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Wine Making: Influence of pH on Physicochemical Parameters of Wine Must Produce from Hot Water Extract of Broom-cluster Fig (*Ficus capensis*) Leaf using *Saccharomyces cerevisiae*

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ABSTRACT: The chemical and biological stability of wine are very dependent on pH value, hence winemakers believe that pH plays a critical role in fermentation and final wine quality. This paper optimized and assessed the influence of pH on different physicochemical parameters of wine must prepared from hot water extract of the leaf of Broom-cluster Fig (*Ficus capensis*) leaf with *Saccharomyces cerevisiae*, ameliorating to 22 ºBrix using table sugar and adding potassium metabisulfite. Then the pH of the must was adjusted as required and subjected to pasteurization. The must was inoculated with yeast inoculum at 0.8g/ml. Soluble solid, alcohol, titratable acidity and pH profile of the wine was monitored daily. After optimization and fermentation physicochemical parameters of the wine were analyzed. It was shown that pH of the must increase and decreased as the fermentation days progressed from day 1-12 for pH 3 - 3.5 and 4-4.5 respectively. Total soluble solid decreased in all the must samples. The alcohol content increased gradually during fermentation. pH 3 and 4 had the highest titratable acidity when compared to pH 3.5 and 4.5. The pH of 4.5 wine had the lowest TSS and highest alcohol content. This study shows that wine can be produced from hot water extract of *Ficus capensis* leaf and the must fermented at lower pH gave highest percentage of alcohol. There is need to optimize and assess the pH of vegetable must before fermentation.

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Wine from fruits (grape, banana, pineapple, mango, kiwi, watermelon, cartus pear) and vegetables (pumpkin and *Hibiscus sabdariffa*) are generally produced by alcoholic fermentation by different strains of yeast, *Saccharomyces cerevisiae* which converts the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters and other chemical compounds that help to preserve the wine (Pradip and Archana, 2016; Ranjitha *et al.*, 2017; Zenebe and Kidu, 2019; Sobowale *et al.*, 2021; Boondaeng *et al.*, 2022). The chemical composition and the sensory quality of the wine are usually influenced by these constituents (Ogodo *et al.*, 2018). Several factors, such as the addition of sulphur dioxide, the temperature of fermentation, pH of the must, composition of fruit juice, inoculation with specific yeasts, and interactions of microorganisms affect alcoholic fermentation but pH is considered the most critical (Bagheri *et al.*, 2020; Krieger-Weber *et al.*, 2020). The preferred wine pH is around 3.6 and the better pH for yeast and lactic acid bacteria is around 4.5 (Galli *et al.*, 2022). However, spoilage bacteria can also grow well at pH 4.5 but, spoilage bacteria do not grow well below pH 3.6 (Shankar *et al.*, 2021). Wine

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yeasts and some lactic acid bacteria can still metabolize in a pH range of 3.3–3.6 (Pradip and Archana, 2016; Capozzi *et al*., 2021). The low pH can prolong the fermentation process due to the slow growth of microorganisms involved (Chidi *et al.,* 2018) while high levels of pH during fermentation could affect the metabolism of yeasts and the concentration of yeast-related compounds such as those involved in the formation of new pigments and new polymeric tannic structures (Angelita *et al*., 2022). In fact, during fermentation, great variations in pH and titratable acidity occur due to the activities of yeasts and bacteria and the precipitation equilibria linked to potassium bitartrate formation (Chidi *et al*., 2018). Furthermore, during fermentation, yeast produces enzymes which bring about various biochemical transformations and without the required pH, temperature and ionic strength, they may become denatured (Drappier *et al.*, 2019). Enzymatic activities and metabolism are very sensitive to pH changes (John *et al*., 2012; Drappier *et al.*, 2019). *Ficus capensis* belongs to the family moraceae and it's an evergreen plant that grows in the wild (Agbelade and Ojo, 2020; Boton *et al.,* 2021). Traditionally, *F. capensis* hot water extract has been taken as tea along with milk to boost blood levels. However, its nutritional, phytochemical properties, and antioxidant activities (Mgbemena *et al*., 2022; Suleiman *et al*., 2022) make it a useful raw material for wine product that can be assessed easily and also serve as a source of income for the masses. Several studies on influence of pH on the physicochemical properties of different fruit juice has been well studied and documented (Starek *et al.,* 2019; Salehi, 2020; Katariya *et al*., 2020; Ousaaid *et al*., 2021). However, no work on hot water extract of broom-cluster fig (*F. capensis*) leaf using *S. cerevisiae* has been reported. Therefore, in this present study winemaking was explored by assessing the physicochemical parameters of wine must produce from hot water extract of broom-cluster fig (*F. capensis*) leaf using *S. cerevisiae.*

MATERIALS AND METHOD

Preparation of Ficus capensis Must: Preliminary operations such as cleaning, sorting was carried out to remove extraneous materials from the *F. capensis* leaf. Hot water extract of *F. capensis* was obtained at a temperature of 100°C for 20 min in a steam jacketed kettle. The water was filtered from the leaves using double folded muslin cloth and 250g/liter of table sugar was added to the juice to adjust the soluble solids from 1.0 to 22°brix. Must was sterilized in an autoclave at 121°C for 20 min. About 1g of sulphur (IV) oxide in the form of sodium metabisulphite was added to the extract to inhibit the growth of bacteria, and wild yeast and as well to prevent fermentation before the addition of the starter culture. The must was distributed into 300 ml aliquots in 500 ml flasks for pH adjustments. Must with following pH ranges (3.0, 3.5, 4.0 and 4.5 in triplicates) were prepared to study the effect of pH on fermentation parameter. Citric and tartaric acid were used to adjust the pH of the must. The flasks containing must were plugged with cotton wool. Must was then kept in room temperature until required (Pradip and Archana, 2016; Ogodo *et al*., 2018).

Preparation of starter culture: This was prepared according to Ogodo et al., 2018, with slight modification. Exactly 0.8g/l of commercial baker's yeast (*S. cerevisiae*) was mixed in 200ml of *Ficus capensis* hot water extract and stilled vigoriously. About 2g of yeast nutrient each (potassium phosphate, ammonium sulphate, and Magnesium sulphate) were dissolved in 100ml of distilled water and added to the mixture. The mixture was allowed to stand for three hours after which it was added to the must for fermentation.

Fermentation of must to wine: The extract was poured into an aspirator (fermenting vessel). It was covered for about 15 to 20 min to allow for the yeast population to build up. The fermenting vessel was covered with a safety lock which has 200 ppm of sodium metabisulphite at the lid of the lock to control oxidation. The must was stirred for 24 hrs intervals and aliquotes were collected for analysis. To monitor the advancement of the fermentation process and to observe the effect of pH on the fermentation profile of must, soluble solids, and pH of each fermenting must be measured on alternate days after 24 hrs of inoculation. Fermentation was stopped when there was no evolution of gas bubbles by keeping the flasks in the refrigerator at a temperature of 3-5 ^{0}C. Three replicates were maintained for experimenting (Ogodo *et al.*, 2018).

Parameters studied: During the fermentation, the decrease in TSS (°B) and the pH, were monitored at the appropriate time intervals. The wines were analyzed for different physicochemical characteristics. The pH of the must and wine were measured with a digital pH meter (Systronics, India), pre-calibrated with buffers of pH 4.0 and 7.0 (AOAC, 1990). Total soluble solid was measured using Abbey's refractometer (0 – 32) in terms of °Brix (AOAC, 1990). Titratable acidity was determined by titrating with 0.1 N NaOH (Amerine and Ough, 1980) and alcohol content was obtained using the difference in potential alcohol method. In this method, the alcohol contents were calculated based on the sugar contents of the must before fermentation and the final sugar level of the fermented must (Jacobson, 2006; Tochukwu and Oyinloye, 2017).

RESULTS AND DISCUSSION

Table 1 shows the changes in pH, total soluble solid (TSS), alcohol content (ALC) and titratable acidity (TTA) of *F. capensis* wine at pH 3 and incubation period of 12days. At pH 3, the maximum alcohol content of 4.499 was recorded in day 12 with titratable acidity of 6.770, total soluble solid of 14.400 and pH of 3.2. Table 2 shows the changes in pH, total soluble solid (TSS), alcohol content (ALC) and titratable acidity (TTA) of *F. capensis* wine at pH 3 and incubation period of 12days. At pH 3.5, maximum alcohol content of 8.170 ± 0.000 was recorded in day 12 with titratable acidity of 5.830, total soluble solid of 8.200 and pH of 3.6. Table 3 shows the changes in pH, total soluble solid (TSS), alcohol content (ALC) and titratable acidity (TTA) of *F. capensis* wine at pH 4 and incubation period of 12days. At pH 4, maximum alcohol content of 8.643 of *F. capensis* wine was recorded in day 9 with titratable acidity of 6.7100, total soluble solid of 7.400 and pH of 4.0. Again, the alcohol content was the same at day 10 and started decreasing gradually. Table 4 shows the changes in pH, total soluble solid (TSS), alcohol content (ALC) and titratable acidity (TTA) of *F. capensis* wine at pH 4.5 and incubation period of 12days. At pH 4.5 the maximum alcohol content of 8.888 was recorded in day 10 with titratable acidity value of 6.760, total soluble solid of 7.000 and pH of 4.0.

Table 1: Effect of incubation period (12days) and pH 3 on pH, total soluble solid, alcohol yield and titratable acidity of *Ficus capensis* wine.

Incubation Period (Days)	**pH**	**TSS (ºBrix)**	**ALC (%)**	**TTA** (g/l)
1	3.4 ± 0.058	20.90 ± 0.000	0.651 ± 0.000	5.040 ± 0.052
2	3.4 ± 0.000	20.933 ± 0.115	0.631 ± 0.479	1.420 ± 0.105
3	3.3 ± 0.058	20.733 ± 0.642	0.750 ± 0.380	4.730 ± 0.736
4	3.4 ± 0.000	21.400 ± 0.000	0.355 ± 0.000	0.900 ± 0.444
5	3.4 ± 0.000	22.000 ± 0.000	0.296 ± 0.000	1.860 ± 0.579
6	3.3 ± 0.000	22.500 ± 0.000	0.000 ± 0.000	5.200 ± 1.786
7	3.3 ± 0.000	24.600 ± 0.000	1.539 ± 0.000	5.733 ± 0.551
8	3.1 ± 0.000	20.000 ± 0.000	1.184 ± 0.000	4.616 ± 0.076
9	3.2 ± 0.000	18.200 ± 0.000	2.250 ± 0.000	7.140 ± 0.060
10	3.2 ± 0.058	17.200 ± 0.000	2.842 ± 0.000	7.470 ± 0.197
11	3.2 ± 0.000	15.600 ± 0.000	3.789 ± 0.000	7.873 ± 0.133
12	3.2 ± 0.000	14.400 ± 0.000	4.499 ± 0.000	6.770 ± 0.017

Values are expressed as mean ± SE of triplicate determinations. TSS- total soluble solid, ALC- alcohol content and TTA- titratable acidity

Table 2: Effect of incubation period (12days) and pH 3.5 on pH, total soluble solid alcohol yield and titratable acidity of *Ficus capensis* wine.

Incubation Period (Days)	**pH**	**TSS (ºBrix)**	**ALC (%)**	**TTA (g/l)**
1	3.8 ± 0.000	20.600 ± 0.000	0.828 ±0.000	3.350 ± 0.087
2	3.4 ± 0.000	18.333 ± 0.115	2.170 ± 0.068	1.590 ± 0.030
3	3.4 ± 0.000	20.266 ±.0.115	1.026 ± 0.068	5.560 ± 0.439
4	3.4 ± 0.000	17.200 ± 0.000	2.802 ± 0.068	1.690 ± 0.165
5	3.4 ± 0.000	16.300 ± 0.000	3.374 ± 0.000	3.600 ± 0.687
6	3.3 ± 0.000	13.000 ± 0.000	5.328 ± 0.000	6.560 ± 0.544
7	3.5 ± 0.000	14.800 ± 0.000	4.262 ± 0.000	7.000 ± 0.458
8	3.5 ± 0.000	11.800 ± 0.000	6.038 ± 0.000	5.346 ± 0.006
9	3.6 ± 0.000	10.000 ± 0.000	7.104 ± 0.000	7.850 ± 0.062
10	3.6 ± 0.000	9.000 ± 0.000	7.696 ± 0.000	6.700 ± 0.321
11	3.6 ± 0.000	8.600 ± 0.0000	7.933 ± 0.000	7.083 ± 0.057
12	3.6 ± 0.000	8.200 ± 0.000	8.170 ± 0.000	5.830 ± 0.086

Values are expressed as mean ± SE of triplicate determinations. TSS- total soluble solid, ALC- alcohol content and TTA- titratable acidity

Table 3: Effect of incubation period (12 days) and pH 4 on pH, total soluble solid alcohol yield and titratable acidity of *Ficus capensis* wine

Incubation Period (Days)	**pH**	**TSS (ºBrix)**	**ALC (%)**	**TTA (g/l)**
1	3.9 ± 0.000	20.60 ± 0.000	0.829 ± 0.000	4.210 ± 0.360
2	3.4 ± 0.000	15.267 ± 115	3.986 ± 0.069	1.660 ± 0.046
3	3.6± 0.058	17.566 ± 0.057	2.626 ± 0.033	5.000 ± 0.516
4	3.6± 0.000	13.733 ±0.115	4.894 ± 0.069	1.810 ± 0.414
5	3.6± 0.000	12.000 ±0.000	5.920 ± 0.000	3.850 ± 0.824
6	3.5± 0.000	10.000 ±0.000	7.104 ± 0.000	5.670 ± 0.300
7	3.6± 0.000	9.400 ± 0.000	7.459 ±0.000	6.410 ± 0.121
8	3.7 ± 0.000	8.400 ± 0.000	8.051 ±0.000	5.190 ± 0.060
9	4.0 ± 0.000	7.400 ± 0.000	8.643 ± 0.000	6.7100 ±0.173
10	3.9 ± 0.058	7.400 ± 0.000	8.643 ± 0.000	6.490 ±0.233
11	3.8 ± 0.000	8.200 ± 0.000	8.170 ± 0.000	7.973 ±0.006
12	3.8 ± 0.000	8.000 ± 0.000	8.288 ± 0.000	5.430 ± 0.000

Values are expressed as mean ± SE of triplicate determinations. TSS- total soluble solid, ALC- alcohol content and TTA- titratable acidity

Table 4: Effect of incubation period (12days) and pH 4.5 on pH, total soluble solid alcohol yield and titratable acidity of *F. capensis* wine.

Incubation Period (Days)	pH	TSS (ºBrix)	ALC (%)	TTA(g/l)
1	4.2 ± 0.000	20.600 ± 0.000	0.829 ± 0.000	3.590 ± 0 .953
2	3.9 ± 0.100	15.000 ± 0.00	4.144 ± 0.000	1.450 ± 0.142
3	3.7 ± 0.058	17.000 ± 0.00	2.960 ± 0.000	5.450 ± 0.154
4	3.8 ± 0.000	12.000 ± 0.00	5.920 ± 0.000	2.680 ±0.211
5	3.7 ± 0.000	11.600 ± 0.000	6.157 ± 0.000	2.920 ± 0.076
6	3.6± 0.000	9.000 ± 0.000	7.696 ± 0.000	6.840 ± 0.364
7	3.7 ± 0.000	9.500 ± 0.000	7.400 ± 0.000	6.540 ± 0.000
8	3.8 ± 0.000	8.200 ± 0.000	8.140 ± 0.000	4.920 ± 0.104
9	3.9 ± 0.058	7.200 ± 0.000	8.762 ± 0.000	7.200 ± 0.238
10	4.0 ± 0.000	7.000 ± 0.000	8.888 ± 0.000	6.760 ± 0.105
11	3.9 ± 0.000	8.000 ± 0.000	8.288 ± 0.000	9.090 ± 0.000
12	4.0 ± 0.057	8.000 ± 0.000	8.288 ± 0.000	5.400 ± 0.000

Values are expressed as mean ± SE of triplicate determinations. TSS- total soluble solid, ALC- alcohol content and TTA- titratable acidity

Table 5 shows the physicochemical changes of *F. capensis* wine at various pH levels. The pH of 4.5 wine had the lowest TSS and highest alcohol content when compared to pH 3, 3.5, and 4. Fermentation pH is a very important factor in cell development as well as alcohol yield (Hossain *et al*., 2017; Mengesha *et al*., 2022). The better pH for yeast and lactic bacteria is around 4.5 although the preferred wine pH is about 3.6 (Pradip *et al.*, 2016). To attain a suitable pH for the preparation of *F. capensis* wine, the effect of different pH (3 - 4.5) was tested on the pH, TSS, alcoholic content and titratable acidity of the must. The increase of pH between pH 3 and 3.5 (Table 1 and 2) of the must recorded at the beginning of the incubation period could be as a result of delayed onset of fermentation process. This also prevented the yeasts from utilizing the sugar for the production of alcohol and carbon (iv) oxide. pH 3 and 3.5 were too low for the must hence prolonging the fermentation process due to slow growth of the yeast involved (Jacobson 2006).

Table 5: Physicochemical Properties of *F. capensis* Must after Fermentation at Different pH

pH	TSS (ºBrix)	ALC (%)	TTA (g/l)
3	17.200 ± 0.000	2.842 ± 0.000	7.470 ± 0.197
3.5	9.000 ± 0.000	7.696 ± 0.000	6.700 ± 0.321
4	7.400 ± 0.000	8.643 ± 0.000	6.490 ±0.233
4.5	7.000 ± 0.000	8.888 ± 0.000	6.760 ± 0.105

Values are expressed as mean ± SE of triplicate determinations. TSS- total soluble solid, ALC- alcohol content and TTA- titratable acidity

Low initial pH have been reported to prolong yeast lag phase, affect accumulated mass loss, change the consumption rate of total sugar, increase final content of acetic acid and glycerol, and decreasing final content of ethanol and l- succinic acid (Liu *et al*., 2015). Total soluble solid has been reported to decrease as incubation period increased (Worku *et al*., 2019). The decrease in TSS (Table 3 and 4) obtained for pH 4 and pH 4.5 shows that fermentation started immediately as well as the yeasts were able to metabolize the sugar and nutrient present in the must to produce high alcoholic content at day 9 and 10 of the incubation period. Titratable acidity decreased as the pH increased. Similar trends has been reported (Onwuka and Awam, 2001; Akubor *et al.*, 2013; Worku *et al*., 2019; Tan *et al*., 2022). The pH of guava must was reported to decrease due to increase in titratable acidity as fermentation progressed (kocher and pooja, 2011). Similar trend was also observed in several work (Okoro *et al* 2007; Alobo and Offonry (2009)). At the end of the optimization process, pH 4.5 was suitably chosen since it yielded a higher alcoholic content, TSS decreased to a reasonable extent as the incubation period increased. Higher pH has also been reported to extract massive phenolic compounds during fermentation (Angelita *et al*., 2022).

Conclusion: This study was aimed at the influence of pH on the physicochemical parameters of wine must produce from hot water extract of broom-cluster fig (*F. capensis*) leaf using *S. cerevisiae*. The physicochemical parameters studied of the must at pH 4 and 4.5 were better than the must at pH 3 and 3.5 after fermentation. However, pH 4.5 was successfully taken since total soluble solid was significantly lower with a higher alcohol content than the others during the incubation period of fermentation.

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