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Quality of Miracle Berry Wine as Influenced by pH and Inoculum Levels

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Abstract *Synsepalum dulificum* (also known as miracle berry) fruit is rich in nutrients, flavour and antioxidant compounds. However, the fruit is underutilized in the sub region and susceptible to post harvest losses. In this study, miracle berry wine was produced and the effects of varying pH and inocula levels during fermentation (at room temperature for 7 days) on the wine parameters (soluble solids, pH, titratable acidity, acid taste index, total phenols and antioxidant activity) were investigated. During fermentation, changes in °Brix and pH were also monitored. Total soluble solids varied between 4.8-20°Brix while total phenols and antioxidant activity (% DPPH inhibition) ranged 300-580 mg GAE/L and 52-86 %, respectively. There was a decrease in °Brix, pH, phenolic content and antioxidant activity for all samples fermented at varying pH after fermentation. There was, however, an increase in titratable acidity (7-14 g/L tartaric acid) and acid taste index after fermentation. Wine samples produced at pH of 3.8, 4.6 and 5.8 using 1% inoculum produced 13%, 10% and 10% (v/v) alcohol, respectively. With respect to varying inocula, there was a decrease in °Brix, pH, phenolic content and antioxidant activity for wine samples produced at pH of 4.6 and inocula of 1% and 2%, respectively, but increased in titratable acidity and acid taste index after fermentation. At the end of the fermentation process, wine sample with pH 4.6 and inocula of 1% and 2% had alcohol content of 10% and 12% (v/v), respectively. The study revealed that it is possible to produce red wine from miracle berry rich in antioxidant with possible health imparting benefits. Again, varying the pH and inoculum levels can affect the quality of the wine produced.

Keywords: *Synsepalum dulificum*, fermentation, red wine, polyphenols, antioxidant activity

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1. Introduction

Wine is an alcoholic beverage traditionally made by the fermentation of grape juice. Its consumption has increased over the past two decades due to some health benefits. A moderate daily consumption of wine has been linked to a reduction in heart diseases, hyperlipidemia, cancers, ischemic stroke, neurodegenerative disorders, aging, hypertension, dental caries, delayed progression of intestinal diseases and many other disease conditions [1-8]. These positive health benefits have been attributed to the presence of phenolics, flavonoids, anthocyanins and other phytochemicals present in wine [5,9-16]. This has made the consumption of wine popular around the globe even in areas where grapes cannot be cultivated. Several authors have investigated the suitability of other fruits as substrates for winemaking. These include African bush mango [17], guava [18], jamun fruit [19], litchi fruit [20], amla fruit [21], tomato [22,23]. These fruits are known to be good sources of vitamins, carbohydrates, mineral, fiber and phytochemicals hence fermentation of juices from these fruits is likely to produce wines of varied nutritional,

phytochemical and sensory qualities [23].

Synsepalum dulificum is a fruit native to West Africa and well noted for its taste modifying ability. It is rich in nutrients, flavour and antioxidant compounds [24,25,26]. The fruit is underutilized in the sub region despite the myriad of nutrients it contains and also susceptible to high postharvest losses. Processing of fruits into products like wine could serve as an avenue to increase the usage, consumption, reduce post-harvest losses and increase the variety of wines available on the market [27,28].

Alcoholic fermentation is indispensable in the production of any alcoholic beverage including wine. The process may start spontaneously by wild yeast or by inoculation with yeast of desirable characteristics [29]. This process is affected by many factors such as sugar concentration, yeast strain, inoculum level, temperature, pH among others. During fermentation, yeasts converts simple sugars in the must to ethanol under anaerobic conditions. The sugar concentration is therefore an important factor in determining the final ethanol concentration of the wine produced [30]. pH affects metabolism and enzymatic activities hence without the requisite pH the fermentation process and the quality of the final product may be compromised [31]. It has been reported that the ethanol

production of mango (*Mangifera indica*) wine at different pH levels using *Saccharomyces cerevisiae*, CFTRI 101 had maximum amount of 7.8% (w/v) at pH 5.0, but minimum amount of 5% (w/v) at pH 3.0 [32]. Yeast strain and inoculum levels are also key in determining the quality of the alcoholic beverage produced after fermentation. The level of inoculum influences the duration of the lag phase, specific growth rate, biomass yield and the overall quality of the final product [33]. Alcoholic strength of the fermented tomato juice produced with 0.01% (w/v) inoculum level was significantly higher ($p < 0.05$) than that produced with 0.02% (w/v). It is imperative therefore to use the right level of inoculum so as to maximize fermentation [23]. The objective of the present study was to investigate the effect of pH and inoculum levels on the quality of miracle berry wine.

2. Materials and Methods

2.1. Source and Sample Preparation

Miracle berries were harvested from a 3-year old miracle berry plant together with the stalks from a farm in Nsawam in the Eastern Region of Ghana, washed with potable water, 2% potassium metabisulphite solution, frozen and transported to the laboratory under frozen conditions. The frozen samples were thawed in a refrigerator for 2 h and proximate analysis were carried out on the sample [34]. The must was prepared by de-pulping the fruit with the hand to remove the lone seed and the pulp together with the skin was mixed with water in a ratio of (1:1 w/v) and blended in a Kenwood blender. The must was then filtered through cheese cloth. Potassium metabisulphite (2%) was added to the must as an antioxidant and an antimicrobial agent. Pectic enzyme (0.5 g/L) was added to break down pectin to improve aroma and colour extraction, and ammonium phosphate ($(\text{NH}_4)_2\text{PO}_4$) also added as a source of ammonia and phosphorus for the growth of yeast. Sucrose (200 g/L) was added to ameliorate the total soluble solids of the must (TSS) to 21.0° Brix.

2.2. Experimental Design

The original pH of the miracle berry must (pH 4.6) was adjusted to 3.8 using tartaric acid and 5.8 using calcium carbonate and inoculated with 1% yeast. Miracle berry must of pH 4.6 was inoculated with 0%, 1% and 2% yeast, respectively. Total soluble solids (TSS), alcohol content, pH, titratable acidity, polyphenol concentration and antioxidant activity were determined. Fermentation was then carried out at room temperature for 7 days. During fermentation, change in TSS and pH were determined at 24 h intervals. After the 7th day, the wine was cold stabilized at 5°C for 24 h and was centrifuged at 5000 rpm for 10 min before analysis. TSS, alcohol content, pH, titratable acidity, polyphenol concentration and antioxidant activities were determined. The experiment was duplicated.

2.3. Analysis of Miracle Berry Wine

The pH of the must was measured using a pH meter (Metler Toledo FE 20; GB/T111165, Switzerland)

after calibration with buffer solutions of pH 7 and 4, respectively. Titratable acidity was determined by using a previous method [35] and the results expressed in g/L tartaric acid. The TSS was measured using a digital refractometer (Reichert digital refractometer AR 200) with temperature compensation. Distilled water was used to calibrate the refractometer, the measurement was done after calibration and the values expressed in degree brix. Alcoholic strength was measured by using an alcoholmeter (nach Gay Lussac-Cartier, Germany) after distillation of the alcohol [36]. The total phenols of the wine samples was assayed using the Folin-Ciocalteu method [37] with gallic acid as the standard. Determination of the Free Radical Scavenging Activity was carried out by the 1, 1-Diphenyl-2-picrylhydrazyl free-radical scavenging assay [38].

3. Results and Discussion

3.1. Proximate Composition of Miracle Berry Fruit Skin and Pulp

The amount of macro and micro nutrients in a food material determines the nutritional value of that particular food item. Proximate analysis is a quantitative method for determining the macronutrient composition of a food sample [39]. The result of the proximate analysis carried out on the skin and pulp of *Synsepalum dulcificum* (Table 1) indicates that it contained $52.04 \pm 0.23\%$ moisture which is less than 72.11%, 92.81% and 84.39% reported for passion fruit, watermelon and pineapple fruit, respectively by [24] and 86.93%, 85.99%, 86.68% and 86.66 % for *Vitis hybrid* (Sheridan), *Vitis labrusca* L (Gerbong), *Vitis labrusca* (Muscat Bailey A) and *Vitis labrusca* B (Campbell Early) respectively, by [40].

Table 1. Proximate composition of miracle berry skin and pulp

Parameter	Percentage (%)
Crude Protein	11.54±0.85
Moisture	52.04±0.23
Ash	3.29±0.71
Crude Fat	5.25±0.05
Crude Fiber	8.32±0.03
Carbohydrate	19.56±0.02

The moisture content suggests that miracle berry skin and pulp will have a short shelf life and thus processing and preservation methods are necessary to prevent wastage. The miracle berry skin and pulp was found to contain high amount of crude protein $11.54 \pm 0.85\%$ compared to 2.57%, 0.47% and 0.24% reported for passion fruit and water melon and pineapple fruit, respectively [24], and 0.69% and 2.30% reported for the pulp and peel of red grape sultana cultivar, respectively [41]. The high content of proteins in *S. dulcificum* implies that the miracle berry wine will be hazy hence vigorous clarification methods should be adopted to get a clear wine after fermentation. The ash content of the extract ($3.29 \pm 0.71\%$) was comparable to 3.83% reported for pineapple [24] indicating that the extract contains some

amount of minerals. The carbohydrate content of the extract ($19.56 \pm 0.02\%$) is also comparable to 17.55% and 17.79% reported for passion fruit and pulp of red grape sultana cultivar, respectively [24,41]. This shows that the skin and pulp contain some amounts of carbohydrates which can be converted to simple sugars during fermentation.

3.2. Physicochemical Properties of Miracle Berry Wine Samples

A decrease in TSS with time was observed for all the wine samples during storage/fermentation (Figure 1). This observation is in line with findings of [42,43] who observed a decline in TSS during fermentation of guava and raspberry juice, respectively. The rate of reduction in total soluble solids (TSS) during fermentation and the amount of alcohol produced at the end of the fermentation process were higher for wine of pH 3.8 followed by 4.6 and 5.8, in that order. This observation can be attributed to the fact that microbial growth, metabolism and enzymatic activities are different under different juice and wine pH conditions [31,44]. According to [45,46], low pH values also favors the hydrolysis of disaccharides and therefore fermentation, leading to a higher the rate of decline in TSS as recorded at low pH of the fermenting must. [47] studied the effect of five pH levels (3.5, 4.0, 4.5, 5.0, and 5.5) on ethanol production from the Siaha Sardasht grapes, and reported maximum and minimum productions for pH 4.5 and 3.5, respectively.

Table 2. Alcohol content of wine samples fermented at different pH and Inoculum levels

Wine Sample	pH	Inoculum level (%)	Alcohol Content (% v/v)
A	3.8	1.0	13±0
B	4.6	1.0	10±0
C	5.8	1.0	10±0
D	4.6	0.0	ND
E	4.6	1.0	10±0
F	4.6	2.0	12±0

ND: Not Detected.

In this study, miracle berry must of pH 3.8 had the highest ethanol content of 13% (v/v) after fermentation followed by pH 4.6 which had 10% (v/v) and 5.8 which also had 10% (v/v). This finding is different from the findings of [47] but consistent with the rate of decrease in TSS with time. During fermentation, yeast cells metabolize sugars in the must and convert it to ethanol [30], hence, the consistency in the rate of production of ethanol to the rate of degradation of TSS as was observed in this study at different pH values.

One of the important quality parameters of wine is its acidity. Organic acids contribute greatly to wine composition, stability and organoleptic qualities [29]. There was a slight decrease in pH for all the wine samples fermented at varying pH (Figure 1). The reduction in pH after fermentation is in line with observations reported previously [32,48,49,50,51] and may be attributed to the production of organic acids such as succinic and lactic acids during fermentation [30,52]. Expectedly, there was an increase in titratable acidity for all the wine samples. Similarly, an increase in TA after fermentation of African bush mango has previously been reported [17]. The reduction in pH and increase in TA after fermentation is desirable because it

can help inhibit growth and spoilage by microbes. Fruit wines of various pH and titratable acidity have been reported by many authors. In this study, miracle berry wine with pH range of 3.3 - 5.8 with corresponding TA range of 8.6 – 13.8 g/L tartaric acid was produced. The pH and titratable acidity values recorded in this study were higher than that reported by [53] for apple wine but was similar to the findings of [54] who produced Korean black raspberry wine of $pH 3.36 \pm 0.01$ and very high TA 11.10 ± 0.07 g/L. The differences in the reported values for different wines may be due to the difference in substrates and the conditions under which the wines were produced.

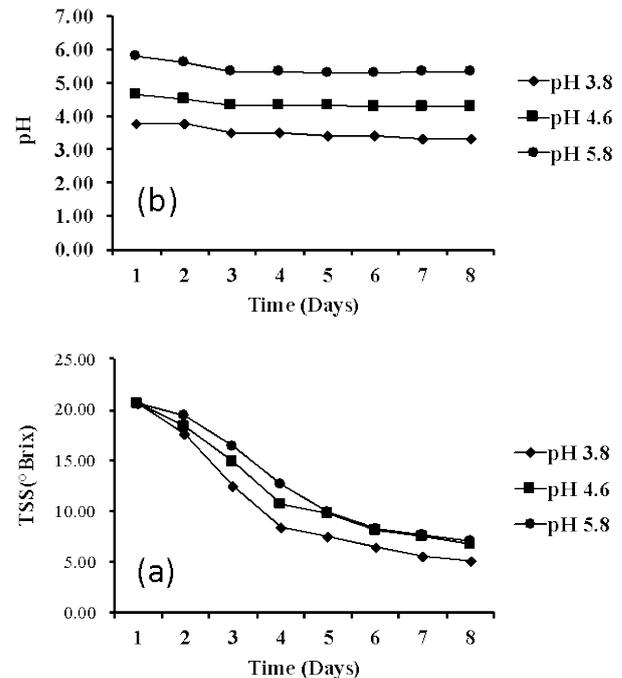


Figure 1. (a) Total soluble solids (TSS) and (b) pH during fermentation at different pH values

Different studies have reported different relationships between polyphenol concentrations and antioxidant capacities. [55] reported low polyphenol but high antioxidant activity for Syrah of 2002 vintage, Syrah of 2003 vintage gave high polyphenol content but low antioxidant activity, and Merlot of 2003 vintage had high polyphenol content and high antioxidant activity. In this study, for all the various wines produced, high polyphenol content gave corresponding high antioxidant activity. This observation is similar to the findings of [56] who found a strong positive correlation between the antioxidant activity and the red wine phenolic constituents such as catechin, myricetin, gallic acid and peonidin-3-O-glucoside.

In this study, the polyphenol concentrations of the various wines produced at varying pH ranged from 300-580 mg GAE/L. These values were lower than what was reported for raspberry wine (977 mg GAE/L) but higher than that of Riesling grapes (250mg GAE/L) reported by [57]. In this study, a decrease in both antioxidant activity and polyphenol content was observed after fermentation. This can be attributed to the decrease in pH after fermentation. This observation is in line with findings of [23,58] who reported on the sensitivities of polyphenols to different pH values since pH affects the structure and stability of polyphenols.

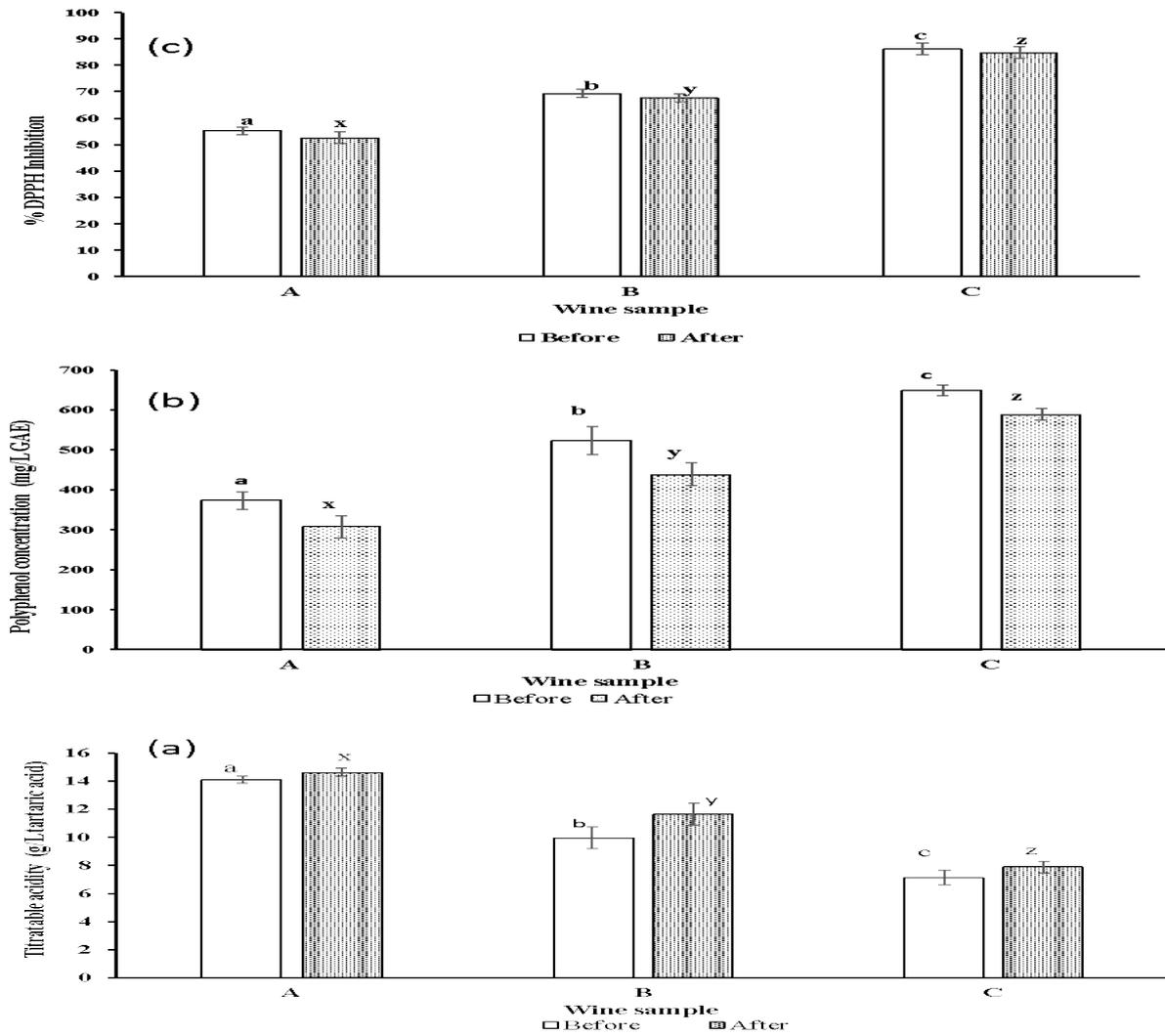


Figure 2. (a) Titratable Acidity, (b) Polyphenol concentration and (c) % DPPH Inhibition of wine sample before and after fermentation at 1% inoculum level and different pH (A= pH 3.8; B=pH 4.6; C= pH 5.8). Bars of the same pattern but different superscript show significant difference ($p < 0.05$).

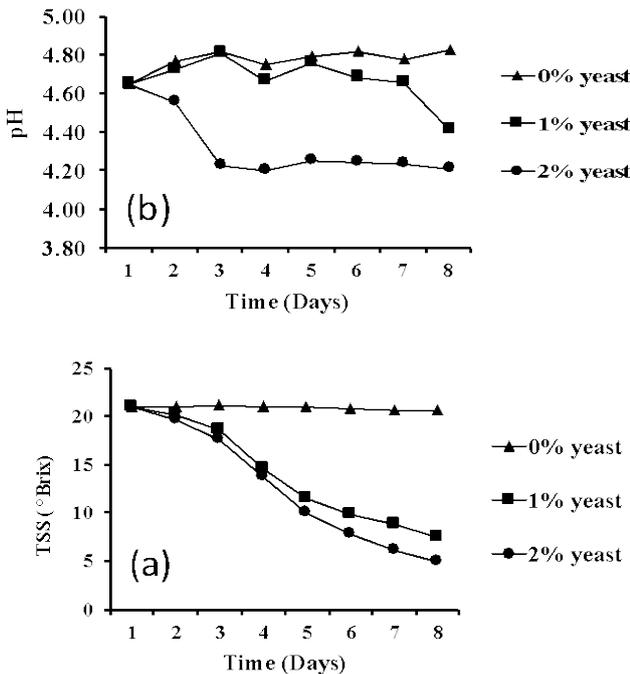


Figure 3. (a) Total soluble solids (TSS) and (b) pH of wine samples fermented using different Inoculum (0-2% yeast)

Miracle berry wine fermented using 2 % inoculum had the highest rate of decrease of TSS and produced high amount of alcohol (12%) followed by 1% and 0%, respectively (Figure 3). This finding is similar to that of [23] who reported differences in alcohol content for tomato wine fermented using different levels of inoculum. The level of inoculum is known to influence the lag phase, specific growth rate and the overall quality of the final product [33]. The higher inoculum (2%) probably shortened the lag phase of the yeasts hence the higher the rate of decrease in TSS with corresponding higher amount of alcohol produced as observed compared to wine fermented with 1% and 0% percent, respectively. Wine sample fermented using 0% inoculum had an extended lag phase hence no active fermentation occurred in the sample resulting in virtually no decrease in TSS and no alcohol detected in the sample at the end of the 7th day of fermentation (Table 2).

After 7 days of fermentation, the pH of must fermented with 0% was higher with a corresponding lower TA value than must fermented with 1% and 2% inoculum, respectively (Figure 3). This finding was similar to the findings of others [23] who reported differences in pH and TA for tomato wine fermented using different levels of inoculum after 8 days of fermentation. Higher inoculum

levels might have resulted in more production of organic acids during fermentation leading to lower pH in the case of wine sample fermented with 2% inoculum than wine fermented with 1% and 0%, hence the variation in pH observed at the end of the fermentation process [23,30].

There was a reduction in both polyphenol and antioxidant activity after fermentation for all the wine samples produced using varying inoculum levels ($p < 0.05$) (Figure 4). This observation can be attributed to the fact that, there was reduction in pH values after fermentation for all the samples except wine produced using 0% inoculum. This observation was similar to others [58,59,61] who reported on the sensitivity of polyphenols to pH change since pH affects the structure and stability of polyphenols. This observation is also similar to that of [60] who observed a decrease in polyphenol concentration particularly flavon-3-ol of apple cider juice after fermentation.

4. Conclusion

Five varieties of wine were produced in this study using *S. dulcificum* (miracle berry) as substrate. pH of the fermenting must and the quantity of starter culture added to the must (inoculum) influenced the wine parameters such as pH, titratable acidity, acid taste index, alcohol content, polyphenol concentration and antioxidant activities of the final product. Hence, these parameters must be carefully studied, monitored, controlled and optimized to produce high quality wines. At the end of the fermentation process, a decrease in pH, antioxidant activity and polyphenol concentration were observed for all the wine samples produced at varying pH. However, an increase in titratable acidity was observed at the end of the study. The alcohol content of the wine samples varied with inoculum level and pH, which implies that controlling the pH and inoculum levels can be used to design and produce miracle berry wine with specific qualities.

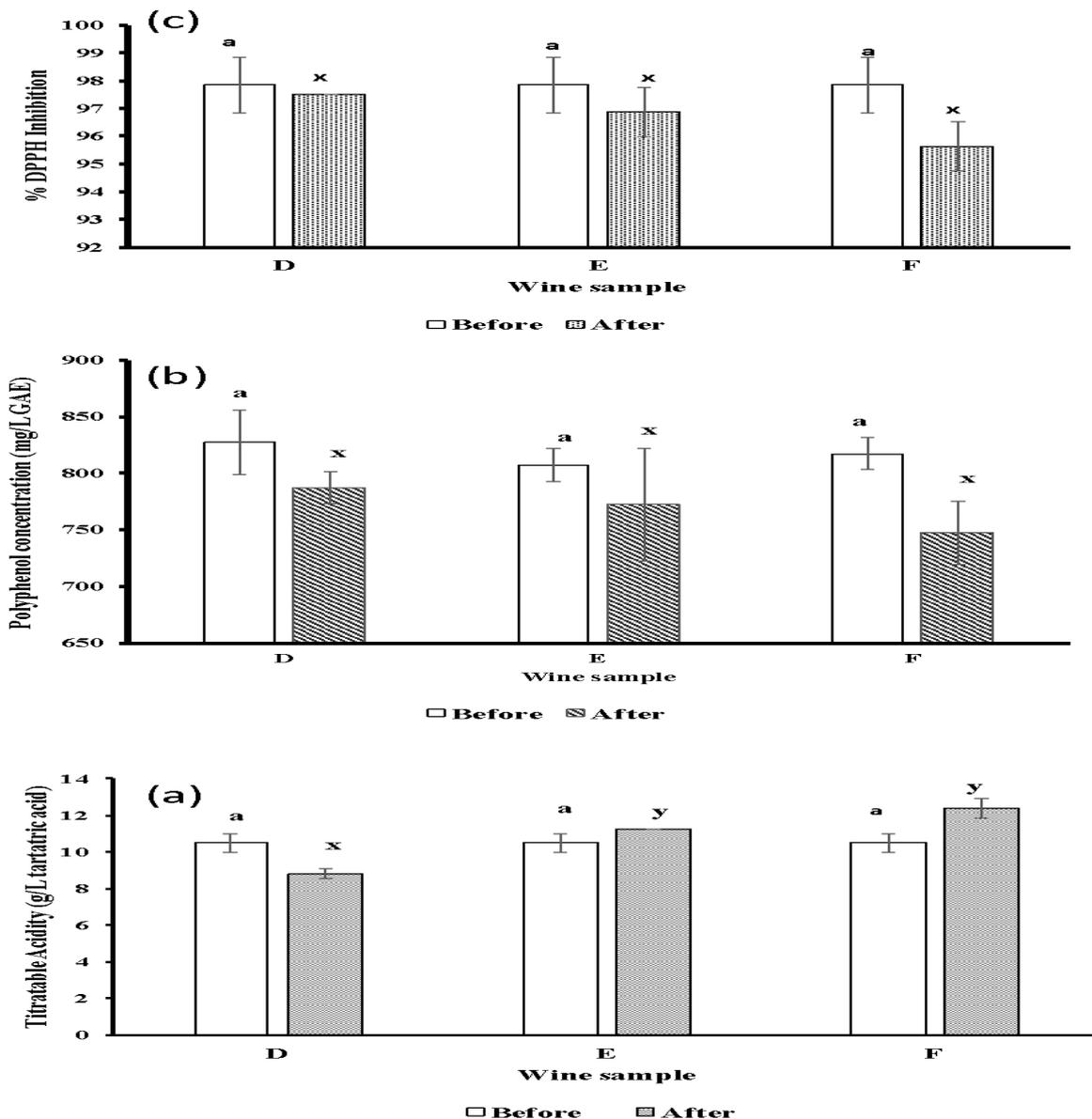


Figure 4. (a) Titratable Acidity, (b) Polyphenol concentration and (c) % DPPH Inhibition of wine sample before and after fermentation of wine at pH 4.6 and at different inoculum levels (D = 0%; E = 1%; F = 2% yeast). Bars of the same pattern but different superscript show significant difference ($p < 0.05$).

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