**Addis Ababa University**
**School of Graduate Studies**
**Faculty of Science**
**Food Science and Nutrition program**

# PREPARATION OF WINE FROM CACTUS PEAR FRUIT/BELES/ (Opuntia spps).

**BY:**
**YOSEF BEYENE**

**A thesis submitted to the School of Graduate studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition.**

**Sep, 2012.**

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**By Yosef Beyene**

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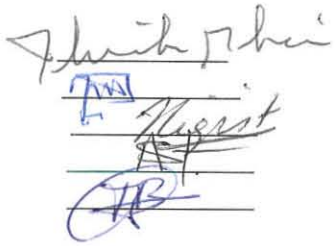

# PREPARATION OF WINE FROM CACTUS PEAR FRUIT/BELES/ (Opuntia spps).

BY
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UNDER SUPERVISSION OF
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# Table of Contents

List of Tables

## List of figures

**List of Abbreviations;**

| | |
|---|---|
| AOAC | Association of official and analytical chemists |
| APC | Aerobic Plate Bacterial Count |
| BGB | Brilliant Green Bile Agar |
| CFU | Colony Forming Unit |
| CSA | Central Static Authority |
| EHNRI | Ethiopia Health and Nutrition Research Institutions |
| FID | Flame Ionization Detector |
| RBA | Rose Bengal Agar |
| TSA | Trypton soya Agar |
| VRBA | Violate Red Bile Agar |

# *Abstract*

*An investigation was conducted on the cactus fruit (**Opuntia spp**) for the physicochemical content, microbial safety and potential of wine production by using both indigenous yeast flora and commercial yeast extract and the acceptability of produced wine.*

*The physicochemical content and the microbial safety of the cactus juice were analyzed with standard methods at EHNRI laboratories. The results of the microbial analysis of the juice were $6.8x10^6$, $3.5x10^3$, $1.5x10^1$ and $2.5x10^2$ CFU/ml of total aerobic bacteria, total coliform, yeast count and mould count, respectively. Fecal coliform, E-coli and Salmonella were not detected in the juice. In this study, the physicochemical analysis of the juice such as; total solids, protein, fat, ash, carbohydrates, fiber contents, titratable acidity, pH, specific gravity of the juice were done and the results were 13%, 0.7%, 0.3%, 0.8%, 8.45%, 2.8%, 0.03%, 5.011, and 1.0325, respectively. Four different compositions of juice in vessels (A; juice + indigenous yeast, B; juice + sugar + indigenous yeast, C; pasteurized juice + commercial yeast and D; pasteurized juice +sugar + commercial yeast) were fermented with indigenous and commercial yeast. The alcohol productions were 4.9, 7.8, 5.3 and 12.2 % v/v after 10 days fermentation. The alcohol produced in vessel D was the highest followed by vessel B. The alcohol produced in Vessel D > vessel B > vessel C > vessel A. There was significant difference between vessel D and vessel (A, B and C), and vessel B and vessel (A, C, and D). There was no significant difference between vessel A and vessel C in terms of alcohol production. The sensory analysis showed no significant difference in attributes such as color and appearance between individual vessels. Vessel D has higher score in terms of aroma, taste and overall acceptability than other vessels.*



*Keywords; Fermentation, Indigenous yeast, Commercial yeast and fruit wine*

# 1. INTRODUCTION

## 1.1. Background

Prickly pears are members of the Cactacceae or cactus family, which include about 97 genera and 1,600 species. The species are found in Europe, Mediterranean countries, Africa, southwestern United States, and northern Mexico. Plants in the genus Opuntia prefer a dry, hot climate and consist of perennial shrubs, trees, and creeping plants. Prickly pear can grow 5 to 8 m in height; its roots are shallow, but the plant can spread up to 40 m in diameter over the ground (DeFelice, 2004).

The prickly pear (cactus plant) is a vegetable and a fruit. It is one of the few plants that are both. The green pods (pads), called napolito, are the vegetable, it is cylindrical in shape. The oval, pear-shaped, purplish colored pear (tuna) of the cactus is the fruit. It is called Cactus pears or Cactus fruit. The fruit is a berry, typically weighs 100 to 200 g (0.2 to 0.4 lb), and consists of a thick fleshy skin or rind surrounding a juicy pulp that contains many hard-coated seeds. It varies in color, size and flavor. The green pods are edible all year round; however, the pears are only available in the summer months (March, 2011, Synman, 2006).

Cactus pear fruits play an important role in human diet in their countries of origin, such as Mexico and Chile, where peeled fresh fruits are commonly consumed at home, in vegetarian restaurants, or in local health-food stores (Pimienta-Barrios, 1994; Sáenz and Sepúlveda, 2001).

The cactus pear (Opuntia ficus-indica) found in Ethiopia particularly in Tigray and Oromiya (Bale). In Tigray, it is viewed as both a blessing and a curse and it has become an integral part of the culture and economy and is utilized in many ways, although not as a vegetable, nor is it processed much. While efforts are also made to exploit value-adding and processing opportunities. However it plays a crucial role in sustaining humans and livestock during drought and famine (Brutsch, 1997).

More than 85% of the Tigary population is reported to be directly dependent on agriculture for a livelihood, but, with long periods of drought and unreliable rainfall compounded by excessive

human and livestock pressures on the land, famines are not uncommon, resulting in high human and livestock mortalities. It is in this context that the cactus pear plays an increasingly vital role as a source of food and animal feed. It is also used as a fuel, as a live fence or hedge, for soil conservation, and its fresh fruit as a limited source of household income (CSA, 2006).

Wine plays almost an indispensable role in the life of man ranging from social, religious as well as economic benefits. It is an alcoholic beverage typically made from fermented juice of different fruits, usually from grapes using yeasts. However it is also made from other fruits, such as apples and berries, and is normally named after the fruit from which it is produced (for example, apple wine or elderberry wine) and is generically called fruit wine or country wine (Johnson, 1989).

Wine is a healthful beverage that has been seen as a natural remedy for man's illness from early days and is said to aid recovery during convalescent period (Jay, 1996; Okafor, 2007). In recent times, home wine production has been practiced with various fruits such as apple, pear and strawberry, cherries, plum, pineapple and oranges (Fleet, 1993; Webb, 1984).

Wine fermentation may be natural with innate wild yeasts or artificial using grown yeast cultures such as Baker's yeast. Virtually, any fruit or sugary plant sap can be processed into an alcoholic beverage. The process is well known being essentially an alcoholic fermentation of sugars to yield alcohol and carbon dioxide. There are many studies in the literature that demonstrate the feasibility of using fruits, such as cacao (Dias et al., 2007), gabiroba (Duarte et al., 2009), kiwi (Soufleros et al., 2001), cajá (Dias, at al, 2003), mango (Reddy & Reddy, 2005) and orange (Selli et al. 2008) to produce alcoholic beverages.

In this study, the potential of cactus pear fruit for the purpose of wine production is investigated.

## 1.2. Statement of the problem

Cactus pear fruits are susceptible to rapid microbial spoilage, especially by yeasts and mesophilic bacteria, therefore feasible transformation and preservation processes are a prerequisite to prevent postharvest losses. The late discovery of their nutritive value may explain that no commercial juice products are available at industrial level and processing of cactus pear fruits in general has only been scarcely investigated (Joubert, 1993; Sáenz and Sepúlveda, 2001).

There is an abundant cactus fruits in Ethiopia specifically in Tigray region with a potential to be used by the food industry. Moreover, the production of fruit crops in Tigray is seasonal, thus, there is a need to preserve and store them from time of harvest through the period of scarcity, for the purpose of retaining them as foods and articles of trade. Different new uses and new methods for processing cactus fruits need to be developed to minimize postharvest losses, generate more profits and promote the sustainable use of biomes. One possible use of these fruits may be production of fruit wines (Dias et al., 2007; Duarte et al., 2009).

## 1.3. Significance of the study

Findings of this study could show the potential of cactus pear fruit for the production of fruit wine; Thus, the study could;

- Be used for beverage industries for production of alternative wine from cactus fruits.
- Be used to provide income and employment to many people especially for producers.
- Be used to decrease postharvest losses of cactus pear fruit.
- Be used as a benchmark for further research works in related production of wine from other fruits.

## 1.4. Objectives

### 1.4.1. General objective;

- To investigate the cactus pear fruit for the purpose of wine production.

### 1.4.2. Specific objectives;

- To determine the potential of cactus pear fruit for alcohol production using indigenous yeast.
- To determine the potential of cactus pear fruit for alcohol production using commercial yeast.
- To determine the physical and chemical characteristics of the wine produced from cactus fruit.
- To evaluate the acceptability of cactus pear fruit wine in terms of flavor, aroma, color and overall quality.

# 2. Literature review

## 2.1. Physicochemical characteristics of cactus pear fruits

The cactus pear fruit, also known as prickly pear, tuna, or fico d'india, is an oval, elongated berry of 67-216 g weight. They offer a wide spectrum of colors from white, yellow, orange, red, and purple based on betalains (Stintzing et al., 2005) and contain about 85% water, 15% sugar, 0.3% ash and less than 1% protein (Mohamed-Yasseen et al., 1996). The thick pericarp is covered with small-barbed spines hosting a juicy pulp with 150-300 nonedible seeds. The latter account for 3-7% on a weight basis, followed by the pericarp and mesocarp (36-48%) and the edible pulp (39-64%) (Felker et al., 2002; Felker et al., 2005; Gurrieri et al., 2000; Hoicke and Stintzing, 1999). The pulp is the most valuable edible part of the cactus pear fruit; it is considered the most interesting fruit portion for food processing.


**Figure 1; picture of cactus pear fruit.**

The chemical and mineral composition described by different authors shows that cactus pears have nutritive value similar to other fruits. Its soluble solids content in the pulp reaches values higher than 17%, mainly constituted by 53% glucose and fructose (Russell and Felker, 1987; Sawaya et al. 1983; Sepúlveda and Sáenz, 1990; Kuti and Galloway, 1994).

Other components in cactus-pear pulp, such as protein (0.21% to 1.6%), fat (0.09% to 0.7%), fiber (0.02% to 3.15%) and ash (0.4% to 1%), are similar to other fruits (Askar and El Samahy, 1981; Paredes and Rojo, 1973; Pimienta, 1990; Sawaya et al., 1983; Sepúlveda and Sáenz, 1990; Rodriguez et al., 1996).

### 2.1.1. Sugars and organic acids

Total soluble solids range between 12 and 17°Brix (Sáenz-Hernández, 1995), with glucose and fructose being the predominant carbohydrates in a ratio of about 1:1, depending on invertase activity (Kuti and Galloway, 1994; Sawaya et al., 1983). The high sugar content of the pulp results in sugar:acid ratios within the range of 90:1 up to 490:1, which is responsible for the bland taste and, therefore, far from a sensory pleasant ratio of 10 to 18 (Felker et al., 2005; Joubert; 1993, Odoux and Domínguez-López, 1996; Stintzing and Carle, 2006).

The pH-value varies between 5.6 and 6.5 in fully ripe fruits (Felker et al., 2005) resulting in a very low acidity of about 0.05% to 0.18% citric acid and thus characterizes cactus pears as a low-acid food (pH > 4.5). Hence, acidification prior to thermal treatment is a prerequisite to allow pasteurization instead of more rigid sterilization, thereby retaining nutritionally important components such as betalains (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001).

Whereas citric acid (62.0 mg/100 g fruit weight) is the major organic acid in cactus pear, followed by malic acid (23.3 mg/100 g), quinic (19.1 mg/100 g), shikimic (2.8 mg/ 100 g), oxalic acids are also typical (Barbagallo et al, 1998). Isocitric, fumaric, glycolic and succinic acids were only found in traces (Askar and El-Samahy, 1981; Stintzing et al., 2001).

### 2.1.2. Vitamins and minerals

Cactus pears have a high level of ascorbic acid, which can reach levels near 40 mg/100 g (Pimienta, 1990; Sepúlveda and Sáenz, 1990; Rodriguez et al., 1996). Cactus-pear fruits are also rich in calcium and phosphorus, 15.4 to 32.8 mg/100 g; 12.8 to 27.6 mg/100 g, respectively (Sawaya et al., 1983; Sepúlveda et al., 1990). Potassium is another important mineral component with values of about 217 mg/100 g for the green-pulp cactus pear (Sepúlveda and Sáenz, 1990).

### 2.1.3. Amino acids

The high level of free amino acids in fruits from O. vulgaris was first reported in 1981 (Askar and El-Samahy, 1981). In a recent study, previous data on fruit analysis could be confirmed

(Stintzing et al., 1999): Free amino acids comprised all essential amino acids. Proline was found to be the predominant amino acid in the pulp of six different cultivars ('Apastillada', 'Bianca', 'Gialla', 'Gymno carpo', 'Morado', and 'Rossa') from Opuntia ficus-indica fruits amounting to 883.4 and even 1929.1 mg/L followed by taurine (323.6 to 407.3 mg/L), glutamine (98.3 to 574.6 mg/L), and serine (130.6 mg/L to 392.6 mg/L) (Stintzing et al., 1999, Kugler et al., 2006).

### 2.1.4. Hydrocolloids

The polysaccharide fraction of cactus pear pulp was only recently reported to be composed of a complex mixture of polysaccharides of which less than 50% corresponded to a pectin-like polymer. The hydrocolloid fraction from the fruit pulp of O. ficus-indica fruits obtained by ethanol precipitation yielded 3.8% and contained 93.5% sugars. Whereas, after saponification, uronic acid content of 42.3% was determined; neither proteins nor nitrogen were detected. After total hydrolysis, the presence of arabinose, rhamnose, xylose, and galactose in the molar ratio of 1.0:1.7:2.5:4.1 was found. However, further studies are required to completely characterize the hydrocolloid fraction of cactus pear fruit pulp (Matsuhiro et al., 2006).

### 2.1.5. Polyphenols

The presence of polyphenols in the pulp has been recently reported (Butera et al., 2002; Kuti, 2004; Tesoriere et al., 2005a,b). Quercetin, kaempferol, and isorhamnetin derivatives were found to be the major flavonoids in cactus pear pulp (Kuti, 2004). However, more in-depth studies should be envisaged to fully assess the phenolic composition of cactus pear fruits neglected so far.

### 2.1.6. Lipids, sterols, and fat-soluble vitamins

It is well known that mesocarp or pulp of fruits generally contain very low levels of lipids ranging from 0.1 to 1.0% (Kamel and Kakuda, 2000). In cactus pear pulp oils, linoleic acid was reported the dominating fatty acid, followed by palmitic and oleic acids. Also, the polyunsaturated fatty acids γ-linolenic and α-linolenic acids were detected in higher amounts (Ramadan and Mörsel, 2003a,c). The major sterol in pulp oils was β-sitosterol followed by

campesterol, together constituting about 90% of the total sterol portion. Interestingly, δ-tocopherol was the predominant vitamin E homologue, followed by α-, β-, and γ-tocopherols in far less amounts (Ramadan and Mörsel, 2003a, c).

### 2.1.7. Flavor compounds

Cactus pear pulp flavor is characterized by a faint melon or cucumber-like aroma determined by alcohols and esters with 2-(E/Z)-2,6-nonadien-1-ol and 2-methylbutanoic acid methyl ester being the key aroma substances (Agozzino et al., 2005; Arena et al., 2001; Di Cesare and Nani, 1992; Flath and Takahashi, 1978; Weckerle et al., 2001). The flavor intensity was found to be strongest for yellow, followed by red, and, finally, white cactus pear fruits (Agozzino et al., 2005).

### 2.1.8. Pigments

Cactus pear pulps offer different colors based on betalains covering a wide spectrum from white to purple with pigment contents of 66 to 1140 mg/kg fruit pulp (Castellar et al., 2003; Stintzing et al., 2005). Cactus pear fruits bear both yellow betaxanthins and red betacyanins in betaxanthin:betacyanin ratios varying between 0 to 11.7 resulting in different color shades (Butera et al., 2002; Odoux and Domínguez-López, 1996; Stintzing et al., 2005). Interestingly, fruits solely containing betaxanthins are not yet known (Stintzing and Carle, 2006).

Table 1; Physicochemical characteristics of purple and green cactus-pear pulp.

| Parameters | Green pulp* | Purple pulp** |
|---|---|---|
| pH | 5.3–7.1 | 5.9–6.2 |
| Acidity (% citric acid) | 0.01–0.18 | 0.03–0.04 |
| TSS (°Brix) | 12–17 | 12.8–14.5 |
| Pectins ($g.100g^{-1}$) | 0.17–0.19 | --- |
| Vitamin C ($mg.\ 100g^{-1}$) | 4.6–41.0 | 20.0–31.5 |
| Ca ($mg.\ 100g^{-1}$) | 12.8–27.6 | --- |
| Color | 18.2–26.7 | 22.4–33.4 |
| L* | 4.2–5.5 | 10.0–18.4 |
| a* | 4.0–6.5 | 1.1–4.3 |
| b* | 5.8–8.5 | 10.1–18.9 |
| C* | 130.2–136.4 | 6.2–13.2 |
| H* | | |
| Viscosity (mPa•s) | 73.9 | 119.2 |

Source: *Askar and El-Samahy (1981); *Pimienta (1990); *Sawaya et al. (1983); *Sepúlveda and Sáenz (1990); *Sáenz (1995);*Sáenz (1996); **Sáenz, Sepúlveda and Moreno (1995);**Sáenz and Sepúlveda (1999)

## 2.2. History of Wine

Wine has a rich history dating back to around 8000 BC and is thought to have originated in areas now within the borders of Armenia, Georgia and Iran. Wine first appeared in Europe at about 4500 BC in the Balkans, and was very common in ancient Greece, Thrace and Rome. Wine has also played an important role in religion throughout history. The Greek god Dioysus and the Roman Bacchus represented wine, and the drink is also used in Catholic Eucharist ceremonies and the Jewish Kiddush ("wine" Encyclopedia, Dec, 2010).

Archaeological evidence suggests that the earliest known production of wine, made by fermenting grapes, took place in sites in Armenia, Georgia and Iran, from as early as 8000-6000 BC. These locations are all within the natural area of the European grapevine *Vitis vinifera* (Berkowitz, Mark 1996).

A 2003 report by archaeologists indicates a possibility that grapes were mixed with rice to produce mixed fermented beverages in China in the early years of the seventh millennium BC. Pottery jars from the Neolithic site of Jiahu, Henan contained traces of tartaric acid and other organic compounds commonly found in wine. However, other fruits indigenous to the region, such as Hawthorn, can not be ruled out. If these beverages, which seem to be the precursors of rice wine, included grapes rather than other fruits, these grapes were of any of the several dozen indigenous wild species of grape in China, rather than from *Vitis vinifera*, which were introduced into China some 6000 years later (Eijkhoff, 2000).

## 2.3. Commercial importance of wine

Wine production starts with vineyards for grape growing.Grape production has developed in to the World's most important fresh fruit crop. In 2002, the worldwide grape production was about 62 millions metric tons. Spain, France, Italy, China and USA have greatest vineyard areas. Approximately 66% of the production was fermented in to wine (OIV, 2005). France and Italy produce the largest volumes of wine. They produce about 50% of the world's wine, and supply 60% of the world wine export. (Glanzel and Veugelers, 2006).

Table 2: Wine sales in the U.S. from 2004 to 2009 in millions of gallons.

| Year | Tabel wine | Dessert wine | Sparkling wine | Total wine | Total Retail value |
|---|---|---|---|---|---|
| 2009 | 670 | 64 | 33 | 767 | $28.7 billion |
| 2008 | 658 | 64 | 32 | 753 | $30.0 billion |
| 2007 | 651 | 62 | 33 | 746 | $30.4 billion |
| 2006 | 628 | 58 | 32 | 718 | $27.8billion |
| 2005 | 609 | 52 | 31 | 692 | $25.8 billion |
| 2004 | 589 | 45 | 31 | 665 | $24.0 billion |

Sources: Volume—Wine Institute, Department of Commerce, Estimates by Gomberg, Fredrikson & Associates. Preliminary

## 2.4. Preparation of wine

The basic process for making wine is pretty straightforward. Fresh fruits are first crushed and separated from the stems. Next the mixture of juice and solids (called *must*) is allowed to ferment with yeast, converting the sugar from the fruit to alcohol and carbon dioxide, and extracting the color from the fruit. Once fermentation is completed, the wine is separated from the fruit solids in a *wine press* and set aside for aging. The wine will be allowed to age and develop its flavors over a year. Over the course of this year, it may be possible to add oak, tannins or a variety of other types of additives to the wine to augment or change its flavor. Also, the wine will be transferred to a fresh container periodically (called *racking*) in order to separate it from the sediments that naturally settle out of the wine during this time. Towards the end of the aging phase, clarifying agent may be add to improve the wine's appearance. Clarifying a wine this way is called *fining* and the additives used to do it are called *fining agents*. Alternatively, filteration is possible to clarify the wine. Generally the preparation of wine is illustrated in flow chart (Figure 2).

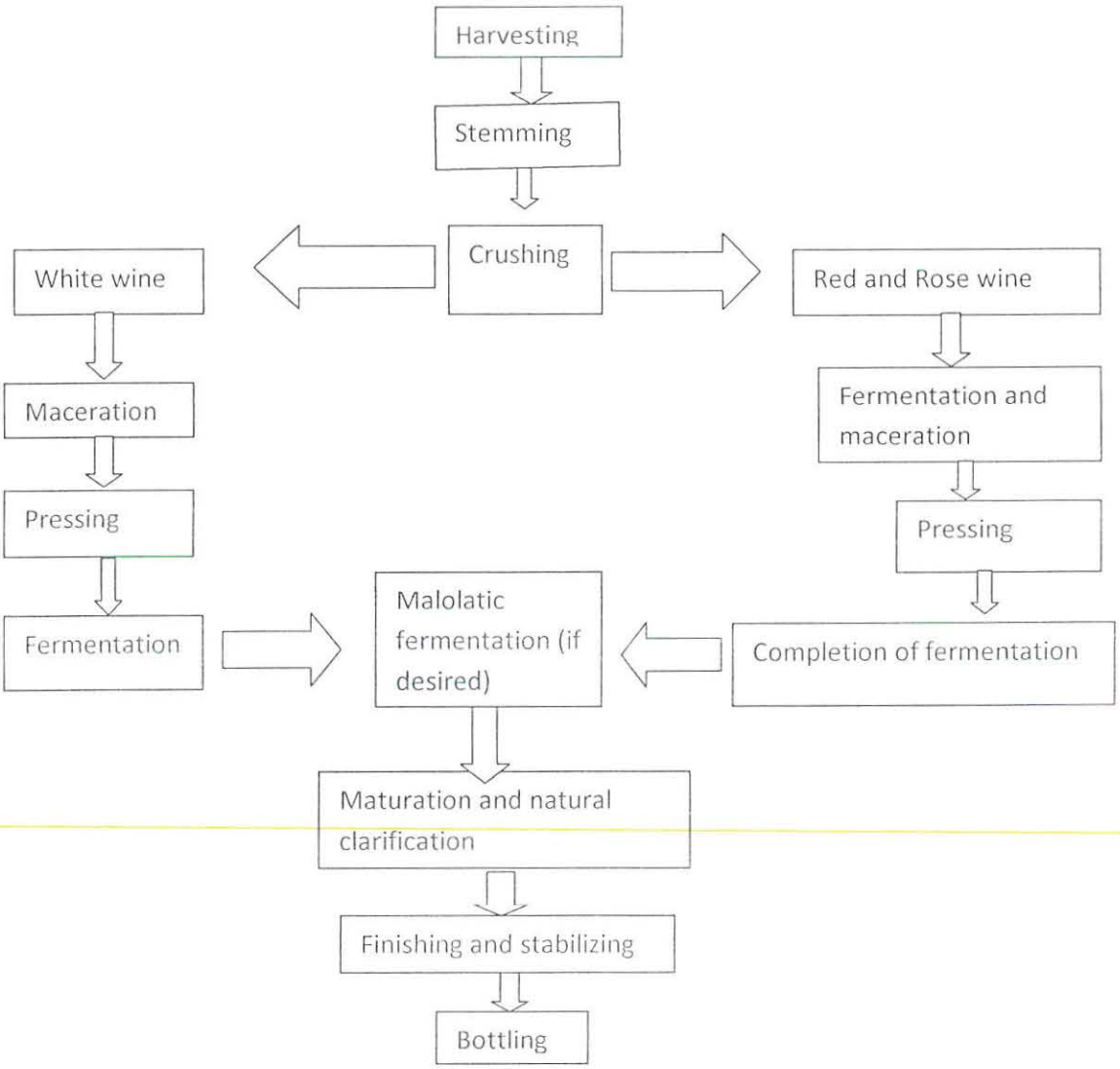



Figure 2: Flow chart of wine preparation.

Source: (Ronalds. Jackson 2008).

## 2.5. Preparation of cactus pear fruit wine

Put prickly pear cactus fruit in large crock or pail. Pour one gallon boiling water over fruit. Wait two minutes (to loosen skin) and drain off water. Allow fruit to cool and carefully peel skin off, being especially watchful not to touch spines. Cut fruit into pieces not larger than one inch, put in pot, add 1/2 gallon water, bring to boil. Reduce heat to maintain gentle boil for 15 minutes. Cover pot and allow to cool to luke warm. Pour fruit and juice into large nylon grain-bag (fine mesh) or sieve and squeeze juice into primary fermentation vessel. Discard pulp. To juice, add sugar, acid blend, yeast and nutrient and stir to dissolve sugar. Cover well and set in warm place for seven days, stirring daily. Siphon off lees into secondary fermentation vessel, top up with water, fit airlock, and let stand three weeks. Rack and top up, then rack again in two months. Allow to clear, rack again if necessary, and bottle. May taste after one year, but improves with age (*Jack, 2006).*

## 2.6. Fermentation

Fermentation becomes active anywhere from 1-3 days after introducing the yeast to the must. An important factor in determining how long it will take is the temperature of the must. Yeast's rate of metabolism is directly affected by temperature: cold musts start fermenting more slowly, while warm musts get off to a quicker start. Once the fermentation has begun, the yeast will proceed to consume the sugars in the must/juice, producing $CO_2$ (bubbles) and alcohol.

### 2.6.1. Fermentation temperature

Every winemaker has a theory on what temperature to ferment at. We have seen great wine fermented from a variety of different temperature schedules. You should pay attention to the temperature. It's definitely a good habit to note the temperature of the must each time you punch down the cap (a good way to do this is to use a floating thermometer (MT400)) for future reference. The actual act of fermentation produces heat and can cause the must to be 10°-15° F higher than the ambient temperature.

**A Typical temperature schedule**

If you have control over fermentation temperatures, a popular red wine schedule is to start slowly at cooler temperatures, such as the low 60s. Next, gradually allow the must to warm up to the desired temperature as the fermentation gets underway. Starting cold allows water soluble compounds in the must such as anthocyanins (color) and agreeable tannins extra time to become fully extracted improving the final wine.

During fermentation the temperature is allowed to rise, often reaching 80°-90° F for a brief period of time. Yeast creates different compounds at different temperatures. How high you allow the fermentation temperature to rise or "spike" is often debated. Some winemakers allow it to go into the low 90° F. However, alcohol toxicity increases as the temperatures rise, so spending too much time in the upper temperature ranges can be damaging to the yeast and they may have difficulty finishing the fermentation. The recommended limits spike to the mid to upper 70° F for safety's sake. Most winemakers agree that a temperature range of 70°-85° F is acceptable.

### 2.6.2. Malolactic fermentation

Malolactic fermentation occurs when lactic acid bacteria metabolize malic acid and produce lactic acid and carbon dioxide. This is carried out either as an intentional procedure in which specially cultivated strains of such bacteria are introduced into the maturing wine, or it can happen by chance if uncultivated lactic acid bacteria are present.

Malolactic fermentation can improve the taste of wine that has high levels of malic acid, because malic acid in higher concentration generally causes an often unpleasant harsh and bitter taste sensation, whereas lactic acid is perceived as more gentle and less sour.

## 2.7. Classification of wine

There is no generally accepted system of classifying wines. For taxation purposes, wines often are divided in to three general categories; still, sparking and fortified. This division recognizes

significant differences, not only in production, but also use. In addition, classification by color provides the purchaser with a rough indication of the wines flavor intensity. Stylice and geographical origin often go hand-in-hand, at least for many European appellations, supplying additional information about the wines likely characteristics.

Initially wines are grouped based on alcoholic concentration. This is indicated by the term "table", alcohol contains between 9 and 14% by volume and "fortified", alcohol contains between 17 and 22% by volume. Table wines are subdivided in to "still" and "sparking" depending on the wines carbon dioxide content (Ronalds. Jackson 2008).

### 2.7.1. Still table wine

Most wines are fall in this category. In the oldest division based on color, wines divided in to Red, white and rose subgroups. In fact, it also reflects distinct difference in flavor, use and production methods.

### 2.7.2. Red wines

Red wines tend to have strong, rich flavors. Depending on the type of grape and the amount of time it's aged, red wine can range in color from dark purplish red to full red to russet brown. To make red wine, "black" grapes (actually purple) are crushed and the juices left in contact with the skin for anywhere from a few days to a few weeks. This skin contact is crucial: the skin gives red wine its color and its structure.

### 2.7.3. White wines

White wines are generally more delicate in flavor than red wines and are typically served chilled. Though called white wines, these wines actually range in color from pale yellow to deep gold or even pale green, depending on the grape and the age of the wine. Most white wines are made from "white" grapes (actually pale yellow or green in color).

### 2.7.4. Rosé wines

Also called blush wines, rosés are made with red wine grapes, but with just a few hours of skin contact. The brief skin contact gives the wine just a pink "blush" and a delicate structure. Rosés are often consumed as "picnic wines" and can be tasty and refreshing when chilled to about 60°F (15°C) and drunk on a hot day.

### 2.7.5. Sparkling wine

Sparkling wine, like champagne, is a type of wine that contains carbon dioxide. To produce this sparkling wine, grapes must be fermented twice. Beverages are most often presented at a formal dinner party or a wedding. Sparkling wine can be red wine, white wine or rose wine.

### 2.7.6. Fortified wine (Dessert Wines)

Because yeast typically can't survive in an environment containing more than 14% alcohol, most naturally produced wines have an alcohol content of less than 14% by volume. Certain winemakers (such as those in Porto, Portugal, where Port is made) found a way around this limitation: they added extra sugar or alcohol to the wine as it fermented, resulting in wines with higher alcohol content. Most, though not all, of these wines are also sweeter than regular wines and are consumed after meals (Ronalds, Jackson 2008).

# 3. Materials and methods

## 3.1. Sources of fruits

Fresh and healthy cactus fruits were collected randomly from open markets of Tigray region, Mekele town and were transported immediately to Addis Ababa. These fruits were refrigerated until required for the analysis.

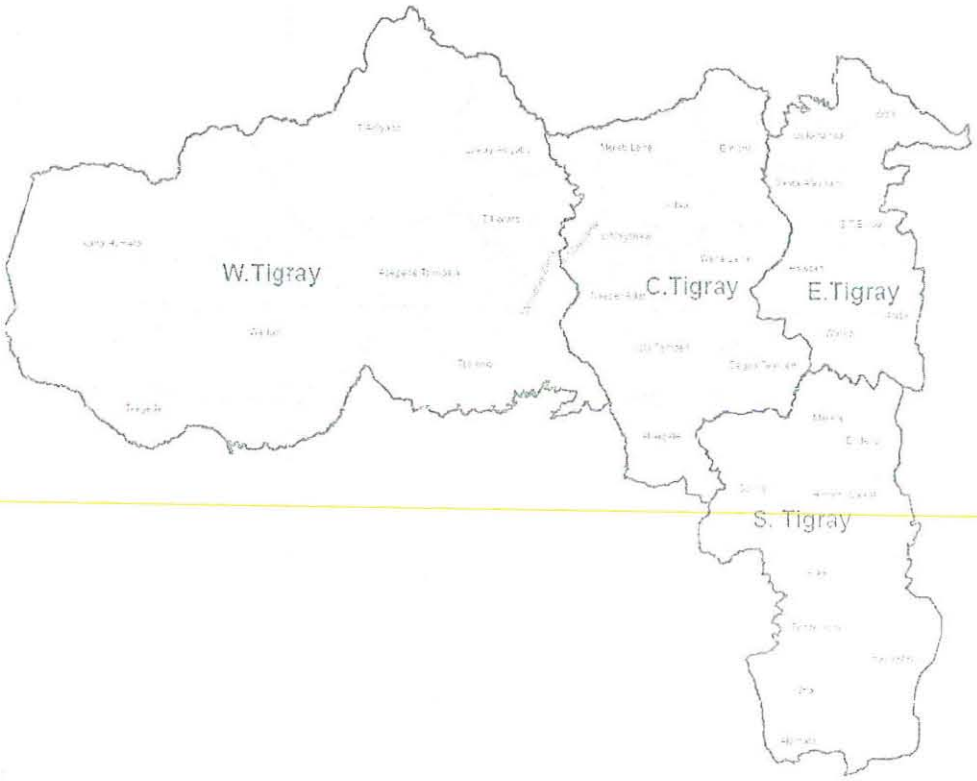



**Figure 3; Map of Tigray Region**

Source: http://www.ocha-eth.org/Maps/downloadables/OROMIYA.pdf

**Location;**

The State of Tigray is located at the northern tip of Ethiopia. The region shares common borders with Eritrea in the north, the State of Afar in the east, the State of Amhara in the south, and the Republic of the Sudan in the west.

## 3.2. Cactus pear juice extraction

About 9 kg of fruits were treated with hot water for five minutes and drained out the water so as to make the skin very thin and make peeling so easy. When the cactus fruits were cooled, peeled the skin off of the fruit and placed the fruit on cutting board. They were sliced into many pieces and blended it with blender machine. Then the juice was squeezed out and the seed were separated by sieving and transferred in to sterile bottles.

## 3.3. Indigenous yeast identification and isolation

The indigenous yeast flora was isolated from two-day fermented cactus pear fruit juice. The juice was aseptically inoculated with streak plate technique on rose Bengal agar incorporated with chloramphenicol. The plates were incubated at 21 $^{0}$C for 48 hours. The yeast present in the plate was identified by looking at the colonies and using a microscope. Furthermore, the identified yeast strain was incubated in pure culture and stored in slant tubes in a refrigerator.

## 3.4. Propagation of yeast

Yeast propagation was performed by inoculating the isolated indigenous yeasts and the commercial yeast in to test tubes containing 15ml pasteurized cactus juice and incubated at 22$^{0}$C for two days before fermentation.

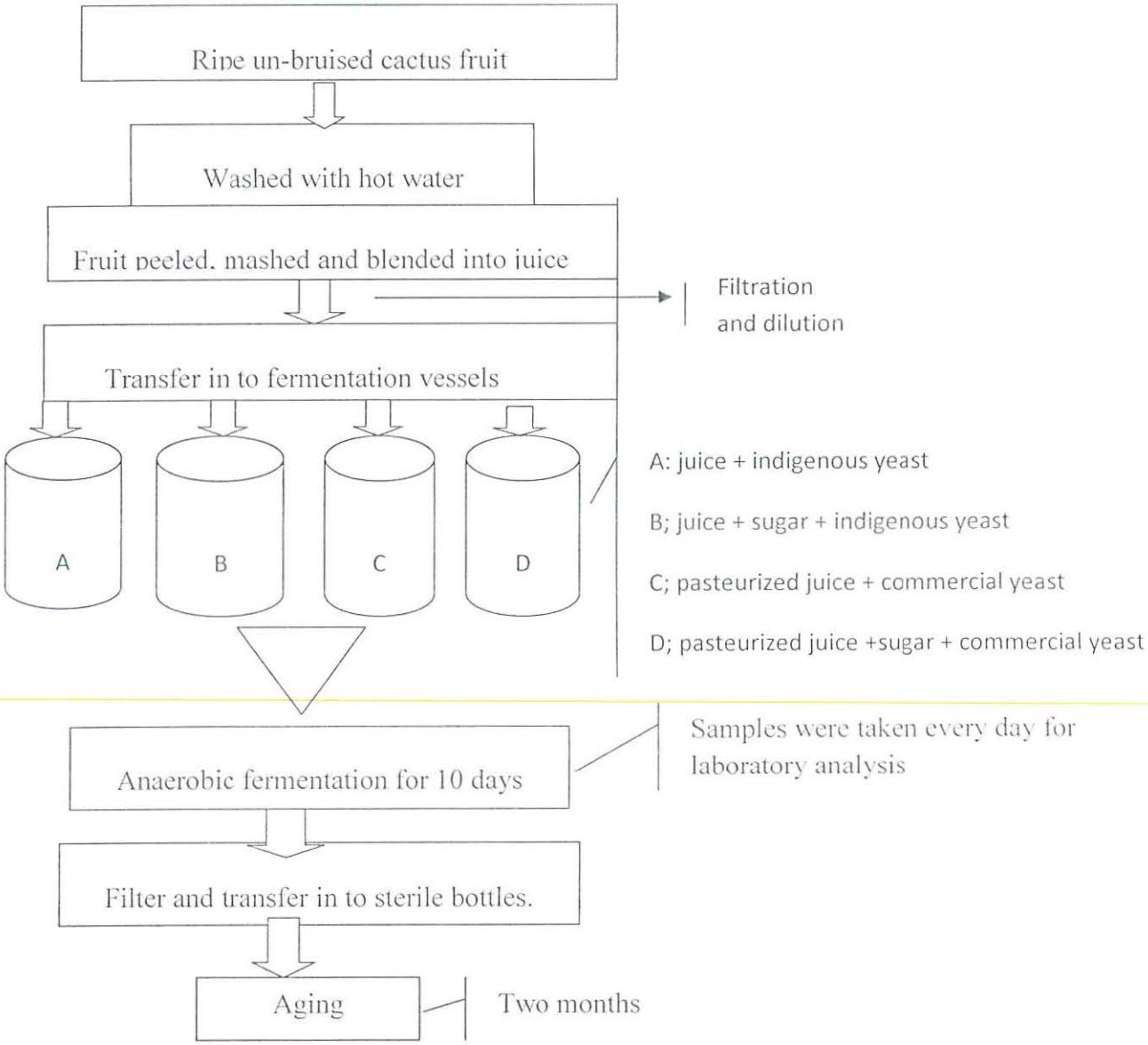
## 3.5. Study design

This experimental study was designed in four different batches to prepare wine from cactus fruit juice and each batch was carried out in duplicates. The batches which were used for each vessel are shown in the Table. 3. Prior to the experiment, dilution of the juice was done in 1:1 ratio to give the desired result.

Table 3; Experimental compositions of the vessels.

| Vessels | Compositions |
|---|---|
| Vessel A | 500 ml of Diluted juice + 15ml propagated indigenous yeast |
| Vessel B | 500 ml of Diluted juice + 15ml propagated indigenous yeast + 50 gm sugar |
| Vessel C | 500 ml of Diluted pasteurized juice + 15ml propagated commercial yeast |
| Vessel D | 500 ml of Diluted pasteurized juice + 15ml propagated commercial yeast + 50gm sugar |

## 3.6. Flow chart of the cactus wine production process



### 3.6.1. Vessels A

In vessels A 1 liter of diluted juice was poured into two 500 ml sterile bottles and 15ml of propagated indigenous yeast was added. The bottles were closed and left to incubate at a temperature of 22°C for about 10 days for fermentation to take place. Samples were taken for yeast count at two-day intervals, and for pH and alcoholic content measurement samples were taken daily.

Finally, the cactus fruit wine obtained at the end of the fermentation was filtered and run off into clean sterile bottles and preserved with 0.02 g/100 ml of sodium benzoate.

### 3.6.2. Vessels B

In vessels B there was an amelioration of the juice with 10 g/100 ml sugar. 1liter of diluted juice was poured into two 500 ml sterile bottles and shaken thoroughly. After thorough stirring of the juice, 15 ml of propagated yeast were introduced into the bottles. The bottles were capped and incubated at 22°C for 10 days for fermentation. Samples were taken for yeast count, PH and alcohol measurement daily.

Finally, the cactus fruit wine obtained at the end of the fermentation was filtered and run off into clean and sterile bottles and preserved with 0.02 g/100 ml of sodium benzoate.

### 3.6.3. Vessels C

In vessels C, the juice was pasteurized in a water bath at 64 °C for 30 minutes, and then rapidly cooled. 1 liter of pasteurized juice was poured into two 500 ml sterile bottles and 15ml of propagated commercial yeast (*Saccharomyces cerevisiae*) was added to each bottle. The bottles were capped and incubated at 22°c for 10 days for fermentation. Samples were taken for yeast count, pH and alcohol measurements daily.

Finally, the cactus fruit wine obtained at the end of the fermentation was filtered and run off into clean and sterile bottles and preserved with 0.02 g/100 ml of sodium benzoate.

### 3.6.4. Vessels D

In vessel D, the diluted juice was pasteurized in a water bath at 64 °C for 30 minutes, and then rapidly cooled. 1 liter of pasturized juice was poured into two 500 ml sterile bottles and 15ml of propagated commercial yeast (*Saccharomyces cerevisiae*) and 50 gm of sugar was added to each bottle. The bottles were capped and incubated at 22°c for 10days for fermentation. Samples were taken for yeast count, PH and Alcohol measurements daily.

Finally, the cactus fruit wine obtain at the end of the fermentation was run off into clean and sterile bottles and preserved with 0.02 g/100 ml of sodium benzoate.

## 3.7. Laboratory analysis

### 3.7.1. Microbial safety of cactus fruit juice

The cactus fruit juice extracted by the above procedure was analyzed for microbial safety. Total aerobic bacterial count, total coliform count, fecal coliform count, E-coli count, isolation of salmonella and sheigella, and yeast and mould counts were determined by using standard method at EHNRI Food Microbiology Laboratory.

#### 3.7.1.1. Total aerobic bacterial count

The aerobic colony count estimates the number of viable aerobic bacteria per gm or ml of a product. 25ml of the juice was diluted with 225ml of peptone water and further dilution was made by transferring 1ml of the diluted sample to a tube containing 9ml of normal saline and further serial dilution made until $1;10^5$. Transfer 1ml from each diluted sample to triplicate sterile Petri dish, add 20-25ml molten Plate count agar and mix well and allow 15mins to solidify and incubate under 30°C temperature for 72 hr. It is assumed that each viable aerobic bacterium will multiply under these conditions and give rise to colonies (Habetemariam B, 2010).

#### 3.7.1.2. Total coliform count

The number of coliform bacteria was determined by diluting 10 ml of the sample in to 90 ml peptone water and further dilution was made 1:10, 1:100, 1:1000. Then 1ml from each dilution was transferred to sterile Petri dish and 5ml TSA was added and mixed. The petri dish was kept at room temperature for 1 hour to activate the stressed coliforms prior to addition of VRBGA, the selective media for coliforms. After setting, the dishes were incubated at 37 °C for 24 hours and then read. Typical colonies were count and selected colonies were confirmed by testing for production of gas in a selective liquid medium containing lactose known as BGB (Habetemariam B, 2010).

#### 3.7.1.3. Fecal coliforms

#### 3.7.1.4. Total coliform count

The number of coliform bacteria was determined by diluting 10 ml of the sample in to 90 ml peptone water and further dilution was made 1:10, 1:100, 1:1000. Then 1ml from each dilution was transferred to sterile Petri dish and 5ml TSA was added and mixed. The petri dish was kept at room temperature for 1 hour to activate the stressed coliforms prior to addition of VRBGA, the selective media for coliforms. After setting, the dishes were incubated at 37 °C for 24 hours and then read. Typical colonies were count and selected colonies were confirmed by testing for production of gas in a selective liquid medium containing lactose known as E.C broth (Habetemariam B, 2010).

#### 3.7.1.5. E-coli count

This test was done because of the ability of *E.coli* type 1 to decompose the amino acid tryptophan to indole which accumulates in the medium at high (44°C) temperature. Indole production was tested by a colorimetric reaction with p-dimetyl-amino-benzaldehyde. The E-coli colonies was tested by transferring the fecal coliform colonies in to nutrient broth and incubated at 44 °C for 24-48 hours then three drops of Kovacs reagent/ p-dimetyl-amino-benzaldehyde/ was added. The presence of purple red ring on the surface of the broth indicates positive to indol test (Habetemariam B, 2010).

### 3.7.1.6. Isolation of Salmonella

The procedure consists of six distinct stages. The initial handling of the food and the non-selective enrichment stage (pre-enrichment) vary according to the type of food examined.

**Non-Selective Enrichment (Pre-enrichment).** The test sample was initially inoculated into a non-inhibitory liquid medium to favor the repair and growth of stressed or sub lethally-injured Salmonellae arising from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations.

**Selective Enrichment**; Replicate portions of each pre-enrichment culture were inoculated into two enrichment media to favor the proliferation of Salmonellae through a selective repression or inhibition of the growth of competing microorganism.

**Selective Plating**; Enrichment cultures were streak onto selective differential agars ( XLD and SSA) for the isolation of Salmonellae (Habetemariam B, 2010).

### 3.7.1.7. Yeast and Mould count

The number of yeasts and moulds in a sample was determined by inoculating a known quantity of the sample on to a selective agar medium RBA incorporated with chloramphenicol. The sample was diluted 1:10, 1:100, 1:1000 ....Then 1ml from each dilution was transferred to sterile Petri dishes then plates were incubated at 21 $^{0}$C for 48 hours. The yeast present was identified by looking at the colonies and by using a microscope (Habetemariam B, 2010).

## 3.7.2. Physicochemical analysis of cactus fruit juice

The moisture, protein, fat, ash, carbohydrates, fiber contents, titratable acidity (g citric acid/100 g), pH, specific gravity and alcohol content of the fresh cactus pear fruit juice was analysed using standard methods of AOAC.

### 3.7.2.1. Total solids analysis

Empty drying dishes (made of porcelain) were dried using a drying oven (Germany, Memmert) for 1 hour at 100$^{0}$C. The dishes were cooled for 30 minutes in a desiccator (with granular silica

gel) and weighed using a digital analytical balance to the nearest milligram. About 25ml of well mixed cactus juice samples (in triplicate) were transferred into dried and weighed drying dishes. The dishes and their contents were placed in drying oven and dried for 5 hours at $100^0$C. The dishes and their contents were cooled in a desiccator to room temperature and weighed. The procedure was repeated until a constant weight was recorded (AOAC, 2005).

Moisture content was expressed as:

$$\%\text{Moisture content} = \frac{\text{M initial} - \text{M final}}{\text{M initial}} \times 100\%$$

Then the total solid is calculated;

$$\text{Total solid}\% = 100\% - \%\text{moisture content.}$$

### 3.7.2.2. Determination of Ash

Porcelain dishes were placed in a muffle furnace (Carbolite, Aston Lane, Hope, Sheffield, England, UK) for 30 min at $550^0$C. The dishes were cooled in a desiccator (with granular silica gel) for about 30 minutes and weighed to the nearest milligram. About 2.5000 g of fresh sample (in triplicate) was placed in the dish. Dishes were placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes with samples were placed inside the muffle furnace at $550^0$C for 5 hours and cooled in a desiccator for 1 hour. The ash was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (AOAC, 2005 and Osborne, D.R. and Voogt, V, 1978).

$$\%\text{ Ash (wet basis)} = \frac{\text{M ash}}{\text{M wet}} \times 100\%$$

*NB: Mash* refers to the mass of the ash, and *Mwet* refer to the original mass of fresh samples.

### 3.7.2.3. Crude protein determination

*Digestion:* About 0.5000 g of dried sample (in triplicate) were taken in a Tecator tube and 6ml of acid mixture (5 parts of concentrated orthophosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed, and 3.5 ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken and placed back to the rack. Three gram of catalyst mixture (ground 0.5000 g of selenium metal with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 minutes before digestion.

When the temperature of the digester attains $370^0$C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1h. The tubes in the rack were cooled in a fume hood; 15ml of deionized water was added, and shaken to avoid precipitation of sulfate in the solution.

*Distillation and titration:* The digested and diluted sample solution was distilled using boric acid and the distillate was titrated using 0.1N sulfuric acid to reddish color using Kjeldehal apparatus (Kjeltec 2300Analyzer unit, Foss Tecator AB, Hoganas, Sweden) (*Tecator manual, 1979*).

mg nitrogen in the sample = V x N x 14

g nitrogen / 100 g sample = mg of nitrogen x 100/ mg sample

Total nitrogen (%) = [(V-Vb) x N x 1.4)]/ W

Crude protein (%) = total nitrogen (%) x 6.25a

Where: V = volume of sulfuric acid consumed to neutralized the sample;

Vb= the volume of acid consumed to neutralize the blank;

N = normality of the acid; 14=Eq. wt of nitrogen;

6.25=conversion factor from total nitrogen to crude protein.

### 3.7.2.4. Determination of crude Fiber content

*Digestion:* About 1.7000 g of fresh sample (in triplicate) was placed into a 600 ml beaker; 200 ml of 1.25% $H_2SO_4$ was added, and boiled gently for 30 minutes while watch glass was placed

over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After exactly 30 minutes heating, 20 ml of 28% KOH was added and boiled gently for further 30 minutes, with occasional stirring.

*Filtration:* The bottom of a sintered glass crucible was covered with 10mm sand layer and wetted with distilled water. The solution was poured into sintered glass crucible and filtered with the aid of vacuum pump (High Performance Vacuum Pump, Robin Air way, SPX Corporation, Montplier, USA). The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to the crucible and filtered.

*Washing:* The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% $H_2SO_4$ and filtered, and then washed with hot distilled water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% $H_2SO_4$ and filtered. Finally the residue was washed with water- free acetone.

**Drying and combustion**: The crucible with its content was dried in a drying oven for 2 hours at 130°C and cooled for 30 min in a desiccators (with granular silica gel), and then weighed (recorded as W1). The crucible was transferred to muffle furnace and heated for 30 min at 550°C. The crucible was cooled in a desiccators and weighed (recorded as W2) (AOAC, 2005).

$$\% \text{ Fiber} = \frac{W1 - W2}{W3} \times 100$$

Where; W1 = weight of crucible with sample after drying.

W2 = weight of crucible with sample after ashing.

W3 = fresh sample weigh

### 3.7.2.5. Determination of crude fat content

Extraction flasks were dried in a drying oven (Memmert, Germany) at 92°C for 30 minutes, cooled in a desiccator (with granular silica gel) for 30 minutes and then weighed. About 2.500 g of fresh samples (in triplicate) were added into extraction thimbles, and then covered with about

2 cm layer of fat free cotton. The thimbles with sample were placed into a Soxhlet Extraction Chamber (Soxtec, Manual extraction unit, Foss Tecator, Hoganas, Sweden). Cooling water was switched on, and seventy ml of petroleum ether was added to the extraction flask through the condenser. The extraction was conducted for three and half hours at $70^0$C. The extraction flask with fat was removed from the Extraction Chamber and placed in the drying oven at $92^0$C for about 30 minutes, cooled to room temperature in a desiccators for about 30 minutes and reweighed (AOAC, 2005; Tecator Manual, 1979).

$$\% \text{ Fat} = \frac{W_1 - W_0}{W_D} \times 100$$

*Where:* $W_1$ = *weight of extraction flask after extraction (wt. of flask and fat).*

$W_0$ = *weight of extraction flask before extraction (wt. of flask).*

$W_D$ = *weight of fresh sample*

### 3.7.2.6. Determination of total Carbohydrate content

Total carbohydrate content was estimated by the difference, as percentage (%) on wet basis (AOAC, 2005).

Carbohydrate (%) = 100-(fat% +protein% + ash% + moisture %)

### 3.7.2.7. Titratable acidity analysis

Titratable acid as Citric acid is analyzed; accurately weigh to ±0.1 mg a 10-g sample of cactus juice. Transfer to a 500-ml Erlenmeyer flask and dilute to 250 ml with deionized water or recently boiled water. Titrate to an end point with a standard solution of sodium hydroxide (0.1N) using either a pH meter for end point detection or by adding approximately 1 ml of phenolphthalein indicator and titrating to a faint pink end point. Record the volume to ±0.05-ml. Calculate the percentage of acid as citric acid by;

Acid as anhydrous citric acid:

$$\% \, Acid = \frac{V \times 0.640}{W}$$

Where

$V$ is the volume in milliliters of 0.1N alkali used. (For other concentrations of alkali, the factor becomes N x 6.4.)

$W$ is the weight in grams of sample (AOAC, 2005).

### 3.7.2.8. pH measurement

An electronic pH meter (**JENWAY MODEL 370 pH/mV METER, England**) was used to measure PH. After calibration using standard solutions at pH 4 and 7and 10 the cactus pear juice was measured at internal temperature of 22°C (AOAC, 2005)

### 3.7.2.9. Measurement of specific gravity

Specific gravity of the sample was measured by a "specific-gravity bottle," i.e., a flask made to hold a known volume of liquid at a specified temperature (usually 20°C). The bottle is weighed, filled with the liquid whose specific gravity is to be found, and weighed again. The difference in weights is divided by the weight of an equal volume of water to give the specific gravity of the liquid (AOAC, 2005).

$$d_t^{t'} = \frac{W_1 - W}{W_2 - W}$$

Where: W; Weight of a pycnometer ("specific-gravity bottle,") previously cleaned and dried.

W1: Weight of a pycnometer ("specific-gravity bottle,") filled with sample.

W2: Weight of a pycnometer ("specific-gravity bottle,") filled with distilled water.

### 3.7.3. Determination of Alcohol concentration during fermentation period using Gas Chromatography

#### 3.7.3.1. Instrument conditioning

1ml of fermented sample was taken and diluted 10:1 with isopropanol (1 g/L, internal standard). Gas chromatography (GC) was conducted on DANI GC 1000 (DANI Instruments Ltd., Italy) with flame ionization detector (FID) under optimized operational condition. Alltech Econo-Cap ECTM-5 capillary (30 m length, 0.32 mm diameter) coated with 95 % methylpolysiloxane (stationary phase) was used as column. The flow rates of nitrogen (carrier gas) and hydrogen (FID) were adjusted to 5 and 0.65 bar, respectively. Injector and detector temperatures were kept at 210°C and 300°C, respectively. The oven temperature was at 80°Cwith a hold time of 4.5 minutes.

0.1% internal standard spiking solution and 1%, 5%, 10% and 15% ethanol analytical standards was prepared, using reagent grade propanol and 200 proof ethanol,.

The internal standard spiking solution was added in the same proportion to every standard or sample analyzed.

This procedure specifies nine parts of a 1 g/L internal standard spiking solution be added to one part sample or standard. Therefore, the internal standard concentration is 0.9 g/L universally throughout this procedure.

The calibration curves were obtained using analytical solutions of pure ethanol in concentration of 1%. 5%, 10%, and 15% in which the expected ethanol concentration of fermented juice lies. Acceptability of linearity data was often judged by examining the correlation coefficient and the $y$ intercept of the linear regression line for the response versus concentration. A correlation coefficient of >0.995 is generally considered acceptable. The $y$ intercept should be less than a few percent of the response obtained from the target level.

Accordingly, the calibration curves were obtained from a running of four point calibration solutions having a concentration range of 1% to 15%. The lowest concentration level in the calibration curve was 1% for the instrument. Linearity was evaluated by the calculation of a four-point linear plots of the peak height (as observed Figure 5) representative chromatography from the four concentration range) against concentration based on linear regression and squared correlation coefficient, $r^2$, which should be > 0.9900. The prepared standard solutions had good linearity.

### 3.7.4. Sensory analysis

The sensory analysis of the wine was done to test the acceptability of the wines. A group of 15 semi trained tasters (males and females all of whom were over 18 years of age) and regular consumers of some kind of wine were selected. The testers were well oriented and instructions were given prior to tasting. The sensory evaluation room was clean and had enough light. Each of the testers sampled 20 ml of each batch of cactus juice wine and Awash red wine to compare the acceptance of the cactus juice wine and served separately in transparent glass cups at room temperature. For sensory evaluations of the acceptability of the wines in relation to the attributes of appearance, aroma, and flavor and to the overall acceptance of each wine. The panelists completed an evaluation form comprising a 9-point hedonic scale ranging from 1 ("I extremely disliked it") to 9 ("I extremely liked it"). The sensory analysis score card is attached in Appendix 2 (Dias et al., 2007).

## 3.8. Statistical analysis

The statistical analysis of ethanol concentration and sensory in each batch were carried out using one way (ANOVA) to compare the mean of each batch. And LSD test to check the significance of the difference.

# 4. Results

## 4.1. Microbial analysis of the cactus pear juice

The results of the microbial analysis of the cactus pear juice were $6.8x10^6$, $3.5x10^3$, $1.5x10^1$ and $2.5x10^2$ CFU/ml for total aerobic bacterial, total coliform, yeast count and mould count respectively. Fecal coliform, E-coli and salmonella were not detected in the juice (Table 4).

Table 4: Results of microbial analysis of cactus juice.

| Tests | Result s |
|---|---|
| Total APC | $6.8x10^6$ CFU/ml |
| Total coliform count | $3.5x10^3$ CFU/ml |
| Fecal coliform count | <10 CFU/ml |
| E-coli count | <10 CFU/ml |
| Yeast count | $1.5x10^1$ CFU/ml |
| Mould count | $2.5x10^2$ CFU/ml |
| Salmonella | Not Isolated/25ml |
| Shigella | Not Isolated/25ml |

## 4.2. Physicochemical analysis of cactus juice

The physicochemical composition of cactus fruit is the determinant factor for the growth of yeast for the production of alcohol, flavor, and aroma. And also the overall quality of the wine produced is directly the result of all the components.

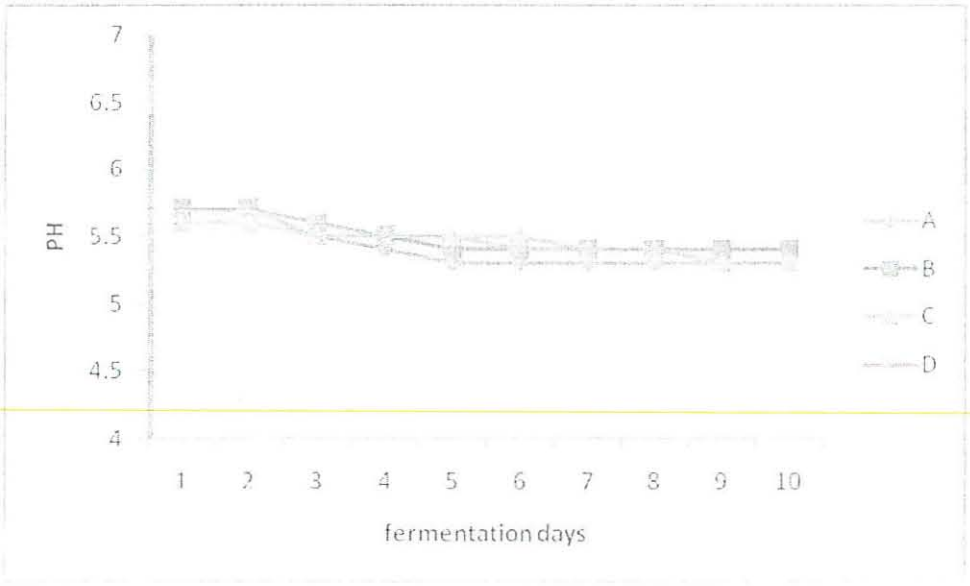
In this study, the physicochemical analysis of the juice such as; Total solids, protein, fat, ash, carbohydrates, fiber contents, titratable acidity (g citric acid/100 g), pH, , specific gravity of the juice was done and the results were 13%, 0.7%, 0.3%, 0.8%, 8.45%, 2.8%, 0.03%, 5.011, and 1.0325 respectively (Table 5).

Table 5; Physicochemical characteristics of the cactus fruit juice.

| Tests | Result |
|---|---|
| Total solids | 13.05% |
| pH | 5.011 |
| Specific gravity | 1.0325 |
| Titratable acidity (g citric acid/100g) | 0.03% |
| Ash | 0.8% |
| Protein | 0.7% |
| Fat | 0.3% |
| Carbohydrate | 8.45% |
| Fiber | 2.8% |

## 4.3. pH of each batches during fermentation period.

The pH in each fermentation batch was measured by using a pH meter each day during fermentation period. The pH was in the range of 5.7 and 5.3 from first to tenth day (Figure 4).

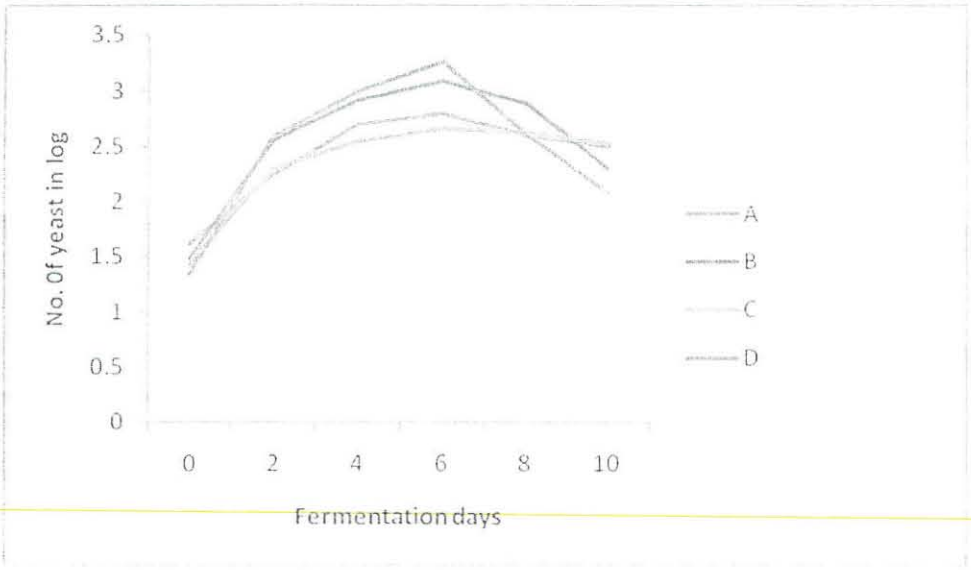



(A, B, C and D; are the compositions of fermentation batches mentioned in Table 3)

**Figure 4:** pH of each fermentation vessel during fermentation period

## 4.4. Yeast count in each batch during fermentation period

The yeast counts increased from the second day to the sixth day where it was highest and later decreased until the tenth day in all fermentation vessels (Figure 5).




(A, B, C and D: are the compositions of fermentation bathes mentioned in Table 3)

**Figure 5:** Number of yeast during fermentation period

## 4.5. Alcohol concentrations during fermentation period

The alcohol concentration in each batch (A, B, C and D) was increased from first day up to day ten fermentation period. After 10 days fermentation period the alcohol concentration were 4.9, 7.8, 5.3 and 12.2 %v/v, respectively. The alcohol concentration was increased from 0% to the concentration of 4.9, 7.8, 5.3 and 12.2 %v/v in vessel A, B, C and D, respectively (Table 6).

**Table 6:** Ethanol concentration in each fermentation vessel during fermentation period.

| Day | Average % Ethanol concentration in each vessels | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1.4 | 2.6 | 2.0 | 3.0 |
| 3 | 1.5 | 2.8 | 2.0 | 6.1 |
| 4 | 2.2 | 3.9 | 2.1 | 6.5 |
| 5 | 2.3 | 4.2 | 2.3 | 7.2 |
| 6 | 2.6 | 4.7 | 2.5 | 8.0 |
| 7 | 2.8 | 5.6 | 2.7 | 9.4 |
| 8 | 3.8 | 6.7 | 3.2 | 9.6 |
| 9 | 4.3 | 7.6 | 5.1 | 10.4 |
| 10 | 4.9 | 7.8 | 5.3 | 12.2 |

(A, B, C and D; are the compositions of fermentation vessels mentioned in Table 3)

## 4.6. Physicochemical analysis of cactus pear fruit wine

The physicochemical properties of the final cactus wine obtained after 10 days of fermentation and 2 month aging were measured. The measurement of pH, titrable acidity and specific gravity in each batch of cactus wine were almost the same. The alcohol concentrations were 4.9 %, 7.8 %, 5.3 % and 12.2 % for batch A, B, C and D respectively. The results are shown in Table 9.

**Table 7: Physicochemical analysis of cactus wine.**

| Tests | Result | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Ethanol % | 4.9 | 7 .8 | 5.3 | 12.2 |
| pH | 5.3 | 5.4 | 5.3 | 5.3 |
| Titrable acidity % | 0.4 | 0.5 | 0.5 | 0.4 |
| Specific gravity | 1.0227 | 1.0225 | 1.0226 | 1.0223 |

(A, B, C and D; are the compositions of fermentation vessels mentioned in Table 3)

## 4.7. Sensory analysis

The results of the sensory evaluation of the acceptability of each batch and awash red wine as reference in relation to the attributes of color, appearance, aroma, and taste and to the overall quality , the mean score are shown in Table 8.

Table 8; Mean scores of sensory evaluation and overall acceptability of the cactus wine and Awash red wine.

| Vessels | Color | | Appearance | | Aroma | | Taste | | Acceptability | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean score | SD | Mean score | SD | Mean score | SD | Mean score | SD | Mean score | SD |
| A | 7.13a | .63 | 7.53a | .59 | 5.4a | .63 | 5.66a | .48 | 5.53a | .63 |
| B | 7.26a | .7 | 7.66a | .51 | 5.6a | .5 | 5.8a | .77 | 5.8a | .77 |
| C | 7.46a | .51 | 7.8a | .51 | 5.6a | .63 | 5.13a | .83 | 5.66a | .48 |
| D | 7.46a | .74 | 7.8a | .67 | 6.26b | .70 | 6.46b | .51 | 6.33b | .61 |
| E | 8.26b | .59 | 8.5b | .51 | 8.2c | .77 | 8.2c | .67 | 7.86c | .63 |

(A, B, C and D; are the compositions of fermentation batches mentioned in Table 3 and E was awash red wine.)

Values within the same column with different superscript letters are significantly different from each other (at $p < 0.05$).

# 5. Discussion

## 5.1. Microbial analysis of the cactus pear juice

The absence of indicator organisms such as fecal coliform and E-coli and pathogenic organisms like salmonella in the juice indicates the safety of the juice which did not show fecal contamination and there was no threat to the health of consumers. The microorganisms such as aerobic bacteria, yeast and mould were present in the juice. The present results are similar to other studies on the microbiological quality of fresh minimally-processed fruits, such as apple, peach, orange, mango and pineapple which harbored small microbial populations consisting of yeasts and moulds, whereas , *E-coli* and *salmonella* were not detected (Abadias, 2008).

In contrast, raw fruit whose internal tissues are normally sterile is considered a potential target for a wide range of microorganisms, including human pathogens (Abadias, 2008). In reality, the incidence of food borne outbreaks caused by contaminated fresh fruit has recently increased. For example, numerous cases of *Salmonella* infection and *Escherichia coli* O157:H7 outbreak by consuming different fresh products are documented (FDA, 2010). Food borne bacterial pathogens commonly detected in juices are coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. (Tambekar Mundhaha, 2006). They are commonly-used bacterial indicators of sanitary quality of foods and water and considered as an indicator of microbial pollution and they are common inhabitants of animal and human guts (Tortora, 1995). *Escherichia coli* are the species associated with faecal contamination and is naturally found in the intestines of humans and warm-blooded animals. In this study those pathogenic organisms were not detected (Table 4).

In this study, the aerobic bacterial count was $6.8 \times 10^6$ CFU/ml (Table 4). This is may be due to the nutritional composition of the juice which is favorable for the growth of microorganisms and the low acidic (pH 5.011) nature of the fruit (Table 5). However, aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality (Aycicek et al. 2004). These organisms reflect the exposure of the sample to any contamination and in general, the existence of favorable conditions for the multiplication of microorganisms. For various reasons, this parameter is useful to indicate if cleaning, disinfection and temperature

control during industrial processing, transportation and storage have been performed sufficiently (Tortora, 1995).

## 5.2. Physicochemical analysis of cactus juice

In this study the total solids of the juice were 13.05% (Table 5) and it was comparable to other studies which were in the range between 12 and 17°Brix (Sáenz-Hernández, 1995), with glucose and fructose being the predominant carbohydrates in a ratio of about 1:1, depending on invertase activity (Kuti and Galloway, 1994; Sawaya et al., 1983).

However, in other similar studies the soluble solids content in the pulp reaches values higher than 17%, mainly constituted by 53% glucose and fructose (Russell and Felker, 1987; Sawaya et al. 1983; Sepúlveda and Sáenz, 1990; Kuti and Galloway, 1994). This high total solid content might be due to the difference in cactus species and / or the area in which it was produced.

The carbohydrate content of the juice was 8.45% from a 13.05% of total solids (Table 5). This high content of sugar was also measured in other similar studies that has sugar : acid ratios ranging from 90:1 up to 490:1. The high sugar acid ratio is responsible for the bland taste in contrast to a sensory pleasant ratio of 10 to 18 (Felker et al., 2005; Joubert; 1993, Odoux and Domínguez-López, 1996; Stintzing and Carle, 2006). The high sugar content of the juice however, has significant role for alcohol production.

In this study, the pH of the juice was 5.011(Tabel 5). These results were consistant with studies of Felker et al (2005) which reported pH ranges of 5.6-6.5 in fully ripe fruits. In general, the cactus juice had high pH-value. The high pH of the cactus juice confirmed that cactus pear fruits are grouped under a low-acid food (pH > 4.5). Hence, acidification prior to thermal treatment is a prerequisite to allow pasteurization instead of more rigid sterilization, thereby retaining nutritionally important components such as betalains (Feugang et al., 2006; Piga, 2004; Stintzing et al., 2001). The low acidic nature of the cactus juice fevers the growth of microorganisms which may accelerate the spoilage of the fruit as well as the growth of pathogenic microorganisms.

The chemical and mineral composition described by different author's shows that cactus pears have nutritive value similar to other fruits. In this study the result of components in cactus-pear juice, such as protein, fat, fiber and ash were 0.7%, 0.3%, 2.8% and 0.8% respectively. These results are similar to other studies which reported that all of the components mentioned above were below 1% except the fiber that varies in different fruits (Askar and El Samahy, 1981; Pimienta, 1990; Sawaya et al., 1983; Sepúlveda and Sáenz, 1990; Rodriguez et al., 1996).

## 5.3. pH of each fermentation vessels during fermentation

In this study, the pH was in the range of 5.7 and 5.3 from first to tenth day. The result indicates that the cactus wine was slightly acidic (Figure 4). These results were incontrast to wine produced from soursop which had pH below 4.5 (Okigbo and Obire, 2009). This high pH of the cactus wine is due to the high pH of the juice itself and low acid production during fermentation period. Thus the wine might be highly susceptible for microbial growth and spoilage.

The result shows that the pH of the cactus wine in each vessel was not significantly different.

## 5.4. Yeast count in each vessel during fermentation period

The results of this study shows that the yeast counts increased from the second day to day six where it was highest and later decreased until day ten in all fermentation vessels (Figure 5). This showed that fermentation was vigorous on day six. There was reduction in the number of yeast cells from eight to tenth day and this might be a result of the increase in the alcoholic content which killed the yeasts and low sugar content. Moreover, the increase in the number of yeasts from the second day to the sixth day indicated that the cactus juice supported yeast growth (Figure 5). The highest yeast count 3.26 log colony forming unit per ml was recorded in vessel D (the pasteurized and commercial yeast treatment) on the sixth day followed by 3.02, 2.79, and 2.65 colony forming unit in log 10 (Figure 5).

## 5.5. Alcohol concentration during fermentation period

It is generally known fact that wine is the alcoholic product of the fermentation of any fermentable fruit or vegetable by action of yeasts. Various tropical fruits have been used for wine production (*Omole JO, 2005 Okigbn, 2003, Osho A*, 1995). Similarly, wine can be obtained from cactus fruit because of its higher sugar content. The culture of yeast used in the fermentation utilizes the sugar present in the juice and converts it to ethyl alcohol and carbon dioxide. During the fermentation process, it was observed that the alcoholic content increased as the fermentation time increased (Figure 6). This occurred as a result of the breakdown of the sugars and subsequent conversion to ethyl alcohol. The cactus juice which was not ameliorated with sugar has the lowest alcoholic content 4.9% (v/v). This was a consequence of low nutrient content of the juice. In addition, since the fruit juice was not pasteurized, it is likely that some other microorganisms were acting on the fruit juice thereby disrupting the yeast activity. When sugar was added, as the only supplement in another fermenting juice, it gave a higher alcoholic content, indicating that the levels of nutrients for the yeasts to utilize have been increased.

The pasteurized juice ameliorated with sugar, and inoculated with commercial yeast gave the highest alcoholic content of 12.2 % v/v (Table 6), making the wine a strong wine and increases its shelf life *(Wineworld. 2002)*. Many factors however, contributed to the high alcoholic level of this wine. Firstly, during pasteurization, many microorganisms were killed so there is no nutrient computation between the yeasts and other microorganisms. The second factor is the addition of sugar which supplied nutrients to the yeast starter. The third factor is probably the commercial yeast which was used for fermentation in this batch had good potential than the indigenous yeast isolated from the cactus fruit. The present results are in contrast with other studies of indigenous yeast isolated from soursop fruit which has contributed to the high alcoholic content for fermentation of soursop juice ( Okigbo and Obire, 2009).

The fermentation of the pasteurized and not ameliorated cactus juice with commercial yeast, gave alcohol content of 5.3% (v/v) (Table 6). The alcohol content is not high when compared with that of the wine obtained from pasteurized and ameliorated juice inoculated with culture of commercial yeast flora which was found to be 12% alcohol (Table 6). This observation coincides

with previous studies on soursop fruits for wine production where the pasteurized and ameliorated juice had higher alcohol content than that of the soursop juice which was not pasteurized and with no sugar added (Okigbo and Obire, 2009).

The results of the present study showed that ethanol concentration of vessel D was significantly different from the other vessels (Table 8). Vessels A and C (no added sugar) were found to have relatively low alcohol content (4.9% and 5.3%, respectively) when compared to those of sugar supplemented vessels B and D (7.8% and 12.2% respectively) (Table 9). The significant difference was due to the addition of sugar in vessels B and D that gave additional nutrition for yeast for alcohol production. There was also significance difference between vessel B and vessel D. This might be the difference of the yeast strain the indigenous yeast strain which was isolated from the cactus juice were might be low potentials of for production of alcohol than the commercial yeast.

**Table 9:** Mean comparison of ethanol concentration of each vessel at the end of fermentation

| Vessels | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| A | 2 | 4.8700 | | |
| C | 2 | 5.2650 | | |
| B | 2 | | 7.7750 | |
| D | 2 | | | 12.1850 |
| Sig. | | .647 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

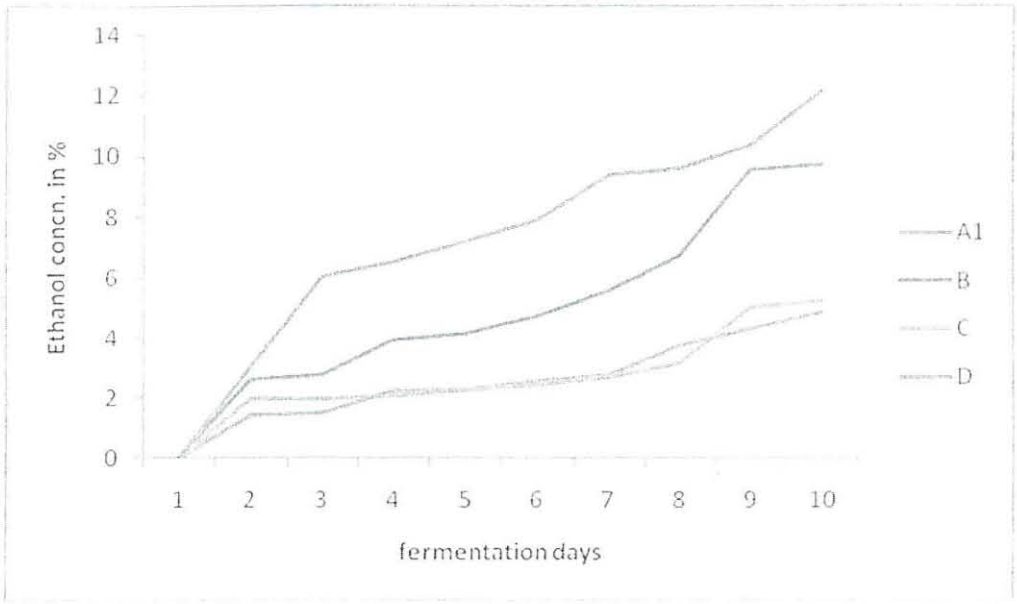



**Figure 6:** Ethanol concentration in each vessel during fermentation period.

(A, B, C and D; are the compositions of fermentation vessels mentioned in Table 3)

Figure 6 shows the production of ethanol in each fermentation vessel during the fermentation period. It was observed that the alcoholic content increased as the fermentation period increased (Figure 6). This occurred as a result of the breakdown of the sugar and subsequent conversion to ethyl alcohol. The graph also shows the alcohol production of vessel B and D were higher than vessel A and C as discussed above.

## 5.6. Physicochemical analysis of cactus pear fruit wine

The pH of the cactus wines were between 5.3-5.4 in all vessels (Table 9). Thus the cactus wine produced in this work has very low acid compared to other fruit wines (<4.5) (Okigbo and Obire, 2009). This might be due to the low acidic nature of the cactus fruit and there was no added acid

during fermentation for this work. This pH and the low titratable acidies of the wine favor the growth of microorganisms therefore it will have short shelf life.

There was a visible color difference between cactus wine and Awash red wine. The color of cactus wine were purple red whereas, the commonly used Awash red wine was bluish red (Appendix 3).

## 5.7. Sensory analysis

The mean score of cactus wine in each vessels (A, B, C and D) and in Awash red wine (E) for color were; 7.06, 7.06, 7.8, 7.7 and 8.2, and for appearance; 7.06, 7.53. 8.33, 8.2 and 8.5, respectively (Table 8). This result shown that there was significance difference in all sensory attributes between the cactus wine and Awash red wine. However, the color and appearance of the cactus wine were acceptable moderately. And there is no significance difference between each vessels of cactus wine in terms of color and appearance. This is due to the color and appearance of the wine in all vessels was almost similar (Appendix 3) and it was not affected by yeast strain and addition of sugar. The color and the appearance of the wine are directly related to the fruit and processing.

The mean score for sensory attributes such as; aroma, taste and the overall quality acceptability for cactus wine of vessel D was found to have high score compared to vessel A, B and C. This might be due to the low alcohol concentrations in vessel A, B and C which favors the growth of microorganisms that cause unpleasant off taste.

# 6. Conclusion

Based on the result of this research work, it was possible to obtain palatable and acceptable cactus fruit wine that was found to have a considerable medium level of alcohol concentration (Vessel D, 12.2% alcohol content). This cactus juice was produced using sugar and standard yeast for fermentation strains. Therefore there is a potential to produce wine from cactus fruit like other fruits.

The fermentation of cactus juice by indigenous yeast and without the addition of sugar (Vessel A), and with added sugar (Vessel B) also produce fairly acceptable wines with low alcohol concentration. Therefore it is possible to produce wine from cactus juice in household level for immediate consumption.

The analysis on nutritional composition and yeast growth of cactus fruit juice indicated that it has favorable properties to support the growth of yeast, and thus it has a potential to produce alcohol. The juice also has a good acceptable flavor, color and other nutritional components like minerals, vitamins etc. However, the low acidic nature of the cactus juice produces low acidic wine.

# 7. Recommendation

The cactus juice has a good acceptable flavor, color and other nutritional components including minerals, vitamins etc and it supports the growth of yeast. Therefore it has a potential to produce wine from cactus fruit like other fruits on a commercial level as well as house holed level. However, further study to increase the quality of the wine and consumer preference taste is recommended.

The low acidic nature of the cactus juice produces low acidic wine therefore the wine could be susceptible for microbial growth and cause spoilage or sour off taste. Therefore, acidification of the juice is recommended to prevent bacterial growth during fermentation as well as after fermentation.

Production of wine from cactus fruit decreases the postharvest losses of the fruits and it will add economical benefits for the farmers and producers.

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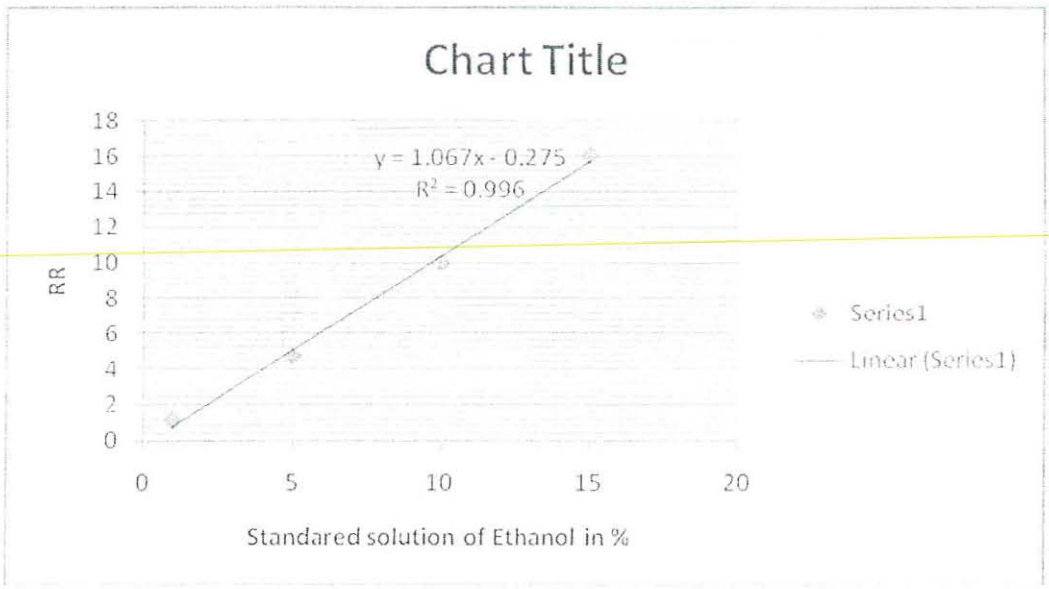
## Appendix 1

Concentration of the standard solutions prepared and the calibration curve.

| ethanol ml/100ml | 1 | 5 | 10 | 15 |
|---|---|---|---|---|
| propanol ml/100ml | 0.1 | 0.1 | 0.1 | 0.1 |
| ethanol peak area mv/s | 221.9393 | 477.911 | 1801.896 | 3591.299 |
| propanol peak area mv/s | 192.1347 | 100.8317 | 179.4792 | 223.4648 |
| AR | 10 | 50 | 100 | 150 |
| RR | 1.155123 | 4.73969 | 10.03958 | 16.07098 |



Date_____

Taster Number _____

Appendix 2

## Sensory Acceptability Test Instructions and score card

Please rinse your mouth with water before starting and between each tasting.

Taste the samples according to the numbers indicated and give value from 1-9 for each attribute based on the key given.

If you have any question please ask the server.

### Keys

9. Like extremely
8. Like very much
7. Like moderately
6. Like slightly
5. Neither like nor dislike
4. Dislike slightly
3. Dislike moderately
2. Dislike very much
1. Dislike extremely

| | Sample code | | | | |
|---|---|---|---|---|---|
| Attributes | A | B | C | D | E |
| Color | | | | | |
| Clarity(appearance) | | | | | |
| Aroma | | | | | |
| Taste | | | | | |
| Over all acceptability | | | | | |

General Comments:_____

_____

## Appendix 3

Appearance of the cactus juice wine from different fermentation batches: A, B, C and D, and Awash red wine E.