S.\ G = \frac{W_3 - W_1}{W_2 - W_1} = \frac{S}{W}$$

Where S= Weight of the sample (W_3-W_1)

W=Weight of the volume of water (W_2-W_1)

2.2.4 Titrable Acidity

Titrable acidity is a measure of the amount of acid present in the given sample. It was estimated by titration method by the use of titrant i.e., Sodium hydroxide (0.1N) which is titrated against sample (wine), in a conical flask 5ml of wine sample and 20 ml of distilled water along with 2 to 3 drops of Phenolphthalein indicator. Phenolphthalein was used as an indicator. (24).

Which is calculated as:

T.A. = amount of NaOH used x 0.75

the Volume of sample taken

2.2.5 Ethanol content

The quantity of ethanol content was estimated by using hydrometer. Generally, ethanol content was increased during fermentation process because of conversion of sugar into ethanol due to yeast activity.

2.3 PHYTOCHEMICAL ANALYSIS

Phytochemicals are non-nutritive plant chemicals that usually have protective or disease preventive properties such chemicals show a rare side-effect on a living being. Many organic compounds present in different parts of plants like in roots, shoots, fruits etc. they impart positive physiological impact on human beings, flavonoid, alkaloid, tannin and many more are such bioactive compound. (25,26). A large group of Phytochemicals have been shown to have an inhibitory effect on all type of microorganism. (27).

2.3.1 Test for Anthraquinone

1 ml of wine sample was added in 1 ml of conc. Sulphuric acid (H_2SO_4) and 1 ml benzene in a test tube. Then 1 ml diluted Ammonia was added. Appearance of rose-pink color will indicate the positive result (28).

2.3.2 Test for flavonoid

Shinoda test

The wine sample was added with few fragments of magnesium ribbon and drop wise concentrated HCL was added if after few minutes pink color scarlet observed. This was taken as the flavonoid presence (25).

2.3.3 Test for protein

Ninhydrin's test: In test tube wine was mixed with 02ml of 0.2% chemical solution of Ninhydrin, presence of amino acid and protein is confirmed if violet color appeared. (25).

2.3.4 Test for carbohydrate

Molisch Test

Wine sample was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. Then 2ml of concentrated Sulphuric acid was poured along the side of the test tube. Appearance of violet color shows the presence of carbohydrate (29).

2.3.5 Test for tannin and phenol

Wine sample was mixed with 2ml of 0.2% $FeCl_3$ solution and the appearance of blue-green or black coloration shows that both the organic component are present. (30).

2.3.6 Test for alkaloid

The wine was added with 2ml of 1% solution of HCL and was gently heated. Then after that Mayer's and Wanger's reagent were added to the mixture. Turbidity of the precipitate was taken as proof for the presence of alkaloid. (25).

2.3.7 Test for saponins

In test tube, few ml of wine was added and mixed with 5ml of distilled water and the mixture was shaken vigorously. The appearance of stable foam was considered as the indication towards saponins presence in the given sample. (31).

2.3.8 Test for Terpenoid

Wine was dissolved in 2ml of chloroform and evaporated to dryness than 2ml of concentrated Sulphuric acid was added and heated for about 2 minutes. A grayish color indicates the presence of terpenoid (32).

3. RESULT AND DISCUSSION

3.1 Physicochemical parameter analysis

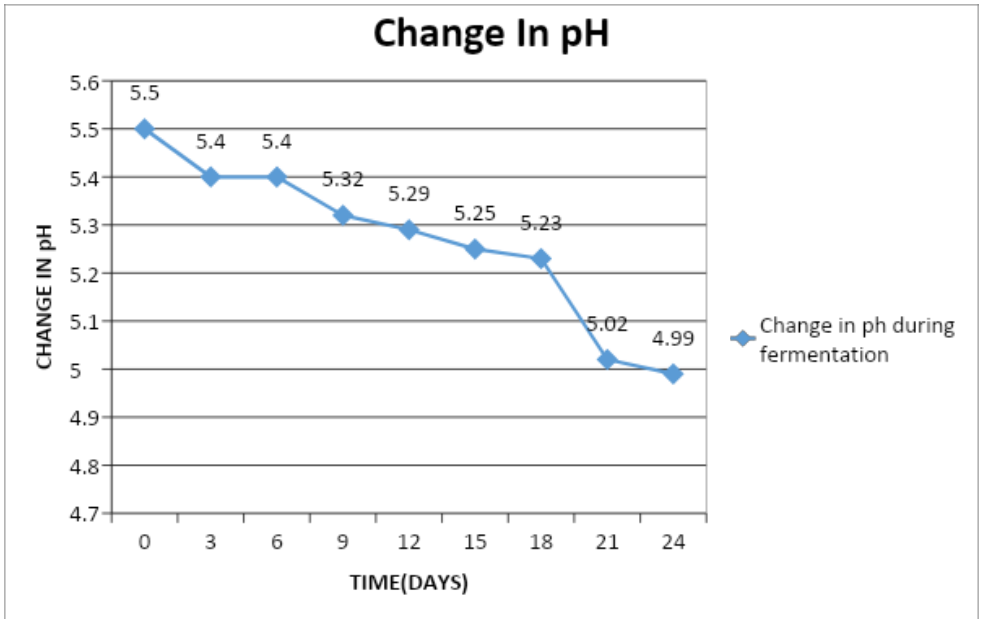
The following table is revealing the result of various parameter analysis determined during the fermentation of "must" after pitching with the yeast culture.

DAYS	Ph	Temp.	S.G.	T.A.	S.C.	E.C.
0	5.5	42	1.0783	0.002	20.5	0
3	5.4	29.9	1.0673	0.36	10	0.361
6	5.4	29	1.031	0.225	8	1.256
9	5.3	27	1.03	0.45	7.5	1.902
12	5.29	28	1.001	0.45	7	2.47
15	5.25	30	1.000	0.46	6.8	3.4
18	5.23	29	0.999	0.49	6	5.33
21	5.02	31	0.962	0.51	5.51	5.4
24	4.99	27	0.944	0.69	5	5.49

Table 1: Represents the record of parameters where Temperature, S.C.,T.A., S.G. and E.C. are temperature , Sugar content ,Titrable acidity, specific gravity and ethanol content respectively.

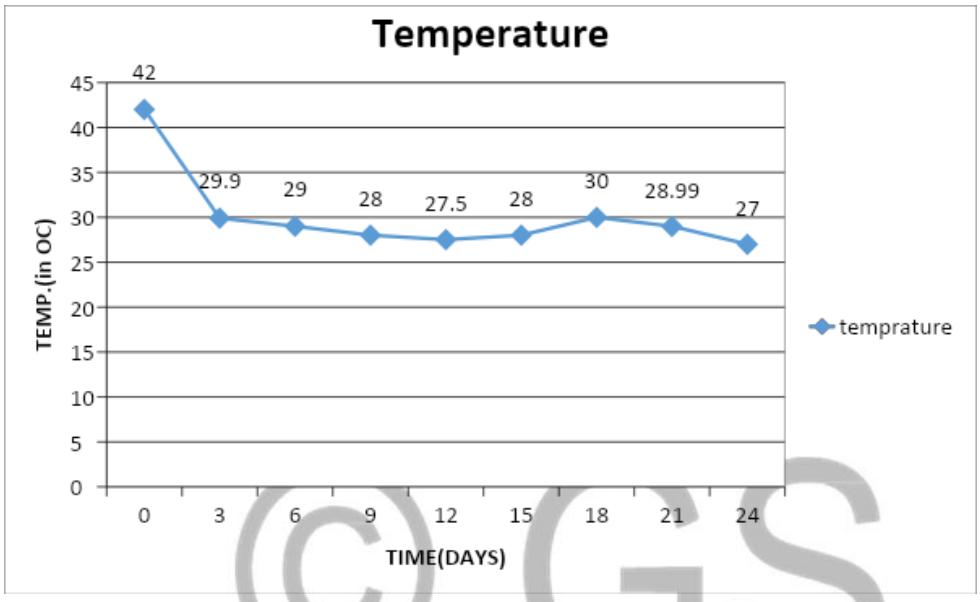
During the period of fermentation, various parameters like pH, temperature, specific gravity , Titrable acidity etc. were observed at a determined interval of time. There were fluctuation in these parameters were noted because of many factors like type of strain used and fermentation conditions during that time. Changes in these parameters are mention in further given graphical presentation.

FIG.3.1 Change in pH



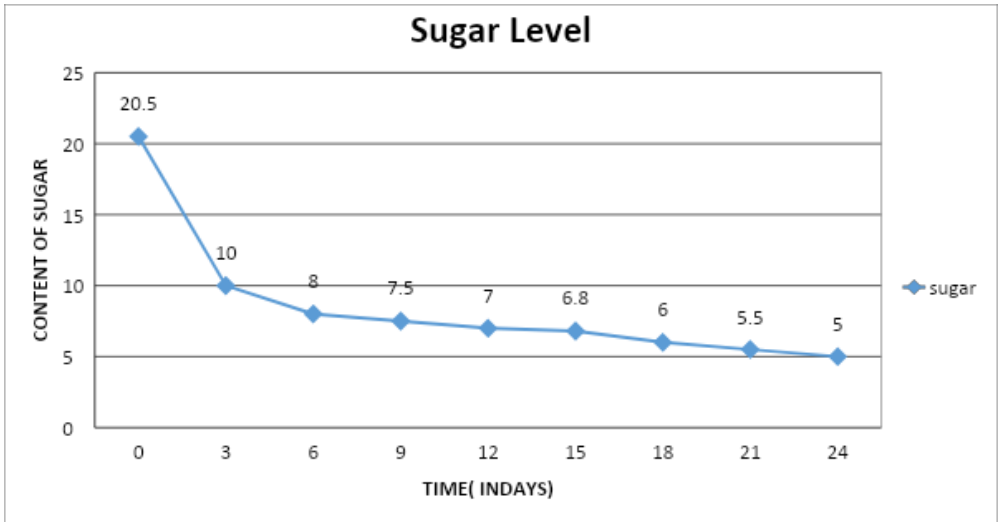
The given graph in figure 4.1 shows a gradual decrease in pH during fermentation of wine, overall we observed from the initial day to 24th day of fermentation, the consumption of sugar by yeast activity and continue falling of pH from 5.5 to 4.99. And we saw a steep decrease from 5.23 to 5.02 on 21 day of fermentation, final day of the process, the observed pH was 4.99. This given graph is showing progressive reduction of pH.

FIG.3.2 Variation in temperature during fermentation



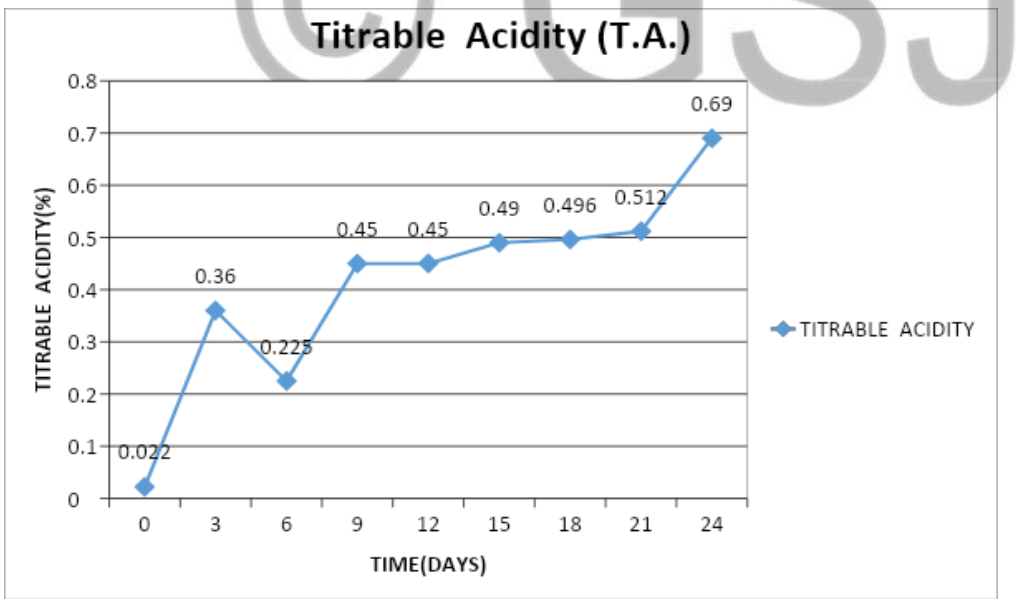
The given graph indicates the change in temperature during must fermentation activities carried out by *Saccharomyces cerevisiae* .On the very first day we have observed the temperature was 42 degrees Celsius and then frequent decrease on the third day of fermentation was recorded . Slight fluctuation in temperature was observed between the15th to 18th day and, on the final day its reached to 27^{O}C. This fluctuation in temperature may was observed due to microbial activities.

FIG.3.3 Given graph is Showing change in sugar content during fermentation of wine from mixed fruits juice.



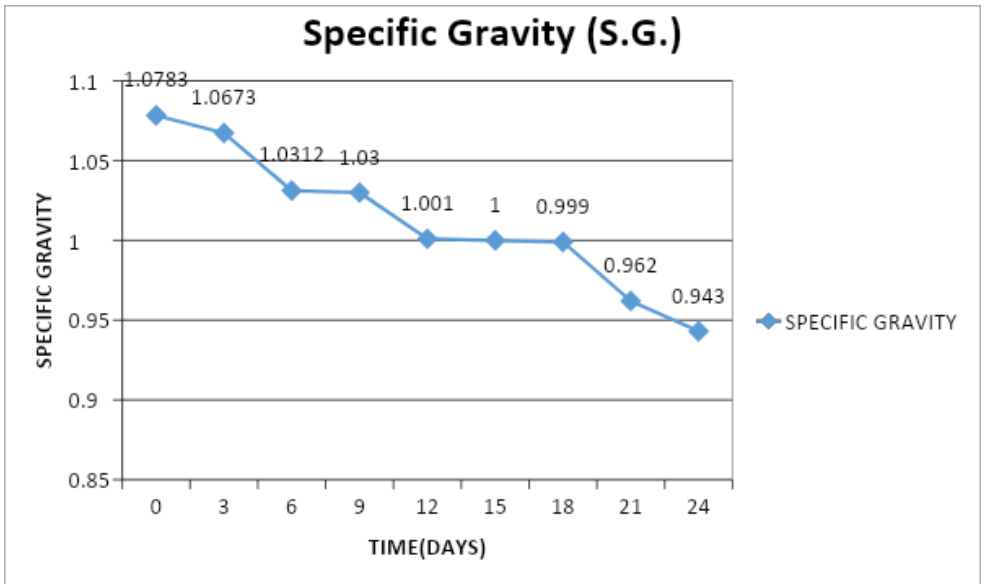
The given graph tells, decrease in the content of sugar of wine from initial stage to final stage. As we have observed reduction in sugar with the passage of fermentation day because of the utilization of sugar by the strain of yeast.

FIG. 3.4 Change in Titrable Acidity during period of fermentation.



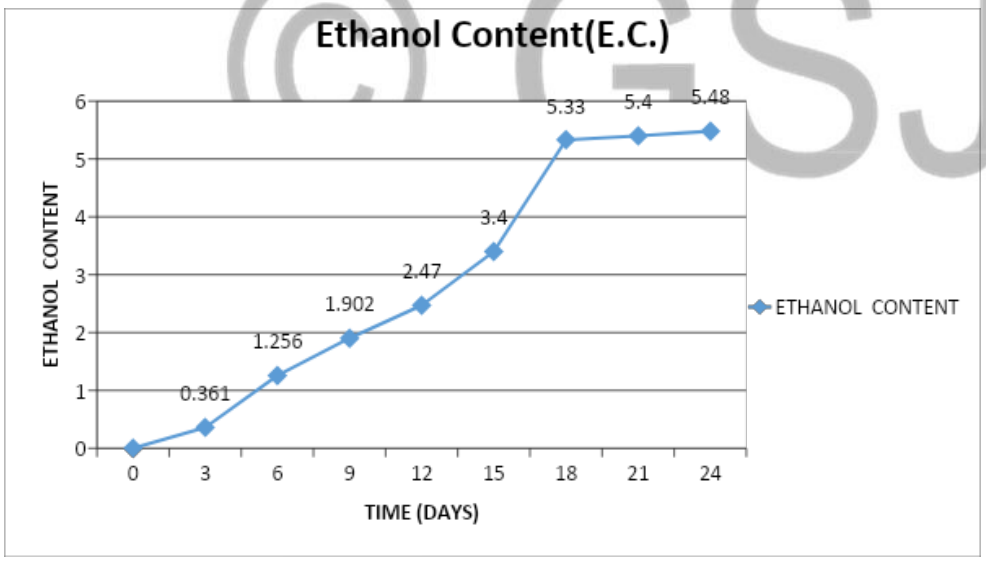
The given graph of titrable acidity shows fluctuation in the value of acidity during the 24 day of fermentation. On the first day acidity was 0.022 and on the last day of this process it reached at the value 0.69.

FIG.3.5 Variation in Specific gravity during the process of fermentation.



This gives the information about the specific gravity observed during wine production .In mixed fruit wine gradual decrease in specific gravity was noticed from 1.0783 -0.944 in 24th day respectively.

FIG. 3.6 Record of ethanol content during the period of wine fermentation.



The given graph information illustrate that the percentage of ethanol is increased gradually with the passage of fermentation process .And on the final day of this process of conversion of sugar to Alcohol and carbon-dioxide by the action of yeast was 5.49%,so we have observed this percentage as the final yield of ethanol content.

The total content of alcohol was increased from initial day to the end of fermentation; this was 0.36% on day one and 5.55% on last day of the fermentation process. This information resembles

with the result of Okunowo, W.O., Okotore,R.O., & Osuntoki,A.A,(2005). The change in the` level of alcohol would be due the change of their Phytochemicals conditions and others factors such as temperature or pH (33,34). It also has been disclosed that the expression of gene-level in yeast is effected by environmental factors such as temperature (35).

CONCLUSION:

Mixed fruit wines are undistilled alcoholic beverages made from basically grapes and other fruits like orange, papaya, carrot etc. Most of the fermented wines have nutritional values and their adequate amount of intake helps in reducing various disease related problems. The best example of such type of wine is red wine. Except for these benefits fermented wine also contain Phytochemicals as well as antioxidant. In this study the result are representing the decrease in content of sugar and simultaneously increase in ethanol content during the period of fermentation. Analysis of Phytochemicals disclosed the presence of alkaloid, flavonoid and terpenoid. The antioxidant and anticancerous study can be pursued and further investigation can be done with this project.

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