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Production and physiochemical analysis of Cantaloupe wine

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Abstract

Cantaloupe is a round melon enclosing moderately sweet orange flesh with good nutritional attributes. Production of wine from this fruit using baker's yeast (*Saccharomyces cerviseae*) increases a wine variety and also reduces its post harvest losses. Different parameters of wine were studied in comparison with control for analysis of quality and efficiency of wine. A gradual decrease in pH ranging from 5 to 3 was observed during the course of fermentation. Carotenoid content was reduced from 0.2724 mg/ml to 0.0048 mg/ml. As the fermentation proceeded, a gradual increase from 0.326 x 10^6cells/ml to 2.8 x 10^6 cells / ml in the yeast count was observed. Alcohol content increased from 2.5% to 8.7%. Specific gravity was decreased from 1.1681g to 1.1339g. Temperature gradually increased from 27.5^{0}C to 29.9 ^{0}C. The Antioxidant content was found to be 61.55% and phenolic content declined from 20.9 mg/ml to 16.3 mg/ml. Reducing sugar decreased from 9.39 mg/ml to 7.21 mg/ml. Electrical conductivity was found to be 6.51 µs. Organoleptic characters observed on 20th day of wine production provided a satisfactory result. Finally FTIR analysis was performed and the functional groups was identified, especially the OH group. The studies had shown that the wine produced was acceptable and of good quality.

Keywords: Alcohol, Baker's yeast, Cantaloupe, Fermentation, Juice, Wine.

Introduction

Wine is an alcoholic beverage typically made of fermented fruit juice. Wine making involves the use of yeast to ferment the 'must' of the chosen fruit for a number of days depending on the winemaker. The main organism which is responsible for alcoholic fermentation usually belongs to the genus *Saccharomyces* (Okeke et al., 2015). Production of wine from these fruits could help reduce the level of post harvest loss and increases variety of wines (Ogodo et al., 2015).

Cantaloupe, also known as musk melon belongs to the family Cucurbitaceae. The botanical name is *Cucumis melo*, which is a round melon with firm, orange, moderately sweet flesh and a thin reticulated light grey rind. (Ensminger and Ensminger, 1993). It has a variety of antioxidants and anti inflammatory properties (Vouldoukis et al., 2004). Phytonutrients including the carotenoids, alpha carotene, beta carotene, lutein, beta–cryptoxanthin and Zeaxanthin (Napier et al., 2006). It also contains flavanoid

luteolin, organic acid- ferulic acid and caffeic acid and two cucurbitacins – cucurbitacin B and cucurbitacin E (De Melo et al., 2000). Cantaloupe is an excellent source of vitamin A in the form of carotenoid and vitamin C (Laur and Tian, 2011). It is also a good source of potassium, dietary fibers, vitamin B1, vitamin B3 (Niacin), Vitamin B6, folate, magnesium, copper and vitamin K. Cantaloupe has the following health benefits such as they improve vision, prevents asthma, prevents cancer, boosts immunity, reduces dehydration, skin and hair care, regulates blood pressure, control diabetes and treats arthritis.

Considering the importance and properties of this fruit, it is more advantageous to use this product for wine production. This will enhance and improve health of the consumers. Therefore the present study focused on the production of cantaloupe wine and determination of physiochemical, sensory properties and FTIR analysis of the respective wine.

Materials and Methods

Collection of substrate

Cantaloupe *(Cucumis melo var. cantalupensis*) used in wine production were purchased from the local market of Cherthala, Alappuzha district, Kerala state, India.

Yeast strain

For the fermentation process, yeast strain *S. cerevisiae* was used. The strain was obtained from culture collection of the Department of Biotechnology and Research, K.V.M. College of Science and Technology, Kerala, India.

Inoculum preparation

Sterilized glucose yeast extract broth (glucose 1%, malt extract 0.3%, peptone 0.5% yeast extract 0.3%, and pH 4.5) was used for culturing *S. cerevisiae* in a rotary shaker for 24 hours at 60 rpm at 30 ^{o}C. Then the cells were separated by centrifugation at 6000 rpm for 10 minutes. The cells were then washed and re suspended in distilled water to obtain a concentration of 10^{8}cells/ml and used as the pre-inoculum. 10 ml of pre-inoculum was transferred into 250 ml conical flask containing 100 ml of the Cantaloupe fruit juice for inoculum preparation. Then the mixture was incubated overnight at 30 ^{o}C in shaking incubator at 60 rpm.

Preparation of must

Fresh ripened cantaloupes (2 kg) were washed under running tap water, peeled and crushed to yield (1.25 kg) pulp. To this 1.5 kg of sugar and 1.5 L of water were added and mixed well. A control was also prepared from the mixture in a conical flask separately, which are then autoclaved at 121^{0}C for 15 minutes, after which they are allowed to cool under room temperature then the prepared inoculums (*S. cerevisiae*) were mixed with the juice except control and allowed to ferment.

Test for pH

pH is an important factor of wine. pH of the wine sample was measured using a pre-calibrated digital pH meter (Eutech Cyber Scan pH 510) as described by (Ochai and Kolhatkar, 2008)

Total carotenoid content

Carotenoid is an important component of Cantaloupe, therefore it is essential to estimate the carotenoid content. Total carotenoid was estimated by the method described by Harborne (1973). The amount of carotenoids were then calculated by using the formula,

$$\text{Amount of carotenoids in 100mg wine solids} = 4 \times \text{OD VALUE} \times \frac{\text{Total volume of sample (10 ml)}}{\text{Weight of fresh plant tissue (100mg)}}$$

Estimation of yeast cell

Optical density is an easy and widely used method to estimate the number of cells in a culture. It was measured using a UV-Vis spectrophotometer (Systronics 117). Optical density is always proportional to the number of cells in the solution. About 5ml of the wine sample was taken in a cuvette along with a suitable blank. The absorbance was read at 600 nm (Ajit et al., 2018).

Estimation of alcohol

Alcohol estimation was done using iodoform test. Here 4 drops of 1N NaOH was added to 1ml of the wine sample containing alcohol taken in a test tube. Concentrated solution of iodine was added drop by drop until a faint yellow colour persisted. The tubes were allowed to stand for a minute. Some amount of

NaOH solution was added if any excess colour developed. The mixture was shaken well and allowed to stand for 2-3 minutes. A yellow coloured precipitate was settled at the bottom of the tube. This precipitate was removed at room temperature and weighed to calculate the amount of alcohol present in the wine sample (Mohan et al., 2018).

Determination of specific gravity

The specific gravity was determined using specific gravity bottle. The bottle (50ml) was cleaned with distilled water. Then, dried in an oven and then cooled. The dried bottle was weighed and the value was recorded as W_1. Then the bottle was filled with distilled water and weighed. The value was recorded as W_2. Then the bottle was filled to the brim with the sample. It is weighed and recorded as W_3. The specific gravity of the sample was calculated as,

$$Specific\,gravity = \frac{W_3 - W_1}{W_2 - W_1}$$

Measurement of temperature

The periodic temperature change during fermentation was recorded using 120 ^{0}C mercury bulb thermometer inserted to the side arm of the fermentation flask through a sterile rubber cork.

Determination of antioxidant activity-DPPH assay

The antioxidant activity of control and wine were determined through the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method of (Villano et al., 2007) with a slight modification. About 5 ml of the wine sample was mixed vigorously with 5 ml of 0.06 mM DPPH dissolved in methanol and was incubated at darkness for 30 minutes. The decrease in the absorption of DPPH after the wine sample was measured at 520 nm. The DPPH radical scavenging activity (RSA) was calculated as follows:

$$RSA\ \% = \frac{\left[1 - (Abs\ control - Abs\ sample)\right] \times 100}{Abs\ of\ control}$$

where, Abs sample is the absorbance of sample (wine) and Abs control is the absorbance of the control (distilled water).

Determination of total phenolic content

Folin-Ciocalteau's method described by (Singleton et al., 1999) was used to estimate the total phenolic content in the wine. To 1.0 ml of the wine sample, 1.0 ml of Folin-Ciocalteau's reagent was added. After 3 minutes, 1.0 ml of saturated Na_2CO_3 solution was added and the final volume was made up to 10 ml with distilled water. The tubes were incubated in dark for 30 minutes. Absorbance was read at 760 nm against a reagent blank.

Reducing sugar assay by DNS method

Dinitrosalicylic acid method described by Miller (1959) was used to determine the concentration of reducing sugar in the wine sample. About 2 ml of wine sample was added to 2 ml of DNS reagent taken in a test tube. Shaken well and heated the mixture at 100°C in a boiling water bath for 15 minutes and added 1ml of 40 % potassium sodium tartrate (Rochelle salt) solution to stabilize the colour. Cooled at room temperature and absorbance were read at 540 nm. Standard curve was plotted using glucose as the standard and the amount of reducing sugar present in the sample was calculated.

Electrical conductivity

The electrical conductivity (EC) was determined using electrical conductivity meter (Systronics 308).

FTIR analysis

The wine sample and control was retrieved and FTIR spectroscopy was done. Fourier Transform Infrared Spectrophotometer analysis can be used to identify the functional groups in the compound. The chemical bonds are detected by analysing the absorbance of specific light wavelengths displayed by the spectrum.

Organoleptic characteristics

Observations were recorded for odour, colour, taste, flavour and general acceptability on a 5 point scale with 5 points for excellent quality and 1 point for bad quality.

Results

Figure 1 represents the pH variation during the wine production. The initial pH was 5 which gradually decreased to 3 during the course. Figure 2 presents the carotenoid content estimated during subsequent days of fermentation. During the course of fermentation, the carotenoid content of the wine increased. The initial value was found to be 0.0048 and the final value was 0.2724. Figure 3 represents the initial yeast cell count value obtained was 3.26×10^6 cells/ml and attained the maximum value of 5.1×10^6 cells/ ml on the 5th day. Then it gradually decreased to a final value of 2.8×10^6 cells/ml on the 20th day. A steady increase in the alcohol content was observed throughout the period of fermentation. The yeast cell utilizes the sugar in the fruit to produce alcohol by the process of fermentation. It was estimated at 2.5% on the 5th day and increased to 8.7 % on the final day (Figure 4). The initial value for specific gravity obtained was 1.1681 and it gets decreased by 1.1339 on the 20th day. The temperature of the wine was found to be increased during the process. The initial temperature was 27.5 ^{0}C on day 5 and it increased slightly overtime. The final temperature observed was 29.9 ^{0}C. The antioxidant content was determined using DPPH assay which was found to be 61.55 % after fermentation. The total phenolic content of the wine estimated using Folin-Ciocalteau's method showed an initial value as 20.43 mg/ml on the 5th day and a final value as 16.3 mg/ml on the 20th day The initial value of reducing sugar was 9.39 mg/ml on the 5th day and it decreased to 7.21 mg/ml on the 20th day (Table 1). The electrical conductivity of the wine sample was found to be 6.51 mS.

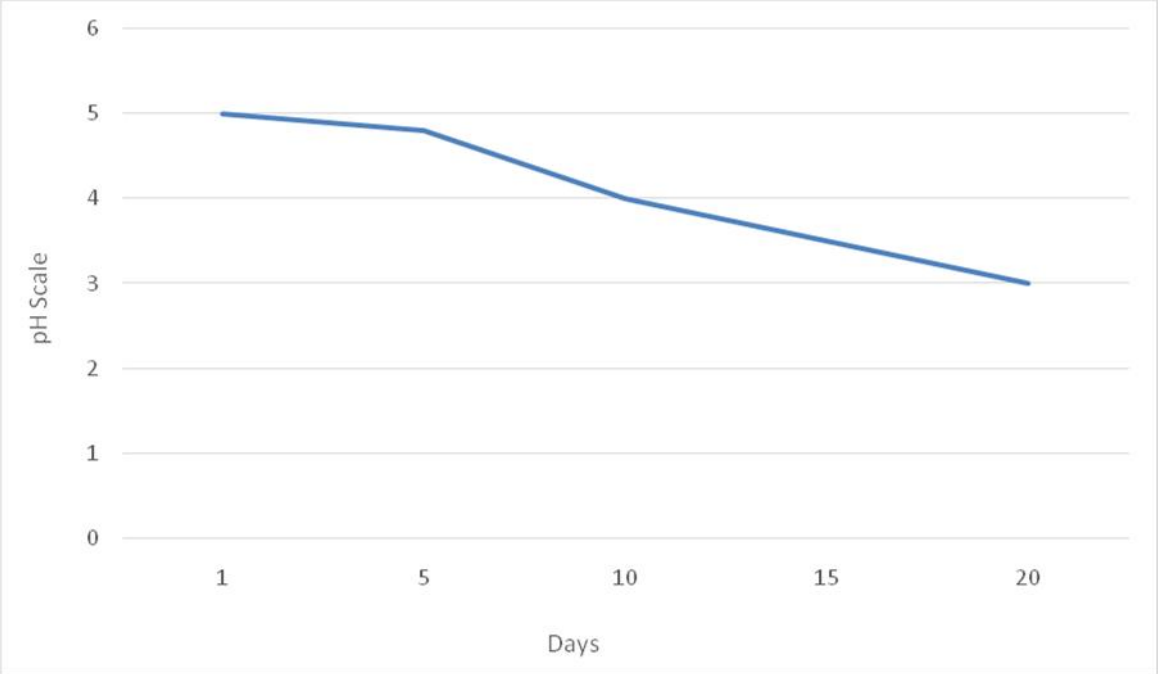

Figure 1. pH of wine sample

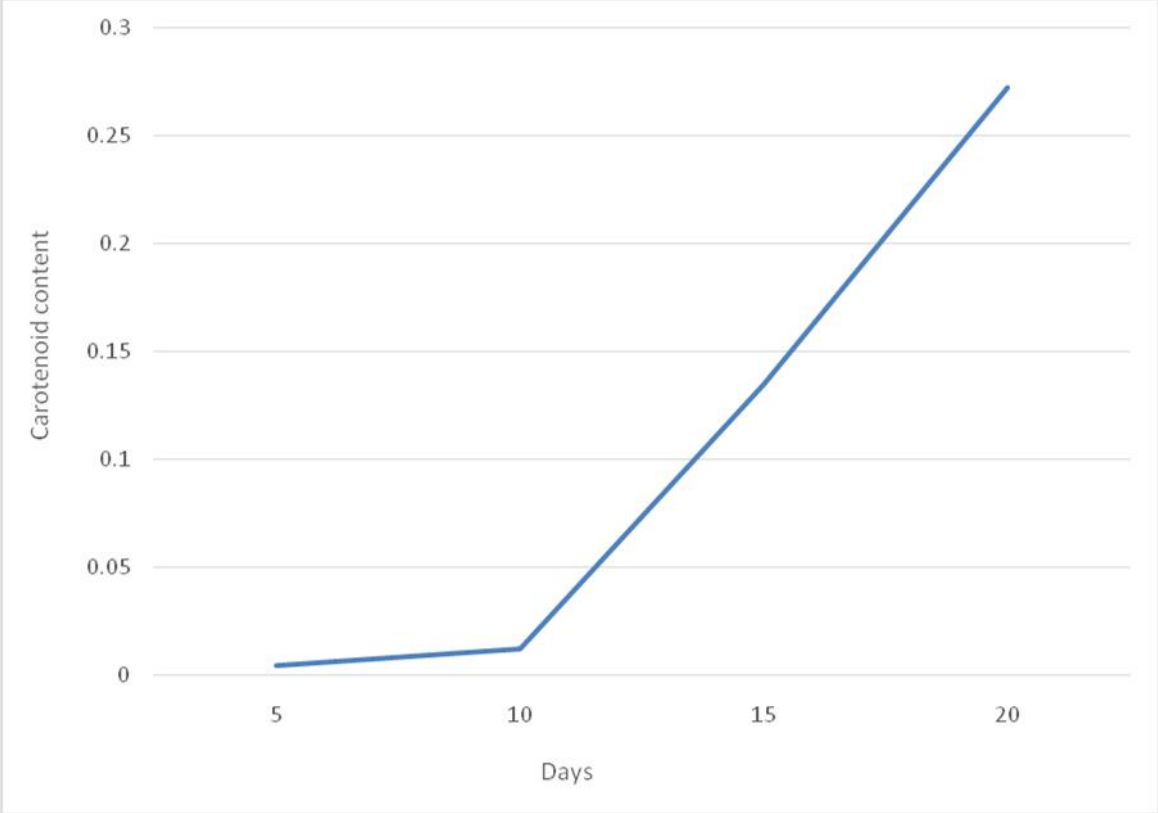

Figure 2. Carotenoid content

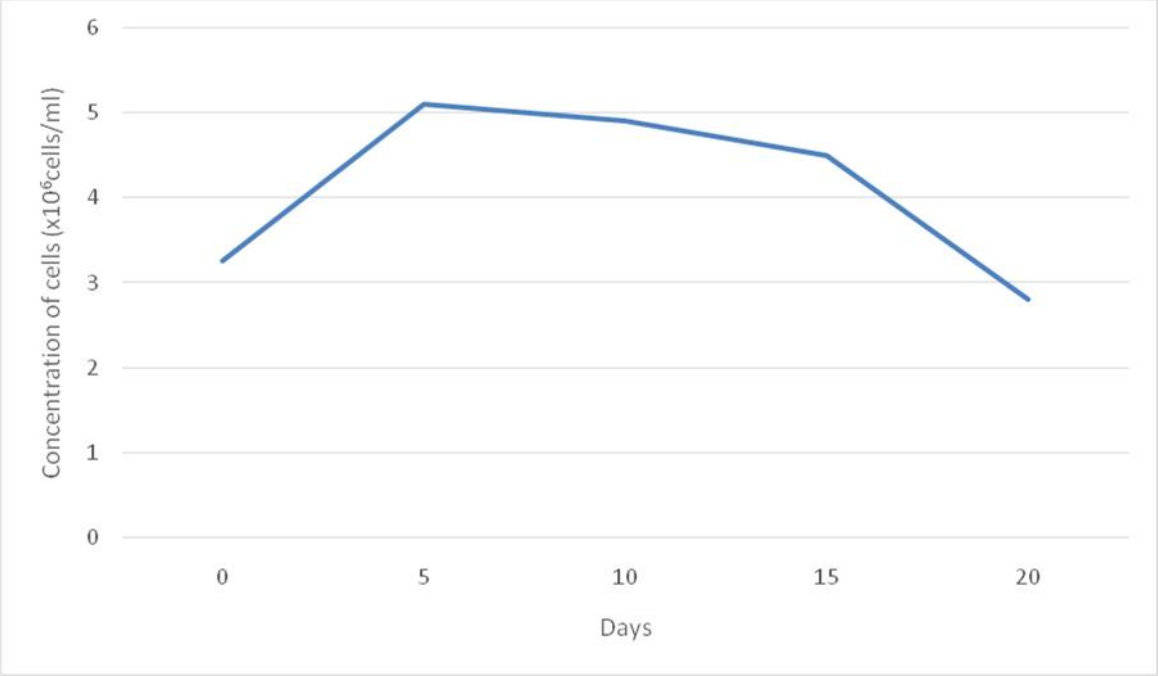

Figure 3. Yeast cell growth

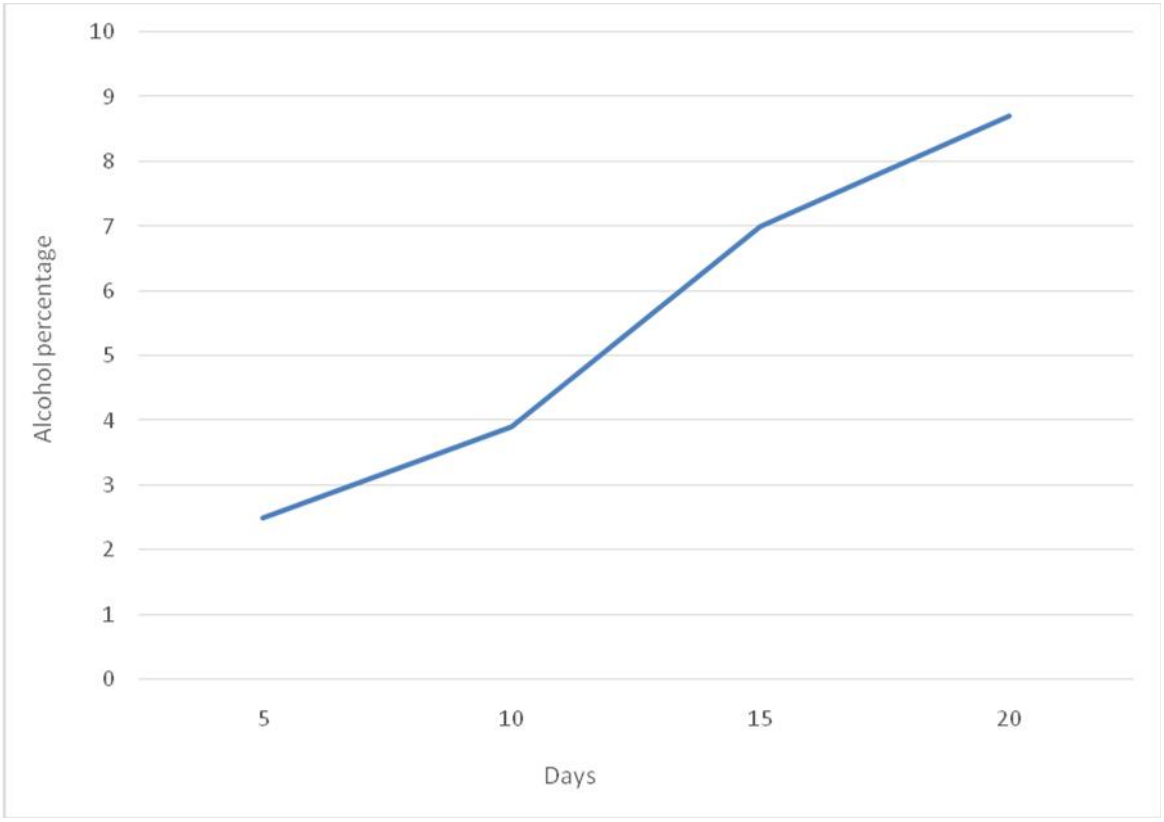

Figure 4. Alcohol content of wine sample

Table 1. Result of physicochemical analysis

Days	5	10	15	20
Specific Gravity (g/cm3)	1.1681	1.1507	1.1428	1.1339
Temperature (°C)	27.5	28.3	29.0	29.9
Total Phenolic content (mg/ml)	20.4	18.1	17.5	16.3
Reducing sugar (mg/ml)	9.39	9.08	8.51	7.21

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed and OH group was detected where the non fermented fruit sample (Figure 5) is rich in carbohydrates as evidenced from absorption bands between 900- 1400 inverse. The peak around 1640 cm inverse represents carboxylate and carboxyl groups. The fermented sample (Figure 6) was found to have considerable amount of ethyl alcohol as is evidenced by presence of a sharp and intense absorption bands at 1055 cm inverse due to C-O stretch of ethanol and C-H stretching vibration of ethyl alcohol at 2936 cm inverse. There is a contribution from OH groups of moisture which corresponds to boarder absorption peak between 3200-3400 cm inverse. Organoleptic assay was determined on the basis of observations recorded for taste, flavour, colour, odour, and general acceptability on a 5 point scale with 5 points for excellent quality and 1 point for bad quality. In terms of general acceptability, the cantaloupe wine has scored an average of 4.7 out of 5 (Figure 7).

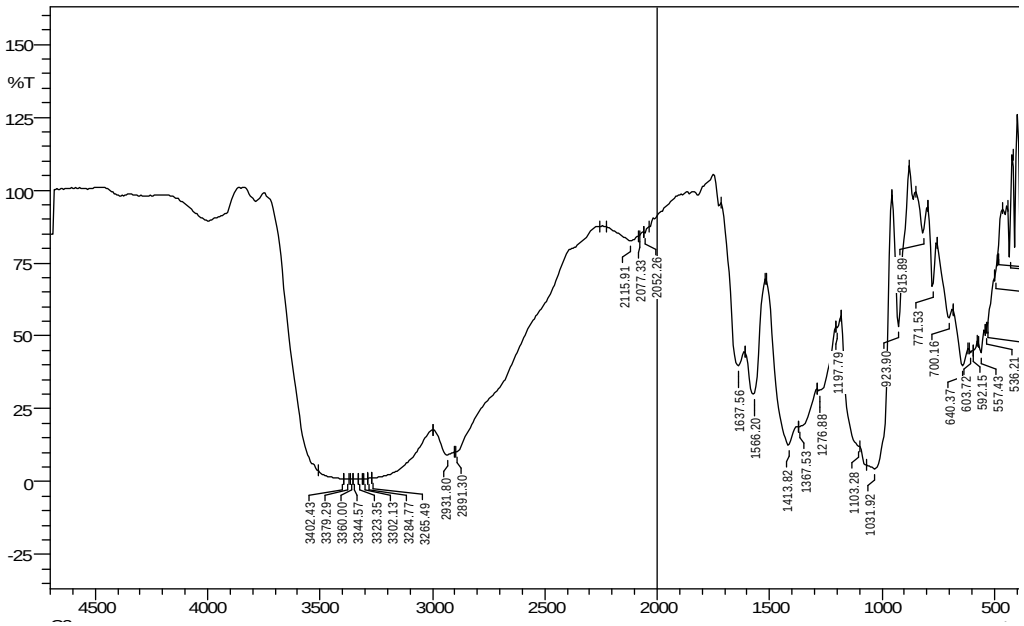

Figure 5. FTIR spectrum of non fermented fruit juice

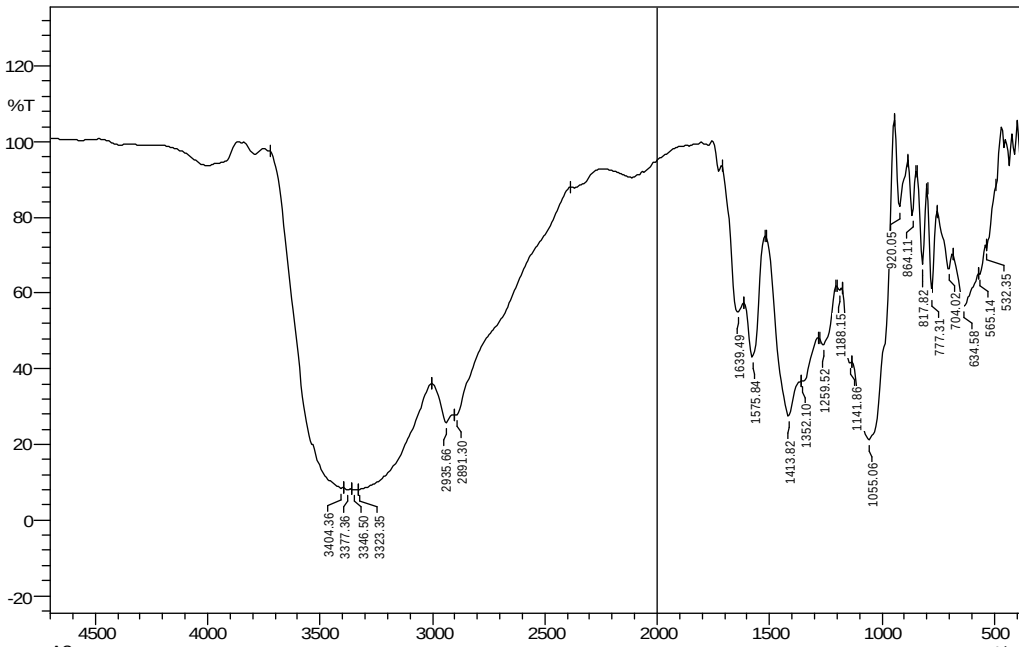

Figure 6. FTIR spectrum of wine sample

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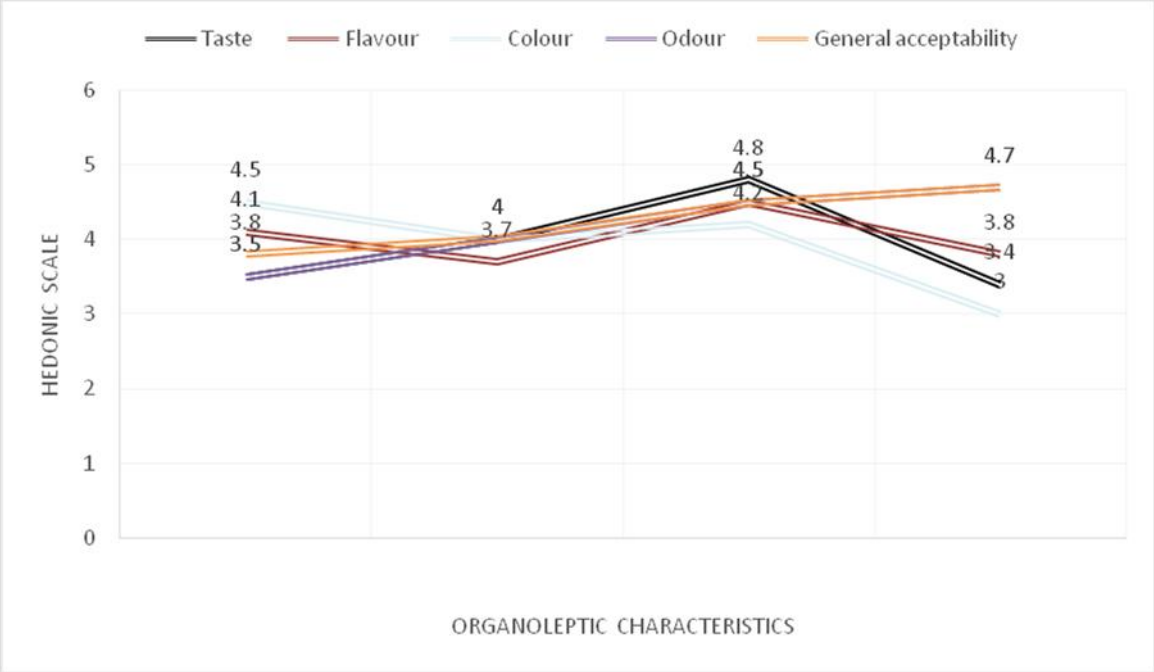

Figure 7. Results of Organoleptic Characteristics

Discussion

Fermentation for the production of beverages like wine depends on the ability and performance of the yeast strains to convert sugar content of the substrates to alcohol and esters. Moreover, the species of yeasts that develop during fermentation determine the final characteristics such as flavour, taste, aroma etc, of the product (Ogodo et al., 2015). Cantaloupe *(Cucumis melo var. cantalupensis*) was used to produce fruit wine in the present investigation using baker's yeast (*S. cerevisiae*). The pH value of the wine was observed to decrease gradually with increasing fermentation period. This observation is similar to the report of (Chilaka et al., 2010) on passion, pine apple and watermelon juices and that of (Obisanya et al., 2002) who reported a steady decrease in pH during the fermentation of mango juice by *S. cerevisiae* and *Schizosaccharomyces* sp. (Ogodo et al., 2015) also reported decrease in pH toward acidity during production of mixed fruit wines of pawpaw, banana and watermelon The decrease in the pH towards acidity could be attributed to production and accumulation of organic acids during fermentation. Moreover, low values in wine have been reported to inhibit spoilage bacteria and creates a favourable environment for the growth of desired organisms (Reddy and Reddy, 2005; Ogodo et al., 2015). Therefore the wines will have good quality. Cantaloupe fruit has a great carotenoid content. During the course of fermentation, the carotenoid content of the wine increased. This observation is similar to report of (Cerillo et al., 2014) on the effect of alcoholic fermentation on carotenoid composition of orange juice. The yeast count initially showed an increase and gradually decreased as the days passed, this is due to the fact that in the absence of oxygen the yeast converts the sugar in the fruit to alcohol and CO_2, through fermentation, while yeast cell die out once the alcohol level increases due to the toxicity of alcohol on yeast cells physiology.

A steady increase in the alcohol content was observed throughout the period of fermentation. The yeast cell utilizes the sugar in the medium to produce alcohol by the process of fermentation. The study showed a decrease in the specific gravity, this is due to microbial utilization of nutrients primarily sugar in the sample for metabolic activities along with the evolution of CO_2 and heat. Temperature play a critical role in fermentation as the temperature increases fermentation rate accelerates. With increased fermentation rate more aromatic compounds are produced because the metabolic intermediates are excreted from the yeast cell, hence increased temperature adds up the quality of the wine. Antioxidants are compounds that inhibit oxidation (Dabelstein et al., 2007). Antioxidant supplementation is widely used to prevent the development of cancer and other chronic disease (Stanner et al., 2004).

Hence antioxidant content determined in the cantaloupe wine enhanced the health benefits of the wine. Phenolic contents have a great role in the release of certain aromas in wine (Dufour and Bayonove, 1999). It also influences the taste, colour, mouth feel, and antioxidant properties of the wine. The phenolic content of the wine reduced as the fermentation proceeded due to the diffusion of phenolic compounds (Othman et al., 2009). As the fermentation proceeds the amount of reducing sugar was declined due to the utilization of the sugar by the yeast cells to produce alcohol. Electrical conductivity of fruit wine depends upon the ionic compounds and is proportional to their contents. Electrical conductivity of wine seems to increase the efficiency of wine. FTIR analysis is based on the fact that functional groups within the sample vibrate upon exposure of IR radiations which helps to determine the functional groups within the sample. The organoleptic characteristics helps to determine the taste, flavour, colour, odour of the studied wine by providing the wine for tasting to different individuals which seems to recognize it as an acceptable wine with health benefits.

Conclusion

The wine produced from cantaloupe fruit pulp was of good quality. The characteristic features of the wine such as the colour, taste and odour as well as the flavour was good. The final alcohol content of the wine was found to be 8.7% at the 20th day and it was of acceptable quality, healthy and more appealing than the fruit.

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