International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:13, No:11, 2019

Exporting Physiochemical Changes during the Fermentation of *Aloe Vera*

Kyaw Hla Myint, Phyoe Wai Htun

Abstract*—Aloe Vera* is a short-stemmed succulent plant which is commonly used in Myanmar traditional medicine. *A. vera* gel was also used as food addictive. This study aims to improve the Myanmar folk medicine to a functional beverage. In this research, *Aloe vera* was fermented with *Saccharomyces cerevisiae* for 6 months. Three different processes were carried out. Process I contains *A. vera* 10%, sugar 30%, water 50%, and starter culture 10%, process II contains *A. vera* 10%, sugar 15%, honey 15%, and water 50%, starter culture 10%; process III contains *A. vera* 10%, honey 30%, water 50%, starter culture 10%. During wine fermentation, the wine parameters such as alcohol content, total soluble solid (ºBrix), pH, color and cell population were analyzed. After 30 days of fermentation, total cell population remained $2.8x10^6$ in P-I, P-II and $3.2x10^6$ in P-III. Total soluble solid content dropped to 15.8 in P-I, P-II and 15.7 in P-III. After 30 days, clear wine was transferred to other vassals for racking. After 6 months of racking, microbial population reached under detectable level and alcohol content was round about 11% but not significantly different among these processes. P-II was found to have the highest color intensity at 450 nm and it got the most taster satisfaction when sensory evaluation was carried out using five hedonic scales after 6 month of racking.

Keywords*—Aloe vera*, fermentation, *S. cerevisiae,* functional beverage, folk medicine.

I. Introduction

WINE is an alcoholic beverage prepared by the fermentation of fruits [1] and consists of two primary ingredients; water and ethanol [2]. Various types of wine are available such as red wine, white wine, light wine, liqueur wine, sparkling wine and aromatized wine [3].

Aloe vera is a stem-less or very short-stemmed succulent plant with the height up to 60–100 cm tall. The leaves are usually green, thick and fleshy. Some varieties have white flecks on their upper and lower stem surface and the margin is serrated with small white teeth. The flowering season is summer and flowers usually occur on a spike. Its height reaches up to 90 cm. Flowers are being pendulous, with a yellow to red tubular corolla of 2–3 cm [4].

Scientific evidence for the cosmetic and therapeutic effectiveness of *Aloe vera* is limited. *A. vera* gel is also used commercially as an ingredient in yogurts, beverages, and some desserts. *Aloe vera* contains various kinds of functional properties against diseases Due to its antiseptic and antibiotic properties; it is highly effective in treating cuts and abrasions [4], [5]. As a traditional remedy, Aloe has been used for coughs, wounds, ulcers, gastritis, headaches, and arthritis since immemorial age.

A. vera helps the protein synthesis in the body and muscle tissues. Three anti-inflammatory fatty acids, cholesterol, campesterol and β-sitosterol were found in Aloe juice. So, it is widely used as food addictive. Moreover, it has anti-viral intestinal cleanings and buffering functions. About 23 polypeptides in the juice can help fight against AIDS, boosts immune function and eliminate cancer tumors, stabilize blood sugar. It can be used to reduce cholesterol in diabetics and reduce high blood pressure [6]. It was proven that *Aloe vera* wine has antimicrobial activities and improves the probiotic bacteria colonization in the gut [7].

Fermentation is the oldest preservation form of foods. Yeasts are facultative anaerobic microorganisms that can metabolize sugars aerobically or anaerobically using two different metabolic pathways: respiration and fermentation [8]. Fermentation is the microbial process in which they consume different substrates for their metabolism requirement and eliminate their metabolites into the medium. These cause changes in flavors, taste of fermentation medium because of fermentative metabolites. Yeasts are unicellular fungi which reproduce by budding or fission, a means of asexual reproduction. Yeast can be isolated in the bloom of fruits, in the nectar of flowers and hence in honey, on grains and seeds and, to the lesser extent, in the soil and floating in the air [2], [8]. *S. cerevisiae* is the primary wine yeast that can convert glucose and fructose into its metabolic energy [9].

Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide. The optimum temperature for growth of most yeast is around 25 to 30 ºC and the maximum about 35 to 47 ºC. Under aerobic conditions, yeasts metabolize sugar and nutrients to increase their populations by respiration but the fermentative yeasts can grow anaerobically. During this anaerobic condition, they convert sugar into alcohol and carbon dioxide by alcoholic fermentation [9], [10]. During fermentation, yeast converts sugar into ethanol and carbon dioxide in the presence of essential nutrients such as amino acids, minerals and vitamins. It produces vest verities of metabolites as byproducts. The common aromatic compounds that make varietal wines distinctive are higher alcohols, sulphur compounds and fatty acid esters [10].

The yeasts that are used for fermenting, whether to make bread, beer, wine or whisky, all belong to the same species, *Saccharomyces*, which means sugar fungus. In the case of wine making, yeasts can cause spoilage of wine if they cannot be eliminated properly [11]. Top yeasts actively ferment and

Phyoe Wai Htun is with Department of Biotechnology, Mandalay Technological University, Mandalay, Myanmar (corresponding author; phone: +95 9 402645243, e-mail: phyoewaihtun@mtu.edu.mm).

Kyaw Hla Myint is with Food and Drug Administration, Nay Pyi Taw, Myanmar. (e-mail: kyawhlamyint@gmail.com).

grow rapidly at 20 ºC. The clumping of the cells and the rapid evaluation of CO_2 sweep the cells to the surface, hence the term top yeast. Bottom yeasts do not clump but grow more slowly. So, bottom yeasts are the most suitable for fermentation at lower temperature (10 to 15º). The absence of CO_2 clumping and the slower growth and evaluation of CO_2 permit the yeasts to the bottom, hence the term bottom yeast. *S. varellipsoidous* is a high-alcohol-yielding variety used for the industrial alcohol production, wine, and distillate liquors [9].

In Myanmar, the combination of *Aloe vera* and sugar as a form of semi-solid is famous for anti-ageing, urinary disorder and intestinal health [12]. But, more scientific researches are still needed for the process development. This study aims to improve the Myanmar folk medicine as functional beverage.

II. Materials and Methods

A. Preparation of Starter Culture

100 ml of sterile 10% sugar solution was heated to 45 ºC for yeast activation. 0.3 g of instant yeast (*Saccharomyces cerevisiae*) was incubated in this warmed sugar solution for 6 hr.

B. Collection of Aloe vera and Preparation of Sample

The *Aloe vera* leaf were collected from the aloe vera cultivation field and washed with distilled water three times. In this experiment, wounded or infected leaf was not used. The leaf gel were collected on the clear tray and cut into small pieces. These pieces were thoroughly washed with distilled water. The wine fermentation processes were prepared with different ratios (Table I). For each process, fermentation media was adjusted to BriX 26% and three different processes were carried out in these experiments. Traditionally, earthen pots were used for fermentation as they can control the environmental changes. In this experiment, earthen pots were used as fermentation tanks so that the process can be standardized.

C. Aloe vera Wine Fermentation

Ingredients were added to the sterile container according to their respective ratio. 10% of starter culture was used in this experiment to override the natural yeast population. The anaerobic fermentation was carried out for one month. After fermentation, the clear wine was separated from the spent yeast cells and other solids. During fermentation, the progress of wine fermentation was monitored and the wine parameters such as alcohol content, total soluble solid (ºBrix), pH, and color and cell population were examined. After 30 days of fermentation, the clear wine was transferred to vessels for racking. After this stage, wine parameters were also studied and physicochemical parameter changes during wine fermentation were explored.

III. Results and Discussion

In this experiment, *Aloe vera* leaf was washed thoroughly with distilled water, collected on the clear tray and the gels were cut into small pieces as shown in Fig. 1. The types of microbes present on wine will have an impact in the wine fermentation, especially in the early stages [2]. The yeast used for fermentation is important to be higher and override other microflora. The commercial baker yeast strain *S. cerevisiae* was used in this research as most of the local wine yards use this type of strain. The colonial morphology and microscopic morphology of *S. cerevisiae* were studied. This yeast strain has round, opaque colony and creamy in color on Potato Yeast Glucose (PYG) agar media shown in Fig. 2. The microscopic examination showed that its size is 8-10 µm in diameter. During the fermentation, CO_2, ethanol and other metabolites were excluded [2]. The production of CO_2 and ethanol inhibits the growth of aerobic spoilage bacteria. The parameter of wine such as alcohol content, total soluble solid (ºBrix), pH, and color and cell population were analyzed. The different kinds of physicochemical parameters of wine fermentation (P-I, P-II and P-III) are shown in Tables II-IV.

TABLE I
Preparation of Wine Sample Fermentation

Process I		Process II		Process III	
Aloe Vera	10%	Aloe Vera	10%	Aloe Vera	10%
Honey	30%	Sugar	15%	Sugar	30%
Water	50%	Honey	15%	Water	50%
		Water	50%		

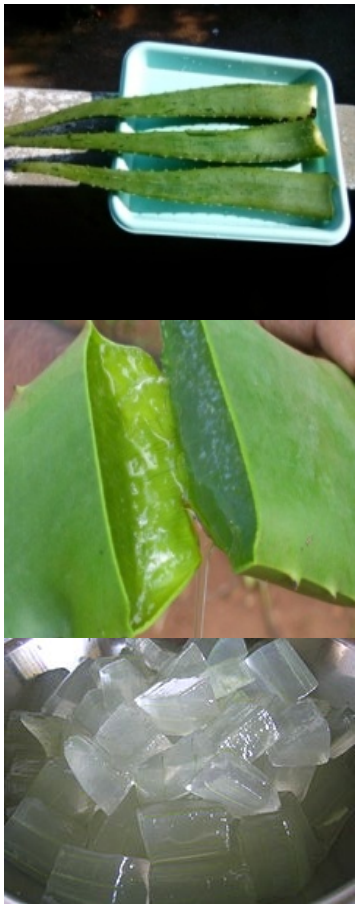

Fig. 1 Preparation of aloe vera leaf gels

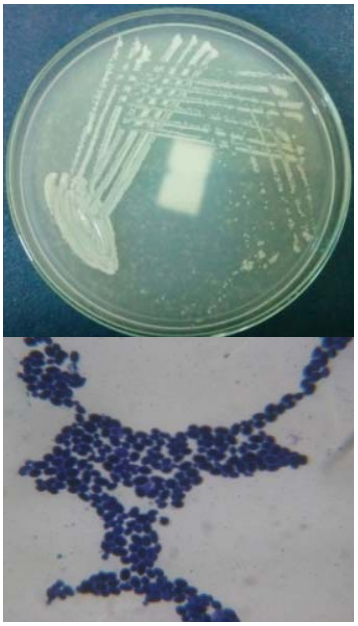

Fig. 2 Cultural and Microscopic Morphology of *Saccharomyces cerevisiae*

TABLE II
PHYSICOCHEMICAL PARAMETERS OF WINE FERMENTATION (P-I)

Time	Cell population (CFU)	pH	Total soluble solid (BriX)	Color (450 nm)
0 hr	$4x10^6$	4.3	26	0.46
6 hr	$7.2x10^6$	4.2	25.9	0.50
12 hr	$4.0x10^7$	4.0	25.7	0.45
18 hr	$4.4x10^7$	4.1	25.5	0.42
24 hr	$4.0x10^7$	3.9	25.3	0.46
48 hr	$3.76x10^7$	3.7	25.3	0.48
72 hr	$3.6x10^7$	3.5	25.4	0.45
4 days	$2.4x10^7$	3.2	25.2	0.45
5 days	$2x10^7$	3.1	24.8	0.58
10 days	$1.5x10^7$	3.0	21.8	0.53
15 days	$4.8x10^6$	3.0	21.0	0.51
30 days	$2.8x10^6$	3.0	15.8	0.42
6 months	ND	3.1	14.3	0.05

TABLE III
PHYSICOCHEMICAL PARAMETERS OF WINE FERMENTATION (P-II)

Time	Cell population (CFU)	pH	Total soluble solid (BriX)	Color (450 nm)
0 hr	$1.6x10^6$	4.5	26	0.32
6 hr	$6x10^6$	4.3	25.9	0.31
12 hr	$3.6x10^7$	4.1	25.5	0.31
18 hr	$4x10^7$	4.2	25.4	0.30
24 hr	$4x10^7$	4.0	25.3	0.30
48 hr	$3.8x10^7$	3.8	25.3	0.32
72 hr	$3.6x10^7$	3.6	25.4	0.37
4 days	$2.8x10^7$	3.5	25.1	0.38
5 days	$1.8x10^7$	3.4	24.8	0.43
10 days	$1.56x10^7$	3.2	21.7	0.47
15 days	$5.6x10^6$	3.0	21.1	0.40
30 days	$2.8x10^6$	3.0	15.8	0.13
6 months	ND	2.9	14.3	0.09

The pH, total soluble solid (Brix) and color changes of all wine samples were examined and the total population of bacterial count in wine samples is counted and is shown in Tables II-IV. In this experiment, 6 hr incubation period of viable counts ($4.8x\ 10^7$ CFU/ml) was used as starter culture in order to dominant the other cell population.

TABLE IV
PHYSICOCHEMICAL PARAMETERS OF WINE FERMENTATION (P-III)

Time	Cell population (CFU)	pH	Total soluble solid (BriX)	Color (450 nm)
0 hr	$3.6x10^6$	5.2	26	0.13
6 hr	$8x10^6$	4.9	25.9	0.15
12 hr	$4.4x10^7$	4.6	25.5	0.12
18 hr	$4.4x10^7$	4.5	25.2	0.10
24 hr	$4x10^7$	4.3	25.3	0.11
48 hr	$3.8x10^7$	4.0	25.3	0.09
72 hr	$3.6x10^7$	3.8	25.4	0.19
4 days	$2.4x10^7$	3.6	25.1	0.15
5 days	$1.6x10^7$	3.4	24.7	0.17
10 days	$1.2x10^7$	3.5	21.8	0.24
15 days	$5.2x10^6$	3.2	21.0	0.20
30 days	$3.2x10^6$	3.1	15.7	0.15
6 months	ND	2.5	14.5	0.08

TABLE V
OVERALL AVERAGE RESULTS OF COLOR, FLAVOR AND TASTE IN 5 HEDONIC SCALE AFTER 6 MONTHS FERMENTATION

General average	P-I	P-II	P-III
Color	4.2	3.8	2.3
Flavor	3.5	3.7	1.5
Taste	3.4	4.3	2.4
Over average	3.7±1.0	3.9±1.2	2.1±0.9

Physicochemical parameter changes were studied for 6 months. All the fermentation processes are carried out under the same environmental condition of 30 ± 5 °C. Wine racking was conducted under cool and dark condition because yeast can cease fermentation over 35 °C [5]. In the process I, bacterial cell population was 4×10^6 CFU/ml at 0 hr while 1.6×10^6 CFU in process II and 3.6×10^6 CFU in process III. These cells' population reached to stationary phase after 12 hr of fermentation and declined slightly after 15 days of fermentation in all processes. After 30 days of fermentation, total cell population remained 2.8×10^6 in P-I, P-II and 3.2×10^6 in P-III. So, it can be assumed that all processes have similar growth patterns. In this experiment, clear wine was transferred to other vassals for racking although there is microbial cell population. Total soluble solid content dropped to 15.8 in P-I, P-II and 15.7 in P-III. The golden wine color was occurred in P-I. The high ration of honey content gives its attractive wine color although the ºBrix was adjusted to the same level. After fermentation, clear wine was transferred to other container for aging.

pH was 4.3 in P-I, 4.5 in P-II and 5.2 in P-III at 0 hr. It was occurred that the more the honey content, the lower the pH value is. This is due to the some fermentation that already took place in honey. pH level dropped slowly during fermentation. It was occurred that fermentation process with honey reached

to pH 3 earlier than others. That means that yeast can convert honey sugar easily and these conditions can prevent other bacteria spoilage. So, it can be pointed out that honey contact is an important factor for *Aloe vera* wine fermentation as it gives not only nutrients to yeast but also color to wine. However, the similar pH level occurred in all treatments after 30 day fermentation. The color changes during fermentation were studied at 450 nm to estimate the level of cloudy and turbidity. Before fermentation, process I showed the highest color density because of honey content. It retained its color density until 30 day fermentation. It occurred that there are no significant changes in turbidity. These showed that the yeast cell population increased and fermentation took place. The clearer wine occurred in fermentation process I of honey only after 6 months of racking. The alcohol content was estimated by alcoholmeter after wine was distilled. 12.5% in P-III and 11.4% in P-II and 10.5% in P-I occurred respectively. So, there will be no significant difference between processes.

After fermentation, sensory evaluation was carried out for the acceptability for color, flavor and taste of *Aloe vera* wine based on five hedonic scales. During fermentation, yeast produced different kinds of metabolites into the media and gave the beverage flavor. 10 volunteer tasters between the ages of 20-35 yr participated for the evaluation of appearance, flavor and aroma. The fermentation of P-II process got more preference (3.9) in color, flavor and taste than other processes but not significantly different to P-I. P-II containing equal ration of honey and sugar attracted the taster preference.

IV. Conclusion

The commercial baker yeast strain *Saccharomyces cerevisiae* can be used for *Aloe vera* wine fermentation. In this experiment, clear wine was transferred for racking after 30 days of fermentation, leaving any sediment behind although there is still microbial population. It can cause microbial cell growth within wine during racking. The microbial cell population cannot be detected after 6 months. The ratio of honey content was important for *Aloe vera* wine fermentation. The fermentation process with equal ration of honey and sugar got the most preference to the wine tasters.

Acknowledgment

The authors are grateful to Myo Myint, Zaw Khaing Oo (Department of Biotechnology, Mandalay Technological University) for their kind suggestion. Special thanks are extended to Dr. Thint Thint Kyawe, Department of Chemical engineering, and Mandalay Beer Brewery for providing technical assistance.

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