PRODUCTION AND QUALITY EVALUATION OF COCOA WINE

BY

ANJITHA BS (2018-06-005)

APARNA V M (2018-06-029)

KAVYA M (2018-06-013)

NAMITHA ASHOKAN (2018-06-015)

SREEDEV V S (2018-06-022)

Department of Processing and Food Engineering

KELAPPAJI COLLEGE OF AGRICUTURAL ENGINEERING AND TECHNOLOGY

TAVANUR-679573, MALAPPURAM

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PROJECT REPORT

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR-679573, MALAPPURAM

KERALA, INDIA

2022

DECLARATION

We hereby declare that this project report entitled “Production and Quality Evaluation of Cocoa Wine” is a bonafide record of project work done by us during the course of project and that the report has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

ANJITHA B S (2018-06-005)

APARNA V M (2018-06-029)

KAVYA M (2018-06-013)

NAMITHA ASHOKAN (2018-06-005)

SREEDEV V S (2018-06-022)

Tavanur

25/02/2022

CERTIFICATE

Certified that this project report entitled “Production and Quality Evaluation of Cocoa Wine” is a record of project work done jointly by Anjitha B S, Aparna V M, Kavya M, Namitha Ashokan and Sreedev V S under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to them.

MS Sreeja R

Assistant Professor

Dept. of Processing and Food Engineering

KCAET, Tavanur

Nighitha M T

Assistant Professor (C)

Dept. of Processing and Food Engineering

KCAET, Tavanur

Dr. Riju M C

Assistant Professor(C)

Dept. of Processing and Food Engineering

KCAET, Tavanur

Tavanur

25/02/2022

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Anjitha B S

Aparna V M

Kavya M

Namitha Ashokan

Sreedev V S

DEDICATED

TO

FOOD ENGINEERING PROFESSION

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SYMBOLS AND ABBREVIATIONS

%	:	Percentage
°C	:	degree Celsius
<	:	less than
°Bx	:	Degree brix
a*	:	Red or green value
b*	:	Blue or yellow value
L^*	:	Lightness
AA	:	Ascorbic acid
AC	:	Antioxidant capacity
AICRP	:	All India Coordinated Research Project
AOAC	:	Association of Official Analytical Chemists
CFU	:	Colony Forming Unit
CFU/ml	:	Colony Forming Unit per millilitre
et al.	:	and others
etc.	:	et cetera
E-nose	:	Electical Nose
E-tongue	:	Electrical Tongue
Fig	:	Figure
G	:	Gram

GA	:	Gallic acid
GC-MS	:	Gas Chromatography –Mass Spectroscopy
g/ml	:	Gram per millilitre
h	:	Hour
H2SO4	:	sulfuric acid
KAU	:	Kerala Agricultural University
K.C.A.E.T	:	Kelappaji College of Agricultural Engineering and Technology
KI	:	Potassium Iodide
mg/g	:	milligram per gram
mg/L	:	milligram per litre
mg/ml	:	milligram per millilitre
MMT	:	Million Metric Tonnes
MT	:	Metric Tonnes
N	:	Newton
NaOH	:	Sodium Hydroxide
n-Butanal	:	Normal butanal
n-Propanal	:	Normal propanal
pH	:	potential of hydrogen
PHET	:	Post harvest engineering technology
RP-HPLC	:	Reverse phase high performance liquid chromatography
Rpm	:	Rotations per minute

SGA	:	Self-generated alcohol
SO2	:	Sulphur dioxide
SPG	:	Specific gravity
TA	:	Titratable Acidity
TLC	:	Thin layer chromatography
TSS	:	Total Soluble Solids
v/v	:	volume per volume
Viz	:	namely
w/v	:	weight per volume
w/w	:	weight per weight

INTRODUCTION

CHAPTER 1

INTRODUCTION

Wine is identified as a potentially beneficial health promoting alcoholic beverages made by the fermentation of the fruit or fruit juices using yeast as inoculum. It is considered to be one of the oldest alcoholic beverages. Fermentation is relatively low energy preservation process which increases the shelf life and decreases the need of refrigeration or any other forms of food preservation technology.

Wines are nutritive, more tasty and mild stimulant. They usually have an alcoholic content ranging between 5-13%.Wines are divided into different categories based on its color, production method, sugar content , origin of production etc. Steps in wine making are picking of fruit, preparation of fruits, fermentation, racking, ageing, fining, filtering, bottling and pasteurization. Moderate consumption of wine lowers the risk of type -2 diabetes, heart attack, artery damage and decreases the level of cholesterol. It also provides many of the antioxidants. Interestingly, red wine likely has higher levels of antioxidants than white wines.

There is abundance of tropical fruits in India which includes cocoa, guava, watermelon, pineapple, plum, orange etc. These fruits are highly perishable, being susceptible to bacterial and fungal contamination, thus leading to their spoilage, mechanical damage and over ripeness (Ihekoroye and Ngoddy, 1985). Hence, these fruits are difficult to keep for long and are utilized either as fresh or processed into juice and speciality products (Oyeleke and Olaniyan, 2007). High rate wastage of these fruits especially at their peak of production season necessitates the need for alternative preservation and post harvest technologies towards their value addition that can reduce the level of post harvest losses besides increasing diversity of wines (Okoro, 2007; Alobo and Offonry, 2009)

Cocoa (*Theobroma cacao* L.) is known world wide for its beans which are used for in the production of chocolate and cocoa butter. It belongs to Malvaceae family. It is a tropical crop, grows within 15-20° latitude from equator and is originated in the

amazon region of South America. The global production of cocoa during 2020-2021 was 4.8 Million Metric Tons (MMT) with major share (76.6%) is from Africa. Globally, cocoa is widely cultivated in Africa, Asia and Latin America. Ivory Coast is the largest producer of cocoa in the world.

Cocoa pulp is one of the by-products resulting from the processing of its fruit and is highly consumed in the preparation of juice, but it has also been proposed for the production of fruit wine. Cocoa pulp, as shown in Figure 1.1, is a white mucilaginous layer, which firmly envelops individual seed of the fruit of *Theobroma cacao* plant (Figure 1.1). It is formed during pod development from endocarp meristem and makes up approximately 40% of fresh seed weight (Biehl and Ziegleder, 2003).

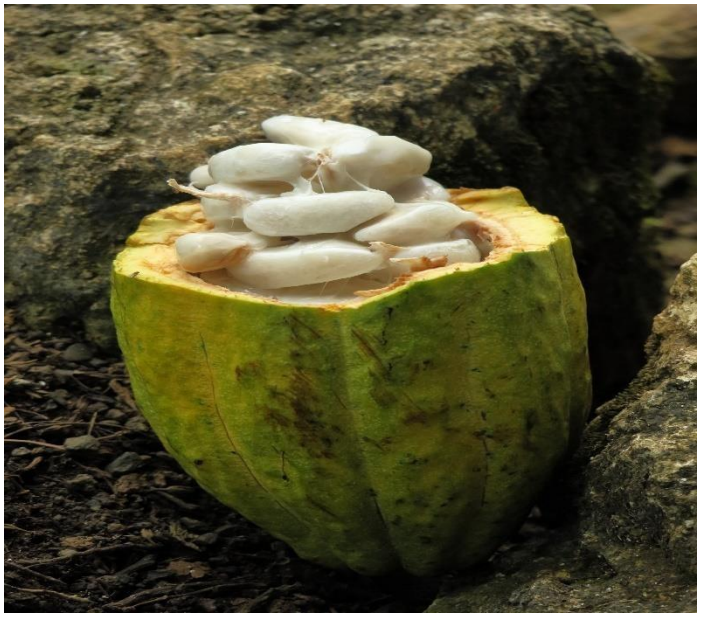


Fig.1.1 Cross section of cocoa fruit

Cocoa pulp mainly consists of water, sugars, acids, and pectin. Sugars in cocoa pulp are mainly sucrose, fructose, and glucose. Pectin, which gives cocoa pulp a thick consistency, presents at approximately 1% on fresh weight basis. Citrate is the major organic acids, which inversely affected the pH of cocoa pulp. Other nonvolatile organic

acids such as malic, tartaric, and oxalic acids are less than 0.1% in cocoa pulp (Pettipher, 1986). The most abundant mineral is potassium, whereas the most abundant vitamin is ascorbic acid, which constitutes 97% of all vitamins present. The concentrations of those nutrients will vary as influenced by different cultivars of cocoa, ripeness as well as growing regions and subsequently, their climate. Because of the composition of cocoa pulp that is rich in macro- and micronutrients, it is a favorable medium for microbial growth and thus, a suitable substrate for cocoa bean fermentation. Having pH of 3.50-3.80, fresh cocoa pulp has a combination of sweet and mildly acidic taste with a note of tropical flavor, popular in cocoa-growing regions.

Although cocoa pulp is an essential ingredient for cocoa bean fermentation, successful fermentation does not necessarily require all of the pulp. In fact, loss of pulp naturally occurs as seeds are spread out and subsequently pulp drains down the fermentation boxes. Up to 20% of pulp (fresh bean weight) can be removed without significant effects on the fermentation process and organoleptic quality of cocoa bean (Lopez, 1979). In certain cultivars, partial removal of cocoa pulp before fermentation has been purposely done to reduce high acidity in cocoa beans. Biehl and Ziegleder (2003) found that strong acidic fermentation (pH<4) produced beans with lower aroma potentials compared to moderate nib acidity during fermentation (pH 5 -5.5). Biehl et al. (1989) also suggested that if the volume ratio of pulp to seed was reduced to below 0.6 from about 1.1-1.3, acetic acid production was significantly reduced. It was reasoned that with thinner pulp layer, anaerobic fermentation phase is shortened causing an accelerated microbial succession, temperature increase and rise in pH on the cotyledon; leading to a rapid progression of fermentation in overall. Nevertheless, the above studies provide information of potential secondary use of cocoa pulp in value-added products instead of a discarded by-product.

Several attempts to further utilize cocoa pulp, extracted before and after bean fermentation, have been made. Dias et al. (2007) were successful in making a fruit wine by fermenting fresh cocoa pulp using *Saccharomyces cerevisiae* strain. Nonetheless, the versatile utilization of cocoa pulp in the aforementioned application may offer a

promising diversification opportunity for cocoa agro industry. This product could become a significant source of secondary income in the cocoa growing communities especially when the demand of cocoa is low.

Cocoa sweatings, the pale yellowish liquid that drains off during cocoa fermentation, is the breakdown product of the mucilage surrounding the fresh cocoa bean, and constitutes about 10% of the weight of the cocoa fruit. This cloudy substance is composed of 85.3% moisture with soluble solids up to 17.78°Bx, pH of 3.43 – 3.5, rich in sugar, minerals, organic acids and phenolic compounds (Efraim *et al.,* 2010; Ouattara *et al.,* 2014). This extract from cocoa mucilage is one of the by-products in cocoa industry with high nutritional value. More than 5,50,000 m^3 of juice from mucilage of cocoa beans are produced and abandoned in farms each year. Therefore, the production of a new product with high economic return from this mucilage will not only solve the pollution problem caused by the by-product but also help in increasing the income of the farmers. It has been found to be a suitable medium for the production of wine and alcohol. There has been a lack of studies on using cocoa mucilage, a byproduct of cocoa fermentation, for the production of wine. Therefore, it is planned to develop a process protocol to produce wine from cocoa mucilage.

Considering above facts, a study had been undertaken at KCAET, Tavanur entitled "Production and Quality Evaluation of Cocoa Wine" with following objectives

1. Determination of physic-chemical properties of cocoa wine
2. Development of wine from cocoa
3. To access the shelf life of cocoa wine

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Alberti *et al.,* 2011 Apple wine processing with different nitrogen contents was done. This work was to evaluate the nitrogen contents different varieties of apple musts and to study the effect of different nitrogen concentrations in apple wine fermentation. The nitrogen content in apple musts was an important factor of growth and fermentation velocity. Brazilian apple must showed average nitrogen concentration above 100 mg/L. At this concentration the alcoholic fermentation occurs without any interruptions. The nitrogen content in apple juice affects directly the yeast growth and fermentation kinetics. However, although in must with low nitrogen content, the fermentation was slower but it occurred until complete exhaustion of the fermentable sugars. The Brazilian apple must not be supplemented with nitrogen for alcoholic fermentation.

In 2012, Kumoro *et al.* conducted study on Preparation of Wine from Jackfruit (*Artocarpus heterophyllus lam*) Juice Using Baker yeast: Effect of Yeast and Initial Sugar Concentrations. This study was to investigate the effect of yeast and initial sugar concentrations on jackfruit juice wine fermentation. Both yeast and initial sugar concentrations affected the production of jackfruit wine. Higher yeast and initial sugar concentrations were found to inhibit the growth of the yeast cell. The use of 0.5% w/v Baker yeast and original jackfruit juice with 14 % (w/w) sugar concentration was adequate to produce jackfruit wine where ethanol concentration of 12.13% v/v was obtained after 9 days of fermentation. No significant difference in ethanol concentration was observed when the yeast concentration was varied from 1.0-2.0 % w/v and wines with ethanol concentrations of about 12-13.5 % v/v were obtained in just 7 days fermentation. All the jackfruit wines obtained from fermentation of original jackfruit juice were clear yellow in color and brought strong jackfruit aroma.

From the study by Idise and Okiemute Emmanuel (2012) wine was produced from pineapple. This wine was produced from pineapple using its innate micro-organisms, granulated sugar and baker's yeast in varying proportions. There was evidence of Malo-lactic fermentation. The wines produced showed no appreciable differences in the tested parameters – pH, temperature, optical density, specific gravity, total aerobic counts, % alcohol (v/v) and % titratable acidity. They could be consumed within 48 h. No chemical preservatives were required. However, there is the need for further research to ascertain the shelf life of the wines.

In 2012 Gavimath *et al.,* had done comparative analysis of wine from different fruits. In this investigation papaya, banana, orange and lime fruits were used. Observations were recorded for acidity, microbial count and alcohol content. All the fruits were suitable for wine production, but in this study banana fruits yield good quantity and quality alcohol when compared to papaya, orange and lime.

From the study by Giri Nandagopal.M.S and Praveen.S.Nair (2013) Production of wine from Ginger and Indian Gooseberry and a comparative study of them over commercial wine was done. Ginger and Indian gooseberry, which are known for its high medicinal and nutritional value are used as the substrate here. Fermentation is carried out with *Saccharomyces cerevisiae* commonly known as baker's yeast. Daily monitoring was done to study the composition and characteristics of the wine. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. During the fermentation period the wines were analyzed for pH, titratable acidity, specific gravity, biomass content, alcohol and reducing sugar on a daily basis. pH show a decreased trend then attains minima and then increased. As the fermentation days proceed, the specific gravity increased and the alcohol percentage increased gradually.

After the fermentation period parameters such as Tannin content, Phenol content, Free and Total SO2, Alcohol content, Total Suspended Solids (°Brix), pH, Titratable Acidity and Specific Gravity were analyzed. These parameters were compared with

that of commercial wine. It was observed that pH (except ginger), specific gravity and alcohol content were higher for commercial wine. But the phenol, tannin content and total suspended solids is higher for homemade wine. By comparing the titratable acidity with commercial wine, ginger wine showed lower value.

In 2014,Joshi *et al*., conducted study on Preparation of plum wine with reduced alcohol content: Effect of must treatment and blending with sand pear juice on physico-chemical and sensory quality. This study was to optimize a technique for preparation of low alcoholic plum wine was made. Fruit of Santa rosa plum cultivar were used to produce wine by different methods, viz. conventional method , must ameliorated with honey , removal of alcohol from wine by distillation , inoculation with *Schizosaccharomyces pombe* to reduce the acidity followed by fermentation with *Saccharomyces cerevisiae* and inoculation with *Schizosaccharomyces pombe* only for deacidifying the must. Conventional method of plum preparation gave the highest rate of fermentation. Amelioration with honey and cane sugar showed similarity in fermentation in terms of TSS, titratable acidity, pH and ethanol production. Plum wine prepared by fermentation with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and blended with 20 % pear juice to reduce the alcohol concentration proved to be the best wine on the basis of physico-chemical and sensory characteristics.

From study by Nzabuheraheza *et al*.(2014) on Golden wine produced from mixed juices of passion fruit (Passiflora edulis), mango (Mangifera indica) and pineapple (Ananas comosus), extraction of three juices, physical and chemical parameters were determined before and during fermentation of the must. These parameters were: wild yeast colony forming units per milliliter (CFU/ml) of fermenting must, total soluble solids (° Bx), pH, alcohol content, titratable acidity in percent, fermentation temperature, sugar content (g/l), and specific gravity. The fermentation of a mixture of juices was done at room temperature, i.e., at 22°C, and the wild yeast used was Saccharomyces cerevisiae, a strain isolated from local traditional banana wine. The quality of obtained yellow wine was a wholesome alcoholic and delicious beverage comparable to imported grape wines. The ideal final pH of yellow/golden

wine should be between 3.0 and 3.3. The high content of fermentable sugars in mixed juice extracted from studied Rwandan fruits affects positively the alcohol content of the yellow wine.

Argade *et al*.(2015) conducted study on Preparation and Standardization of Amla Wine by Using *Saccharomyces cerevisiae* Yeast as a Fermenter. In the present study, the pharmacologically active marker constituents from amla was isolated and its identification was done by TLC and RP-HPLC methods. The amla wine was prepared by using Saccharomyces cerevisiae as a fermenter with addition of jaggery. The self generated alcohol (SGA) content as ethanol was observed to be 9.29%, also the higher alcohol like n-butanol, n-propanol, iso-butanol were also found in trace amount as a byproduct of alcoholic fermentation. The TLC and RP-HPLC analysis strongly suggested the presence of AA and GA in Amla wine formulation, its presence in the Amla wine formulation plays the important role in the treatment and prevention of infectious diseases and also used as radical scavengers.

In 2016, Alagesan *et al.* conducted an investigation to find out the optimal condition for the efficient conversion of papaya juice in to wine using Saccharomyces cerevisiae. Optimization of parameters by the conventional method involves changing one dependent variable while unchanging all other at a fixed level. Pre –preparation of wine was carried out in usual method and in order to determine the optimum TSS, pectinase, inoculum level , temperature, PH the experiment were carried out by incubating the appropriate number of inoculated flask at different TSS(18,20,22,24,26°Bx),pectinase(1ml5ml),inoculumlevel(2ml,4ml,6ml,8ml,10ml),pH value (2,3,4, and 5) and temperature(15,20,25 and 35°C) . The evaluation of wine was done after 5 days of fermentation. The optimum process condition/maximum ethanol yield for this fermentation process were 24°Bx TSS, 26°C temperature, 5ml of pectinase enzyme, 10% inoculum and pH of 4.5. Corresponding to these optimum condition, a predicted value of ethanol production was found to be 11-12% which was experimentally verified.

From the study by Hongmei Denga *et al.,* (2016) Preparation and chemical characterization of banana/orange composite wine was done. In this study banana/orange composite wine was brewed in the lab by liquid fermentation using angel yeast and lactic acid bacteria as the fermentation strains, and characterized by chemical analyses. It was found that the best ratio of banana juice to navel orange juice was 1:2, and the optimum alcohol fermentation parameters were as follows: 28~30°C, 22% initial sugar content, 6% yeast, and 6 days of fermentation to reach an alcohol concentration of 11.63% v/v. The free amino acids in the composite wines were tested by automatic amino acid analyzer, and the flavor components of the composite wine were determined and analyzed by gas chromatography-mass spectrometry (GC-MS). Seven types of trace elements in the composite wine were measured by atomic absorption spectrometry. Results showed that there were 17 free amino acids, and their total concentration was up to 897.6 mg/L. A total of 16 key compounds were identified in the composite wine, 11 of which were ester, 4 of which were alcohols and 1 of which was acid. Magnesium, iron, copper and manganese elements were relatively rich in the wine, while lead was extremely low.

From the study by Lingua *et al.*, (2016) they mentioned the evaluation of phenolic compound and their relationship with the antioxidant capacity(AC) of sample was taken along the wine making process of three *Vitis vinifera*. L (red grapes) syrah, Merlot, Cabernet, saubignon grown in Argentina were studied. Extraction of phenolic compound from whole grapes and pomaces was carried out with minor changes. Grape and pomaces sample were lyophilized and their moisture percentage is calculated by weight difference before and after freeze drying. After lyophilization, sample were frozen using liquid nitrogen and until obtaining fine powder. A portion of 1g of treated sample was extracted with 15ml of acidified methanol in a blender. The obtained homogenate was incubated with agitation for 2h at 4°C and then centrifuged. Then the combined extracts were filtered and stored at 80°C until phenolic and antioxidant activity determination. Wine (1 after alcoholic fermentation)and wine 2(after stabilization) samples from the three varieties under study, were filtered using whatman

no :1 filter paper, fractionated in 125ml poly ethylene bottles and stored at -80°C until phenolic and antioxidant activity analysis determination. The result presented in this study highlight that the phenolic composition of the grapes is greatly affected by the wine making process.

In 2017, Lan *et al.*, conducted study on Evaluation of antioxidant capacity and flavor profile change of pomegranate wine during fermentation and aging process. The total phenol content and radical scavenging activity exhibited a slightly decrease in the end edge. Punicalagins and gallic acid were revealed to be the most abundant phenolic compounds, followed by ellagic acid and vanillic acid. These constituents were mainly responsible for the effective antioxidant capacity of pomegranate wine. The major changes of flavor qualities occurred in the initial stage, particularly 0–4 day of fermentation. Fermentation significantly reduced the relative content of aldehydes, ketones, heterocyclic and aromatic compounds, but promoted the generation of esters and alcohols. This is the first time of using E-nose and E-tongue to monitor odour and taste changes in the brewing process of pomegranate wine. The study may provide a promising instruction for improving functional features and quality control of the pomegranate wine.

Tinha *et al.*, (2016) conducted study on wine fermentation from mucilage of cocoa beans (*Theobroma cocao* L.). This research was to ferment cocoa bean mucilage to produce a beverage with alcohol concentration of 11.2%. The raw cocoa mucilage had a distinct aroma, slight viscosity (8.823Cs), soluble solid content of 17.78°Bx. The total sugar content of the raw material was sufficient and addition of 20% sucrose was required. The microbe species for fermentation was *Saccharomyces cerevisiae* with a density of 2x107 cfu/ml, which accounted for 5% and the commercial yeast powder addition was of 1%. The fermentation included two stages. Primary fermentation was at 28°C in 7 days and secondary fermentation was at ambient temperature in 30 days. The wine produced from cocoa mucilage had an alcohol content of 10 to 11.5 and sensory quality was evaluated.

In 2017, Soibam *et al*. conducted study on Preparation and Evaluation of Wine from Sugarcane and Beet Juice. Sugarcane (*Saccharum officinarum L.*) juice which contains high amount of sugar was used as raw material and an attempt was made for the standardization of the process of preparing wine from sugarcane- beet (*Beta vulgaris Linn.)* juice blending. It was concluded that wine of good colour, flavor and overall acceptability can be prepared from sugarcane beet juice non-pasteurised blended at 50%, TSS 24.4 0Bx, 26°C and pH 4.5 during fermentation followed by storage. This could be one of the alternative post harvest management which provides the room for further research on the characterization of antioxidant and flavouring compound(s) and value addition of wine by blending.

Yusufu *et al*. (2018) conducted a study on Production and Quality Evaluation of Wine from Watermelon Juice and Ginger Extract. This research was done to control postharvest loses in watermelon and ginger and also to develop health promoting and acceptable wine using watermelon (*Citrullus lanatus)* and Ginger (*Zingiber officinale).* During the fermentation process, the pH, specific gravity (SPG) and alcoholic content was monitored on a daily basis. It was indicated that an acceptable, enhanced physico-chemical and mineral with improved antioxidant properties wine could be produced from blends of watermelon juice and hot water ginger extract.

In 2018, Sarkar *et al*. conducted study on Development of Health Functional Wine produced from *Emblica officinalis* and *Phyllanthus niruri* and a Comparative Study of them over Commercial Wine. Bhumi Amla and Indian gooseberry were used as the substrate here. Fermentation is carried out with Saccharomyces cerevisiae. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. After the fermentation period the wine was analysed for pH, total soluble solids, alcohol and antioxidant profile, post aging. The wines with *Phyllanthus niruri* and *Emblica officinalis* displayed high quantum of tannins, phenols and free radical scavenging activity. After the aging period parameters such as Antioxidant profile, Alcohol content, Total Suspended Solids (°Bx), pH, Titratable Acidity were analysed. These parameters were compared with that of commercial wine. Thus the studies

showed that the pH, TSS, Tannin and Alcohol content were higher for commercial wine. But the phenol, reducing sugar and % inhibition of free radicals is higher for homemade wine.

In 2019, Tamrakar *et al.*, conducted Qualitative analysis of wine prepared from banana and orange. Banana and orange both were taken as a substrate with different properties and nutritional value which resulted in the production of different concentrations of alcohol along with TSS, pH and titratable acidity although same yeast culture was used. From the result obtained through this study, there was no significant difference in the wine prepared from banana and orange. Fermentation of banana and orange juices were carried out in the presence of wine yeast for about a month in a natural condition to obtain wine.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The various physico.-chemical properties and microbial count of prepared wine are discussed in this chapter. The method of wine preparation and optimization of wine under various parameters are also mentioned in this chapter.

3.1 RAW MATERIALS

Matured cocoa fruit (*Theobroma cacao L.)* were procured from Cocoa Research Center, KAU, Vellanikkara. Materials for the preparation of wine were purchased from a local store in Tavanur. Fresh cocoa pulp obtained immediately after pod breaking were collected. The cocoa mucilage separated from the pulp using the cocoa pulp extractor were used in this study. Fresh mucilage were collected in steel containers and transported to the laboratory with care. In the laboratory, mucilage were stored at refrigerated conditions for conducting the experiment.

3.2 COCOA MUSCILAGE EXTRACTION

Fresh cocoa fruits, collected from Cocoa Research Center, KAU, Vellanikkara for the study. The cocoa fruits were broken manually. The cocoa pulp was fed into the cocoa pulp extractor. The cocoa pulp extractor was fabricated to remove the required percentage of pulp from the bean and hence create a healthy environment for the fermentation process. The cocoa pulp extractor consists of two concentric cylinders. The inner cylinder composed of uniform holes in the body which is rotating at fixed rpm using a robotic motor. The percentage of pulp extracted varied with the variation in rpm of inner cylinder. The optimum concentration of pulp for the production of high quality cocoa beans was estimated as 80%. In cocoa pulp extractor the 20% extraction of pulp was obtained at 25 rpm of inner cylinder. The pulp extracted using cocoa pulp extractor was then collected in a steel container and then transported to laboratory. The physico-chemical qualities of the cocoa pulp were evaluated prior to wine preparation.

plate 3.1 Cocoa pulp extractor

plate 3.2 Cocoa pulp

plate 3.3 Cocoa drippings

3.3 YEAST INOCULUM PREPARATION

Hundred millilitre distilled water was taken in a 250ml conical flask. Then 4g of chloramphenicol yeast glucose agar was weighed and transferred into this conical flask. 5g of dry yeast was weighed and transferred into this conical flask and mixed well.

This conical flask was then placed in shaking incubator at 100rpm for 2 hours.

3.4 WINE PREPARATION

One litre of collected cocoa drippings was taken in a glass jar. The initial TSS of the collected cocoa drippings was 20°Bx and pH was 5.3. To this, sugar was added and diluted with water .The sample was then pasteurized at 85°C for 5 min and cooled to room temperature. Then it was inoculated with yeast broth. 4g of spices were added to provide specific flavor. After that the sample was tightly covered and kept for 7 days of primary fermentation in a dark place. After 7 days of primary fermentation, this sample was filtered using muslin cloth and physico-chemical properties of these 6 different sample were analyzed. This sample was then kept for 30 days for secondary fermentation in dark place. After secondary fermentation, the wine was taken out and physico-chemical properties were analyzed and quality of the wine was analyzed by sensory evaluation.

Cocoa wine preparation flow chart

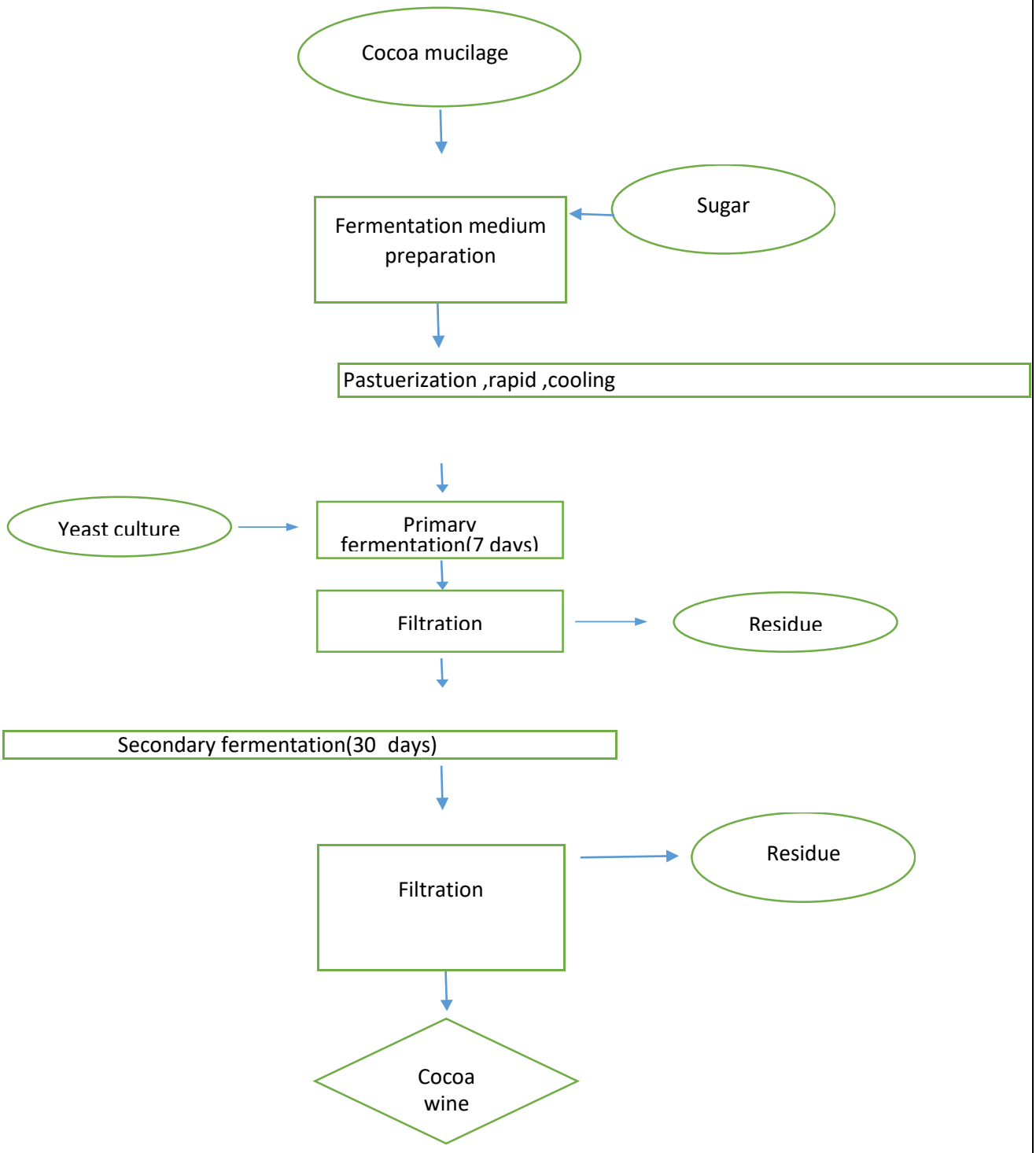


Figure 3.1 flowchart for cocoa wine production

3.4.1 Pretreatment

Tinh *et al.*(2016) found that the highest alcohol content was observed in wine prepared with 22°Bx. To evaluate the results treatments were carried out with 20°Bx, 22°Bx and 24°Bx. The highest alcohol content of 12.82% (v/v) was observed in wine prepared with 22°Bx.

3.4.2 Treatments

The four samples were treated with this optimum pH of 22°Bx.

Treatment 1: Cocoa drippings with an initial TSS of 20°Bx was made in to 22°Bx by adding 3.85 g of sugar .The sample was diluted with 7.7ml of water and then pasteurized at 85°C for 5 minutes. The sample was then cooled and inoculated with 5 ml of the previously cultured yeast. 0.4 g of spices were also added to this sample to provide specific flavor to the cocoa wine.

Treatment 2: Cocoa drippings with an initial TSS of 20°Bx was made up to 22°Bx by adding 3.85 g of sugar. The sample was then diluted with 7.7 ml of water and then pasteurized at 85°C for 5 minutes. The sample was then cooled and inoculated with 10ml of previously cultured yeast. 0.4 g of spices were then added to this sample.

Treatment 3: Cocoa drippings with an initial TSS of 20°Bx was made into 22°Bx by adding 3.85 g of sugar and then diluted with 7.7 ml of water. This sample was inoculated with 10ml of previously cultured yeast. 0.4g of spices were added to this sample.

Treatment 4: Cocoa drippings with an initial TSS of 20°Bx was made into 22°Bx by adding 3.85 g of sugar and diluted with 7.7 ml of water. This sample was inoculated with 5ml of previously cultured yeast.0.4g of spices were added to this sample.

plate 3.4 Prepared wine

3.5 DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF COCOA WINE

Physico-chemical properties of the cocoa wine such as PH, TSS, titratable acidity, vitamin C, colour were determined by standard methods as explained in the following section.

3.5.1 pH

Plate 3.5 pH meter

The pH is of importance as a measure of the active acidity which influences the flavor of the end product. The pH of liquid foods was measured by using digital pH meter (AOAC, 2000).

3.5.2 Total soluble solids TSS

Plate 3.6 Refractometer

Total soluble solids (TSS) content of samples as °Brix was determined by using a digital refractometer (AOAC, 2000).

3.5.3 Titratable acidity

Five milliliter of dripping was dissolved in 100 ml of water. From the makeup, 10 ml of sample was pipette out into a 100 ml conical flask and few drops of phenolphthalein indicator was added. The solution was titrated against 0.1 N NaOH till the color turned to pale pink color. This procedure was repeated to get concordant values. The percent acidity was calculated as follows.

$$Acidity\ (\%\ malic\ acid) = \frac{volume\ of\ titrant\ (ml) \times Normality\ of\ titrant \times 0.067}{Sample\ weight\ (g)}\ x\ 100$$

3.5.4 Ascorbic acid

Standardization of dye was carried out by taking 5 ml of standard ascorbic acid solution and titrated against the 2,6 dichlorophenol indophenol dye till the pink color persists for 10 s. Ten milliliter of cocoa drippings was taken and made in to 100 ml with 4% oxalic acid solution and filtered. Ten milliliter of filtrate was taken and titrated against the dye solution. The dye factor and ascorbic acid content were estimated using the formula.

$$\text{Dye factor} = \frac{0.5}{\text{Titrable value}(V_1)}$$

$$\text{Ascorbic acid}(\text{mg}/100\text{g}) = \frac{0.5\text{mg}}{V_1\text{ml}} \text{x} \frac{V_2}{5\text{ ml}} \text{x} \frac{100\ ml}{\text{Wt. of the sample}} \text{x}100$$

3.5.5 Colour

Plate 3.7 Spectrophotometer

The colors of the drippings were found using a Hunter lab color flex meter (Hunter Association laboratory, Inc., Reston, Virgina, USA; model: HunterLab's Color Flex EZ). It reads the color of sample in terms of L, a and b values where, luminance (L) forms the vertical axis, which indicates whiteness to darkness. A transparent glass cup filled with sample was placed over the port of the instrument and an opaque cover which act as a light trap to exclude the interference of external light was placed over the cup. Before actual measurements color was calibrated by fixing the definite colors like white and black tiles. After calibration, the sample was placed over the port and values of 'L', 'a' and 'b' were recorded.

Table 3.1 Physicochemical properties of cocoa mucilage

Physicochemical properties of cocoa mucilage	
pH	5.3
TSS	20
Titratable acidity	0.182mg/100ml
Ascorbic acid	7.272mg/100ml
Colour L* a* b*	 44.34 6.11 62.25

3.5.6 Determination of alcohol content

Four milliliter of fermented sample was pipetted out into a 100ml volumetric flask made up with distilled water. 5ml of diluted sample was transferred in a screwed conical flask and 100ml of 0.05M potassium chromate was added. 20ml of 50% H_2SO_4 added to each flask. Each flask capped loosely and heated in a water bath at 50°C for 60 minutes. The flask removed from water bath and 10ml of 0.5M KI was added, this content titrated with 0.1M sodium thiosulphate solution. When brown colour of the solution gets a green tinge, a few drops 1% starch indicator was added which was prepared in boiling water. The addition of sodium thiosulphate was continued until the solution gets a clear green blue colour.

3.6. DETERMINATION OF MICROBIAL COUNT

The bacterial and yeast and mould population in fruit juices were analyzed by different microbiological methodologies that includes enumeration of the

microorganism in selective media for different dilutions of samples, incubation of plates and counting the number of colonies present. The media generally used for enumeration of bacteria is nutrient agar medium, whereas, for yeast and mould enumeration chloramphenicol yeast glucose agar media was used (Allen 1953). The fruit juice sample of 1ml was pipetted using a sterile pipette into a test tube containing 9ml of sterile water which give 1:10(10^{-1}) dilution. The test tube were shaken well for 10-15 minutes for uniform distribution of microbial cells in the water blank. Then 10^{-2} dilution was prepared by pipetting out 1ml of (10^{-1}) dilution to 9ml. The process was repeated upto 10^{-9}dilutions with serial transfer of the dilutants. One milliliter of 10^{-4} to 10^{-9} dilutions were transferred to one of the sterile petri dishes for the enumeration of bacteria and the other petri dishes for the enumeration of yeast and mould.

Approximately ,15-20 ml of molten and cooled(45°C) respective agar medium was added to each petri dish containing the sample dilutions and the plated were rotated in clockwise and anticlockwise directions foe thorough mixing of dilutants and the medium. The plates were incubated at 35°C (room temperature). After the incubation period, the colonies were counted and the number of organisms (total bacteria and yeast and mould) per gram of sample was calculated using the equation.

Number of colony forming unit (CFU)per gram of the sample

$$= \frac{\text{Mean number of CFU} * \text{Dilution factor}}{\text{Quantity of sample on weight basis}}$$

RESULTS AND DISCUSSIONS

CHAPTER IV

RESULT AND DISCUSSION

This chapter deals with the optimization of cocoa wine and results obtained from various tests conducted to determine the physico-chemical properties of cocoa wine and quality evaluation of cocoa wine.

4.1 EFFECT OF TSS ON FERMENTATION

Tinh *et al*.(2016) found that highest level of alcohol content was obtained in cocoa wine treated with 22°Bx. Hence to investigate the effect of TSS on fermentation, the experiment was carried out with the following treatments: 20°Bx, 22°Bx and 24°Bx. The primary and secondary fermentation was carried out at room temperature at 7 and 30 days respectively.

Table 4.1 Alcohol content of pretreated samples

Sample	Alcohol content
1	6.8
2	12.82
3	10.23

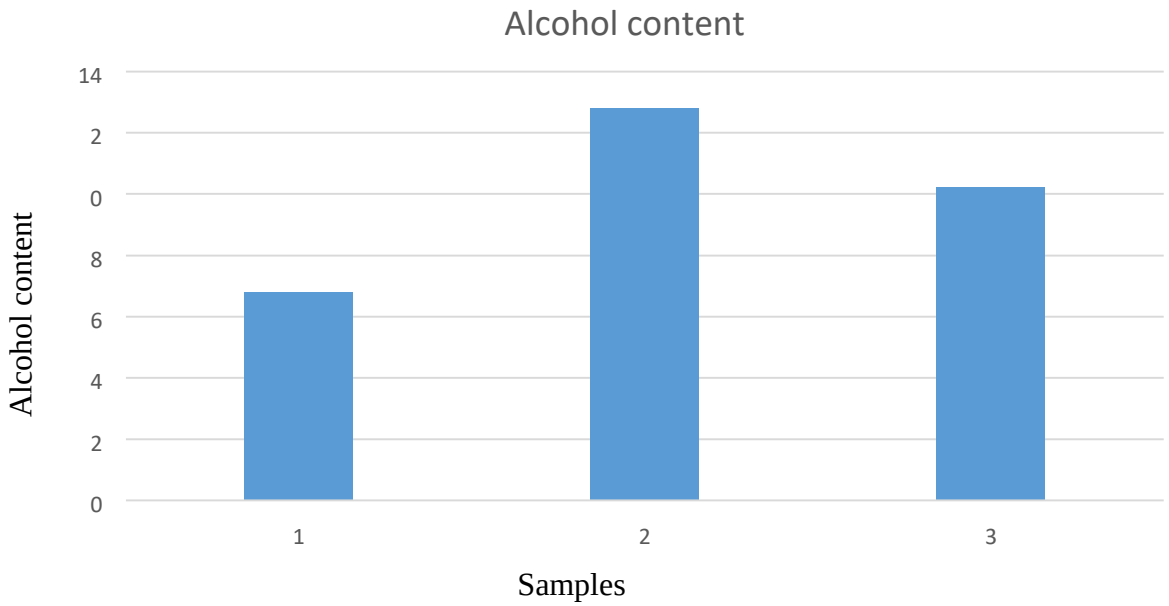


Figure 4.1 Effect of TSS on fermentation

Oxidation to Alkanal Method was used to determine the alcohol content after fermentation. Based on the result showed in the Figure 4.1, we found that wine treated with 22°Bx had the highest alcohol content of 12.82.

4.2 EFFECT OF RATE OF YEAST ON FERMENTATION

Tinh *et al.* (2016) found that, the highest level of alcohol content was obtained in the sample were 5 ml of yeast broth was added. So, investigate the rate of yeast on fermentation ,the experiment was carried out with 5 ml and 10 ml yeast broth at 22 °Bx and pasteurized at 85 °C for 5 min.

Table 4.2 Alcohol content of fermented cocoa wine

Sample	Alcohol content
1	12.82
2	11.02

From above Table 4.2 it was observed that the sample prepared with 5 ml yeast broth had highest alcohol content and it was found to be better wine.

4.3 EFFECT OF PASTERIZATION ON FERMENTATION

To investigate the effect of pasteurization on fermentation , treatments were carried out for 5 ml and 10 ml yeast broth at 22 °Bx in pasteurized and unpasteurized conditions. The unpasteurized sample was found to be better.

Table 4.3 Effect of pasteurization on fermentation

	5 ml yeast broth		10 ml yeast broth	
	Pasteurized	Unpasteurized	Pasteurized	Unpasteurized
Alcohol content	12.82	14.02	11.07	13.65

4.4. PHYSICO-CHEMICAL PROPERTIES OF COCOA WINE

The results of physico-chemical properties *viz* TSS ,pH, titrable acidity, vitamin C, colour and alcohol content are presented and discussed in this section.

4.4.1 TSS

Table 4.4 TSS of different samples after primary and secondary fermentation.

Sample	TSS(°Bx)	
	Primary Fermentation	Secondary Fermentation
1	10	9.5
2	8	7
3	8.2	8
4	9.2	8.6

From the above table, the initial TSS of 22 °Bx of the sample was decreased to 10°Bx after primary fermentation. After 30 days of secondary fermentation, TSS of the sample was further decreased to 9.5 °Bx. Similar changes were observed for the other 3 samples. A gradual decrease in TSS during must fermentation is due to the consumption of solid content by *Saccharomyces cerevisiae* (Nzabuheraheza *et al*., 2014).

4.4.2.Ascorbic acid

Table 4.5 Ascorbic acid of different samples.

Sample	Ascorbic Acid (mg/100 ml)	
	Primary Fermentation	Secondary fermentation
1	7.272	10.9
2	3.6	14.5
3	10.9	10.9
4	14.5	12.72

The initial ascorbic acid content of cocoa mucilage was 7.272 mg /100 ml. Here the sample 1 and 2 are pasteurized and sample 3 and 4 are unpasteurized. During the fermentation period, an increase in ascorbic acid content was observed .This increase was due to the microorganisms. During primary fermentation, this trend was observed in the unpasteurized sample while one of the pasteurized sample showed same ascorbic acid content as before and the other sample showed a decrease. This is because heat treatment leads to loss of ascorbic acid. After secondary fermentation, the pasteurized samples showed an increase while it was decreased in the unpasteurized sample. Researchers at the University of California, Davis and University of Adelaide,

Australia identified an enzyme in grapes that converts ascorbic acid into tartaric acid.This may be the reason for decrease in ascorbic acid content.

4.4.3 Titratable Acidity

Table 4.6 Titratable acidity of different samples after primary and secondary fermentation

Sample	Titratable acidity(mg/100ml)	
	Primary Fermentation	Secondary Fermentation
1	0.1876	2.211
2	0.201	1.675
3	0.2546	5.762
4	0.2814	7.638

Titratable acidity of sample 1 was increased after the primary fermentation. The increase in titratable acidity of the sample 1 was also observed after secondary fermentation. Similar changes were observed for the other 3 samples. The presence of different organic acids such as tartaric, malic, citric, acetic, ascorbic, butyric, sorbic, succinic acid could be responsible for high TA and lower the pH (Nzabuheraheza *et al*.,2014).

4.4.4 pH

Table 4.7 pH of different samples after primary and secondary fermentation.

Sample	Primary fermentation	Secondary Fermentation
1	4.31	3.5
2	4.23	2.98
3	4.96	3.62
4	4.50	3.14

In this study, there was a decrease in pH for all the samples throughout the fermentation. As per the study of Idise *et al.* (2011), wine tends to get more acidic as

the fermentation process gets underway because sugar is utilized for the growth of microorganism. Another study conducted by Sweta *et al.* (2016) in Ambernath states that the pH of banana wine reduced from 3.5 to 3.3 with increase in fermentation time.

4.4.5 Colour

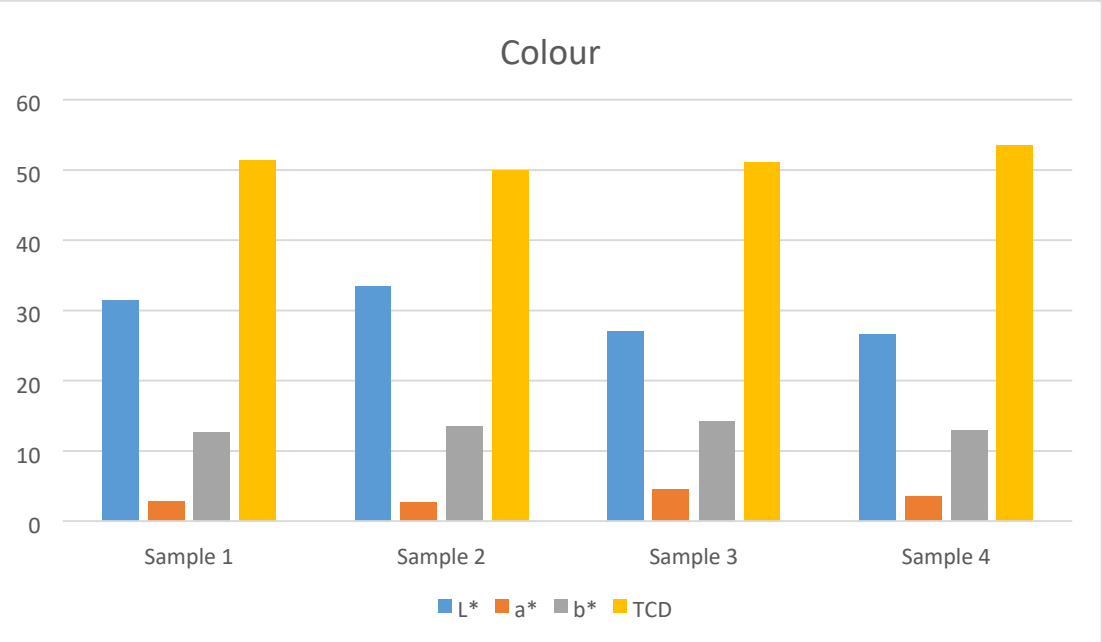


Figure 4.2 Colour of different samples

From the above Figure 4.2, higher L* value was observed for the sample 2.This indicates that this sample tends to be more light. Higher a* and b* values of sample 3 indicates that it was more darker wine compared to other samples.

4.4.6. Alcohol content

The alcohol content in wines from other studies were 12% v/v (9.6% w/v).According to Dias *et al.* (2007) , a wine product should have an alcohol content of 7.5% w/v. From the Table 4.8, below the alcohol content of the prepared wine measured by Oxidation to Alkanal method was in the range of 10-15 % v/v.

Table 4.8 Alcohol content of different samples

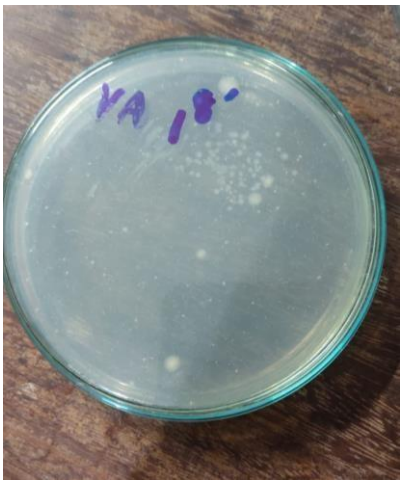
Sample	Alcohol content
1	12.82
2	11.07
3	13.65
4	14.02

4.6. Microbial count

Table 4.9 Microbial count of different samples

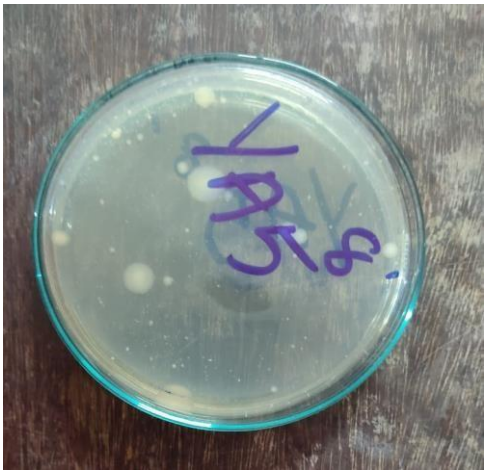
Sample	Microbial count (CFU/g)
1	5.5*10 ^8
2	4*10^8
3	6.3 *10^8
4	3.2*10^8

From the Table 4.9, it is observed that microbial count was higher for sample 3 and 4 which was unpasteurized. In the case of pasteurized sample lower microbial count was observed.



(a)

(b)



(b)

(d)

Plate 4.3 Microbial count of (a) sample 1 (b) sample 2 (c) sample 3 (d) sample 4

SUMMARY AND DISCUSSION

CHAPTER V

SUMMARY AND CONCLUSION

Cocoa (*Theobroma cacao L.)* is a tropical crop and native to Amazon region of South America. It grows in tropical environment within 15-20° latitude from equator. The primary cocoa growing regions are Africa, Asia and Latin America. The global production of cocoa during 2018-19 was 4.8 MMT. Cocoa is a commercial plantation crop in India. It is mainly cultivated in Kerala, Karnataka, Andhra Pradesh and Tamil Nadu. The annual production of cocoa in India during 2018-2019 was 20,000 MT from an area of 78,000 ha. Cocoa is the main raw material in the production of chocolates, cosmetics, health drinks, pharmaceuticals etc.

Cocoa sweatings, the pale yellowish liquid that drains off during cocoa fermentation, is the breakdown product of the mucilage surrounding the fresh cocoa bean, and constitutes about 10% of the weight of the cocoa fruit. This liquid has a sweet-sour flavor and is rich in sugars and bioactive compounds (Efraim *et al.,* 2010; Ouattara *et al.,* 2014). It has been found to be a suitable medium for the production of wine and alcohol. Its rapid collection in high yields and quality is the first step to its utilization on a commercial scale. The cocoa sweating is usually extracted by pressing the cocoa pulp.

The commercial utilization of cocoa sweating nonetheless will require the availability of high quality and hygienic pulp through an efficient extraction process. A mechanical method of extraction has been developed to hygienically extract cocoa pulp. The cocoa pulp extractor has been developed under AICRP on PHET to extract the cocoa pulp for the wine production. The cocoa pulp extractor could efficiently remove the pulp at 10, 20 and 30 percentage levels. The optimum concentration of pulp removal for efficient fermentation process was found to be 20 per cent.

The sweat water from the extracted cocoa pulp was collected and used for wine preparation. The quality parameters of wine were evaluated at 7 th and 30 th days of

storage. The alcohol content in wine was recorded as 12.82 % and 14.02 % at 30 th day of storage in wine prepared from pasteurized and un pasteurized cocoa sweating with 22°Bx and 5 ml yeast broth respectively. This level is under the acceptable limit of maximum permissible ethanol content accepted for fruit wines.

From this study, wine prepared with 22°Bx, 5 ml yeast broth and in an unpasteurized condition was found to be the best wine with alcohol content of 14.02 %. The quality parameters such as pH, TSS, ascorbic acid content and titratable acidity of wine were recorded as 3.14, 8.6°Bx, 12.72 mg/100 ml and 7.628 mg/100 ml .

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REFERENCE

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PRODUCTION AND QUALITY EVALUATION OF COCOA WINE

BY

ANJITHA BS (2018-06-005)

APARNA V M (2018-06-029)

KAVYA M (2018-06-013)

NAMITHA ASHOKAN (2018-06-015)

SREEDEV V S (2018-06-022)

ABSTRACT OF PROJECT REPORT

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Department of Processing and Food Engineering

KELAPPAJI COLLEGE OF AGRICUTURAL ENGINEERING AND TECHNOLOGY

TAVANUR-679573, MALAPPURAM

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ABSTRACT

The research paper depicts the production and quality evaluation of cocoa wine. Cocoa is an important plantation crop grown for chocolates manufactures around the world. Cocoa mucilage, a by product of cocoa bean processing, constitutes 10% of total cocoa beans. Cocoa sweatings, the pale yellowish liquid that drains off during cocoa fermentation is the breakdown product of the mucilage surrounding the fresh cocoa bean. The cocoa sweating from the extracted cocoa pulp was collected and used for wine production.

The aim of our project is to produce best quality wine and to optimize the conditions for producing good wine. The utilization of the cocoa mucilage which is mostly wasted during the cocoa processing for the production of wine helps to increase the market value of cocoa.

The matured cocoa fruits for wine production were procured from Cocoa Research Centre, KAU Vellanikkara. Mucilage collected from the pulp obtained after pod breaking was then transported to laboratory and used for the production of wine. The primary and secondary fermentation were carried out for 7 and 13 days respectively. Wine was being prepared by varying TSS, amount of yeast and in pasteurized and unpasteurized conditions. The physico chemical properties and microbial count were analysed.