BYPRODUCT UTILIZATION OF COFFEE CHERRY AS AN ALCOHOLIC BEVERAGE

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Abstract

Coffee pulp, mucilage and mucilage with beans of ripe coffee cherries (*Coffee Arabica* L) from the Bhirgaun, Dhankuta, Nepal were subjected to alcoholic fermentation with the objective to utilize the byproduct of coffee cherry as an alcoholic beverage. Coffee pulp, mucilage and beans with mucilage were used for developing the alcoholic beverages. 45.3 % pulp, 54.7% mucilage with seed and 9.4% mucilage only were obtained during the wet processing of coffee. Must were prepared for all to TSS 18 °Bx and fermentation was carried out for 6 to 8 days until TSS decreased to 5 °Bx at 30°C. Phenolic characteristics, chromatic structures, chemical parameters and sensory characteristics were analyzed for the prepared alcoholic beverages.

Alcoholic beverage from pulp was similar to commercial red wine where other two were similar to white wine. Among three preparations utilization of pulp is more feasible due to more yields. Methanol content, esters content, aldehyde, alcohol, total acidity, caffeine, polyphenols, flavonoids, chromatic structure and hue of the alcohol beverage from the pulp was 335 mg/L, 70.58 ppm, 9.16 ppm, 8.86 ABV%, 0.41%, 30.94 ppm, 845.7 mg GAE/g, 440.7 mg QE/g, 0.41 and 1.71 respectively. An alcoholic beverage from pulp was superior to commercial red wine and alcoholic beverage from mucilage with beans was superior to beverage from mucilage and commercial white wine in terms of taste, aftertaste and overall acceptability. All the chemical compounds in alcoholic beverage were within the range of standard wine paramaters. There is possibility of developing wine like beverage from pulp of coffee cherry and mucilage but further research is necessary for quality of the beans that were obtained from the fermentation with the mucilage.

Key words: Coffee, pulp, mucilage, mucilage with beans, alcoholic beverage

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List of abbreviations

Abbreviation	Full form
ADY	Active Dry Yeast
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
AR	Analytical Reagent
ATP	Adenosine triphosphate
ºBx	Degree Brix
BOD	Biological Oxygen Demands
ºC	Degree Centigrade
COD	Chemical oxygen demands
EMP	Embden-Meyerhof glycolytic Pathway
GDP	Gross Domestic Product
KG	Kilogram
LSD	Least Significance Difference
Masl	Meters above sea level
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
TSS	Total Soluble Solids

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Part I

Introduction

1.1 General introduction

Recently, the global demand for ethanol has been steadily increasing. The global ethanol production was about 90 billion Ls in 2013 and increased to 115 billion Ls in 2019 (Reidy, 2010). Sugarcane is the readily used plant for ethanol production. However, the demand for obtaining ethanol could not be achieved from sugarcane alone due to its cost and raw materials are restricted to areas with special soil for it (Yadira *et al.*, 2014). In order to meet the ethanol production demand, alternative materials should be explored to reduce the burden in sugarcane.

Coffee plant (*Coffea arabica* L), the source of coffee drink, is one of the most commonly consumed beverages in the world. It is the Sond most traded commodity after oil and due to the demand for this product, large amounts of wastes are generated (Yadira *et al.*, 2014). Coffee bean is naturally fruiting cherry mainly composed of hard dicotyledon seed covered by silver-skin, parchment, mucilage and pulp. Coffee pulp constitutes 29-50% of dry weight of cherry which is obtained during wet processing of coffee (Aristizabal Marulanda *et al.*, 2017; Roussos *et al.*, 1995). The covering materials are removed during processing (L. *et al.*, 2017). Pulp and mucilage, being relatively rich in sugars, are used for microbial growth. For example, *Aspergillus niger* was used for solid-state fermentation of coffee pulp (Antier *et al.*, 1993). *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Candida parapsilosis, Pichia caribbica, Pichia guilliermondii* and *Saccharomyces cerevisiae* were used as potential starter culture for enhancing the coffee fermentation process (Silva *et al.*, 2013).

Coffee pulp has been used for the extraction of caffeine, protein, pectic enzymes, fertilizers, biogas and coffee pulp molasses (Braham and Bressani, 1979; Silva *et al.*, 2013). Mucilage is rich in both simple and complex sugars which can be used in fermentation. Few studies have been conducted for producing ethanol from mixture of coffee pulp and mucilage. For example, the ethanol yield was reported to be equivalent to 77.29% of the theoretical yield (an ethanol yield of 25.44 kg/m^3, resulting from the 64.40 kg/m^3 of total sugars) from a mixture of coffee pulp and mucilage, commercial baker's yeast and panela

(Navia *et al.*), which showed that the production of ethanol is viable in small coffee farms using readily available raw materials. Orrego *et al*. (2018) achieved bioethanol yield of more than 90% of theoretical yield from coffee mucilage. However, alcoholic fermentation of by-products of coffee, such as coffee pulp and mucilage has rarely been studied. This study utilizes coffee wastes during processing, i.e. coffee pulp and mucilage for the production of alcohol.

Having high sugar content (2.6–31.26 g/L), the Ethiopian coffee pulp has found to produce 7.4 g/L ethanol (Kefale, 2020). It has been reported that the bio-ethanol yield was found to be 0.46 g/g of sugar in wet coffee pulp (Shenoy *et al.*, 2011). In addition, coffee pulp is also a good source of natural antioxidant and it contains hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acid) (Torres Mancera *et al.*, 2011). Coffee pulp is one of the food wastes which cause environmental problems. In order to reduce its environmental impacts, several types of researches are focused on the extraction of active ingredient and its utilization as animal feed or compost. Non-fermented drink known as *Kisher* is produced in Yemen and Somalia from ripe fruit berries. Coffee pulp is also used for the production of beverage called *cascara* (also called coffee cherry tea) due to its bioactive components (Heeger *et al.*, 2017). Mucilage and pulp from processed coffee were used in ethanol production in Rwanda (Hirwa *et al.*, 2016). It has been reported that dry white wine has been produced from coffee pulp in Central Americ (Clarke and Macrae, 1987). Even though it contains several beneficial compounds such as minerals, amino acids, polyphenol, and caffeine (Torres Mancera *et al.*, 2011), there are limited works on utilizing coffee pulps and mucilage human consumption. In addition, the alcohol produced from biological way by fermentation of sugars, can be a strong candidate for replacing fossil fuels. The advantages of biologically fermented alcohol over fossil fuels are: they are clean, renewable, have a more complete combustion and less waste (Breisha, 2010). Therefore, this study aims to utilize the pulp and mucilage from wet processing of coffee in preparing the alcoholic beverages like wine instead of leaving them as wastes.

1.2 Statement of the problem

Coffee is one of most consumed beverage in the world (Lenka *et al.*, 2017). Due to its processing, large amount of waste is produced from its byproduct that can directly and indirectly effect the environment. A natural way of mucilage removal, fermentation, can produce a large number of wastes in the environment. It can decrease dissolved oxygen in the water resources and can affect the aquatic plants. Pulp which is nonutilized part of the coffee cherry during its processing can also create environmental pollution. It is being one of the main cash crops in some of the hilly regions of Nepal. Its production is being increasing gradually in Nepal (NTCDB, 2075). On very large production, the byproduct also increases. This will affect the environment if those parts are not controlled or if not turned into some useful products. Some research has been carried out in Gulmi, Nepal in utilization of the pulp of coffee to produce alcoholic beverages. But appropriate technologies were not applied and analysis were not done (NARC) So the lead content and the methanol content were found to be out of range.

Most of the coffee nowadays is processed using the wet method with variations to lower water consumption and control over the drying process (semi-dry, natural pulping etc.). Nevertheless, relatively large amounts of biomass are still dumped once the coffee beans are separated from the coffee cherries. About 45% of the coffee cherry biomass is discarded. In other words: for every kilogram of roasted coffee that we consume, an equal amount of waste is left behind in the producing countries. This is a major problem of the coffee industry.

The pulp and process water used to separate the mucilage from the bean in the wet milling factories have a high BOD, which threatens water sources. Due to the high contents of organic acids, caffeine, catechins, and tannins, the coffee pulp and process water pose a serious environmental problem in the regions where production takes place. Furthermore, where the pulp is discarded in a landfill or other disposal site, rotting pulp may lead to unpleasant smells.

There has been research in the use of coffee pulp as feed for dairy cattle in Central and South Americas. Nevertheless, these practices use only a small percentage of the whole stream due to the anti-nutritional and toxic compounds in the biomass. There has been research in the use of coffee pulp as bioethanol (Orrego *et al.*, 2018). The production of

bioethanol from mucilage was performed both in shake flasks and 5 L bio-reactors. The highest yields of flasks and 5 L bio-reactors were 0.46 g ethanol/g sugars, and 0.47 g ethanol/g sugars after 12 h, respectively, which were equal to 90% and 94% of the theoretically achievable conversion yield of ethanol (Orrego *et al.*, 2018). Coffee byproducts can be blended with other compounds and hence can be a useful alternative to produce biofuels in the rural areas (Diana *et al.*, 2011). There are very few works on research of the coffee pulp byproduct utilization in Nepal. So, this study could be a life changing for the people who are economically involved in its production.

1.3 Objectives of the study

1.3.1 General objectives

The general objective of this study was to study utilizability of coffee cherry as an alcoholic beverage.

1.3.2 Specific objectives

The specific objectives of the study were as follows:

1. To carry out physio-chemical analysis of the coffee pulp and mucilage.
2. To prepare alcoholic beverage of pulp, mucilage and mucilage with seed.
3. To study the fermentation kinetics of prepared alcoholic beverages.
4. To carry out the sensory and chemical analysis of prepared alcoholic beverages.

1.4 Significance of the study

The proposed work will focus on the utilization of coffee cherry byproducts. This study will be helpful in developing wine like beverage from coffee pulp and mucilage. So, the environmental problems that is being created by its byproduct can be minimized. The idea can be helpful to every coffee processing industry. The work can reduce the cost of main product. Similarly, the data obtained from this research will be helpful for the other researchers, scientists and scholars who are carrying researches related to it.

In context of Nepal, the production of the coffee is increasing. People are attracted on this business day to day. Among the various cash crops for commercialization, coffee is

emerging as a likely agro-enterprise with great potential to provide farm employment and income generation opportunities in the mid hills of Nepal (CoPP, 2008). Some Districts like Gulmi, Palpa, Argakhanchi, Lalitpur, Tanahu, Kavre, Sindhupalchowk, Lamjung, Kaski, Gorkha, Syangja, Parbat, Baglung are successfully growing and producing coffee beans. So, if the byproducts of such grown production of coffee cherries are utilized then people will know more about the economic importance of the coffee farming. The value of the coffee cherries increases .

1.5 Limitations of the work

The limitations of the work were as follows:

1. The quality of the beans that were obtained from the fermentation with the mucilage was not examined.
2. Fermented beverages were not subjected to clarification.

1.6 Delimitation of the work

The delimitation of the work were as follows:

1. Coffee cherries were collected from single place.
2. Hand pulping was practised for pulping the cherry.
3. Bakers yeast was used for fermentation.
3. Commercial grape wines: Red wine and White wine were used to compare the sensory characteristics of prepared alcoholic beverages.

PART II

Lature review

2.1 Historical background of alcoholic beverage

Alcoholic beverages are believed to have originated in Egypt and Mesopotamia some 6000 years ago (Jones, 1985). Despite this early application of microbiology, the ability of microorganism to stimulate the biochemical changes was demonstrated several years later. Alcoholic fermentation was first identified by Gay Lussac in 1810, but at that time yeast was recognized as causative organism. Schwan in 1835 demonstrated that yeast could produce alcohol and carbon dioxide when introduced in sugar containing solution. He termed yeast *Zuckerpilz* meaning sugar-fungus, from which the name *Saccharomyces* originated (Prescott *et al.*, 1949). *Saccharomyces* group possesses almost all the credit of producing alcoholic beverages (Tannanhill, 1973).

As stated by Sir john Malcon in his first account of Persian that during the reign of King Jamshed viniculture flourished and it is he who is credit with the discovery of fermentation (Andrew, 1980). According to Finish Foundation of Alcohol Studies (1977), alcohol consumption increased rapidly after the Second World War in most of the European Countries. During the year 1991-1998, Italy was the top wine producing country in the world whereas France was Sond in number.

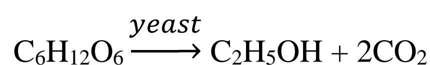
In Nepal, the technique of alcoholic beverage production remains largely traditional. The method has been practiced from very ancient time but the exact principle involved in the process is not yet known. However, it is widely in use and it is difficult to say when and from where the technology had come to Nepal. It is said that the custom of worshipping Gods and Goddess was by *Tantric* process and alcoholic beverage were offered during worship (Karki, 1986). In Nepal, the local producers don't have an idea about the broad dimensions of microbial biochemistry or their complex mechanisms. In fact actual nature of fermentation is still not fully known to them (Gubhaju, 2006).

2.2 Alcohol

The name "alcohol" came to Europe from the east. It is a word of Arabic origin and is composed of the article 'al' plus the term 'khul' (kahala= to stain), with the meaning powder, a term applied to fine powder used by eastern beauties for painting their eye brows and eyelashes.

There are many different kind of alcohol, but when the term is used loosely by wine makers, it applies to the potable alcohol called ethyl alcohol or ethanol, the common ingredients of alcoholic drinks of all type (Austin, 1968). Ethyl alcohol (C_2H_5OH) is completely miscible with water and form a constant point mixture with it at 96% alcohol by wt. It is clear, colorless, inflammable liquid with a density of 0.7939 at 100% alcohol or at 200 °proof. It is good solvent for essential oil, ester, tannins, various organic acids and certain other organic compounds .

Ethyl alcohol is produced when zymase acts in simple sugars (Austin, 1968). There are three main classes of alcoholic beverages wines, malted beverages and distilled liquors. The essential step in all the fermentation processes is the conversion of glucose into alcohol by yeast (Manay and Shadashawaswany, 1987). The intermediate products are methyl glyoxal (CH_3C: OCH: O), Acetaldehyde (CH_3CHO) and pyruvic acid (CH_3: OCOOH). Fig. 2.1 shows the breakdown of glucose by yeast to ethanol and carbondioxide.



$$C_6H_{12}O_6 \xrightarrow{yeast} C_2H_5OH + 2CO_2$$

Fig. 2.1 Breakdown of glucose by yeast to ethanol and carbondioxide

2.2.1 Alcoholic fermentation

Alcoholic fermentation is simply the production of alcohol by using carbon and nitrogen substrate (Kaushik and Yadav, 1997). Sugar and nitrogen compounds are the principal substrates for alcohol fermentation (Prescott *et al.*, 1949)**.** Fruit juices have the highest sugar concentration among the many substrates used for the production of ethanol by fermentation. The TSS in cherry wine decreased upto 8 days (Jia *et al.*, 2019). Here, the decrease in the TSS is due to the conversion of sugars into alcohol (Kasture and Kadam, 2018). The level of ethanol is among the highest seen and the importance of substrate and ethanol inhibition

of growth cell viability and maintenance activity is far greater than those of brewing or other fermented beverages. The wine fermentation is a good example of a fermentation in which there are major contributions due to both cell growth and the resting phase maintenance activity. In much white wine fermentation, the growth of cell mass has essentially ceased at a point at which one-half to one-third of the sugar remains. The remainder of the fermentation is conducted by the maintenance or turnover activity of non-growing but viable and fermenting cells (Boulton, 1998).

According to Butnariu and Butu (2019), Alcoholic fermentation of the must is a spontaneous or induced biochemical oxidoreduction process by which, under the action of yeast enzymes, carbohydrates convert to ethyl alcohol and CO_2 as the main products accompanied by several by-products. This process is exothermic, and for achieving one degree of alcohol between 15.7 and 18 g of sugar/L must be used. The decomposition of carbohydrates into alcohol and CO_2 takes place inside yeast cells. The sugar solution penetrates the cells membrane, and the resulting products (alcohol, CO_2, etc.) are diffused in the environment. The process is determined by yeast activity as they contain the enzymes needed to carry out the fermentation. Under the action of the yeast enzyme complex, the sugar in the must turns into a phosphoglyceral aldehyde, after absorption into the cells and formation of the phosphoric esters. The enzyme classes of yeast include: oxidoreductases, hydrolase, transferases, lysases, isomerases, ligases, and synthases. In the fermentation process the following enzymes are involved: hexokinase, aldolase, dehydrogenase, phosphohexoisomerase, phosphohexokinase, triose isomerase, pyruvate kinase, pyruvate decarboxylase, aldohydrogenase, etc. Enzymes interfere in the fermentation process in successive stages, acting specifically, through their active components, coenzymes (Butnariu and Butu, 2019). Many co-enzymes also take part in alcoholic fermentation (Butnariu and Butu, 2019). By oxidation-reduction reactions and quantified release of potential energy, 2 mol of ATP, one mole of triose, and the main products of fermentation are formed. These include CO_2, obtained by decarboxylation of pyruvic acid and ethyl alcohol, obtained by reduction of acetic aldehyde in the presence of dehydrogenase.

2.2.2 Yeasts

A yeast is a unicellular fungus which reproduces asexually by budding or division, especially the genus *Saccharomyces* which is important in food fermentations (Walker, 1988). In the tropics, *Saccharomyces pombe* is the dominant yeast in the production of traditional fermented beverages, especially those derived from maize and millet (Adams and Moss, 1995)

Yeasts and yeast-like fungi are widely distributed in nature. They are present in orchards and vineyards, in the air, the soil and the intestinal tract of animals. Like bacteria and molds, they can have beneficial and non-beneficial effects in foods. Most yeasts are larger than most bacteria. The most well-known examples of yeast fermentation are in the production of alcoholic drinks and the leavening of bread. For their participation in these two processes, yeasts are of major importance in the food industry (Battcock and Azam-Ali, 1998).

Some yeasts are chromogenic and produce a variety of pigments, including green, yellow and black. Others are capable of synthesizing essential B group vitamins.

Although there is a large diversity of yeasts and yeast-like fungi, (about 500 species), only a few are commonly associated with the production of fermented foods. They are all either ascomycetous yeasts or members of the genus *Candida*. Varieties of the *Saccharomyces cerevisiae* genus are the most common yeasts in fermented foods and beverages based on fruit and vegetables. All strains of this genus ferment glucose and many ferment other plant derived carbohydrates such as sucrose, maltose and raffinose. In the tropics, *Saccharomyces pombe* is the dominant yeast in the production of traditional fermented beverages, especially those derived from maize and millet (Adams and Moss, 1995).

Yeast play an important role in wine fermentations, which can strongly affect the quality and flavor of final product (Querol and Fleet, 2006). Among several yeast, *Saccharomyces cerevisiae* and *Saccharomyces bayans Var.Uvarum* are the most important species present during fermentation process (Pretorius, 2000; Querol and Fleet, 2006).

2.2.3 Biochemistry of alcohol fermentation by yeast

The organism uses EMP pathway, generating 2 ATP per mole of glucose converted to ethanol, plus CO_2. Ethanol, which is the end product, is primary metabolite. In an industrial fermentation, the basic strategy is to maintain Crabtree effect during the fermentation. Fig. 2.2 shows the simplified pathway of alcohol synthesis by yeast.

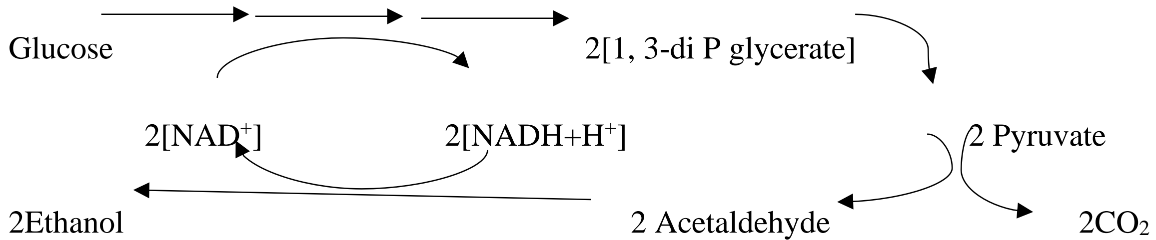


Fig. 2.2 Simplified pathway of alcohol synthesis by yeast

2.2.4 Conditions necessary for fermentation

According to Battcock and Azam-Ali (1998) Yeasts can ferment sugars to alcohol and carbon dioxide in the absence of air but require oxygen for growth. They produce ethyl alcohol and carbon dioxide from simple sugars such as glucose and fructose. In conditions of excess oxygen (and in the presence of acetobacter) the alcohol can be oxidized to form acetic acid. Yeasts can grow in a pH range of 4 to 4.5 (Mountney and Gould, 1988). As describes in beer fermentation, pH value is decreased up to two days of fermentation and then it remains constant until the complete fermentation (Guymon *et al.*, 1961). Normal yeasts require a minimum water activity of 0.85 or a relative humidity of 88%. Yeasts are fairly tolerant of high concentrations of sugar and grow well in solutions containing 40% sugar. There are only a few yeasts that can tolerate sugar concentrations of 65-70% and these grow very slowly in these conditions (Board, 1983). Similarly, temperature plays important role on fermentation. Above 38ºC the yeast will certainly be killed; at low temperature it will ferment only very slowly (Berry, 1996). For white wine the temperature is 10-15ºC and that for the red wine is 20-30ºC. There is possibility of stuck fermentation if it is carried at higher temperature. On the other hand, low temperature may delay onset of fermentation. At high temperature, the loss of alcohol and aroma substance takes place. In general temperature of primary fermentation should be 20ºC, temperature of Sondary fermentation should be 15 ºC and finish wine storage temperature should be 10ºC (Berry, 1996).

It should be noted that it is not solely sugars that impact on yeast fermentation performance. Yeasts also require appropriate supplies of other major, minor and trace nutrients, together with water, in order to efficiently carry out fermentation. Most S. cerevisiae strains can grow if supplied with glucose, ammonium salts, inorganic ions and a few growth factors. Macronutrients need to be supplied at millimolar concentrations, and these comprise sources of carbon (i.e., sugars), free amino nitrogen (amino acids, small peptides and ammonium salts), oxygen, Sulphur, phosphorus, potassium and magnesium. Micronutrients are only needed by yeast at micromolar concentrations, and they comprise trace elements such as calcium, copper, iron, manganese and zinc (Walker, 2016).

2.3 Fruit Wines

Fruit wines are undistilled alcoholic beverages usually made from grapes or other fruits such as peaches, plums or apricots, banana, elderberry or black current etc. which are nutritive, more tasty and mild stimulants. These fruits undergo a period of fermentation and ageing. They usually have an alcohol content ranging between 5 to 13%. Wines made from fruits are often named after the fruits. No other drinks, except water and milk have earned such universal acceptance and esteem throughout the ages as has wine. Wine is a food with a flavor like fresh fruit which could be stored and transported under the existing conditions. Being fruit based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of amino acids and other nutrients from yeast during fermentation. Fruit wines contain 8 to 11 % alcohol and 2 to 3% sugar with energy value ranging between 70 and 90 kcal per 100 ml. A typical wine contains ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, minor constituents like flavoring compounds etc. (Amerine *et al.*, 1980).

2.3.1 Compounds in fruit wines

I Aldehyde and ketones

Acetaldehyde constitutes more than 90% of the total aldehyde content of wines, occurring at 50–100 mg/L Nykanen and Suomalainen (1983). The aldehyde content is, however, low, and this may be explained by the fact that the Sulphur dioxide added to wine reacts with

aldehydes to form α-hydroxysulphonic acids, which reduce the free aldehyde content. Furthermore, aldehydes can be chemically bound to ethanol and higher alcohols as acetals. Minor amounts of other aliphatic aldehydes and ketones are also present in wine. According to Osborne *et al.* (2006) aldehyde enhances the color of red wine by assisting the polymerization of anthocyanins and phenolics.

Two vicinal diketones, 2,3-butanedione and 2,3-pentanedione, may be of importance to the flavor nuances, although they occur at low levels (IARC, 1988). High levels of acetaldehyde are undesirable, but at low levels in wine acetaldehyde gives a pleasant, fruity aroma (Jaarsveld and Francois, 2015). Their higher concentration gives undesirable green, grassy, nutty and apple like aroma. But this is found to be desirable if their concentration is high for some drink like sherry (E and RM, 2010). High levels of acetaldehyde also increase the wine color as it binds the bleaching agent like sulphur dioxide. Although the higher concentration of acetaldehyde can pose several health related problems, no legal limits for concentration of acetaldehyde in wines are currently imposed (Salaspuro, 2011).

II Polyphenols

According to Niculescu *et al.* (2018) Polyphenols play an important role in the quality of wines, due to their contribution to the wine sensory properties: color, astringency and bitterness. They act as antioxidants, having positive role in human health. They can be divided into non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavanols). Anthocyanins are responsible for the color of red grapes and wines, hydroxycinnamic and hydroxybenzoic acids act as copigments, stilbenes as antioxidants and the flavan-3-ols are mainly responsible for the astringency, bitterness and structure of wines, being involved also in the color stabilization during aging.

III Other alcohols and phenols

Apart from ethanol, glycerol and 2,3-butanediol are the principal alcohols in wine. The glycerol content has been reported to range between 2000 and 36000 mg/L in sound wines (Nykanen and Suomalainen, 1983)

Besides phenolic alcohols, aldehydes and acids, red wines contain small amounts of phenol, *m*-cresol, guaiacol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol, eugenol and 2,6-dimethoxyphenol.

IV Carboxylic acid

Acetic acid is the most abundant of the volatile acidic constituents of wine, although yeast is known to produce only minor amounts of acetic acid in fermentation under anaerobic conditions (IARC, 1988).

The largest group of flavor compounds in wines consists of esters of the aliphatic monocarboxylic acids. Esters are formed due to the reaction between the fatty acids and alcohol (Saerens *et al.*, 2007). Ethyl acetate and many of the long-chain esters in wine are formed by yeast principally by enzymic reactions during fermentation and not in chemical reactions between ethanol and corresponding acids (Nykanen and Suomalainen, 1983). Ethyl acetate is the principal ester component. Postel *et al.* (1972) found 44–122 mg/L ethyl acetate in white win Amines are probably formed mainly by bacterial decarboxylation of amino acids, but small amounts may also occur as the result of enzymic reactions of yeasts and 78–257 mg/L in red wines (IARC, 1988). The level of volatile acidity in wine is 0.19 g/L to 0.41 g/L (Martinez *et al.*, 1998).

V Methanol

IARC (1988) found methanol is not a by-product of yeast fermentation but originates from pectins in the must and juice when grapes and fruits are macerated. In general, the methanol content of commercial alcoholic beverages is fairly small, except in those produced from grapes in prolonged contact with pectin esterase and in some brandies produced from stone fruits, such as cherries and plums. The methanol level in red wine that can be accepted by human body is 400 mg/L (Hodson *et al.*, 2017).

VI Caffeine

Caffeine (1,3,7- trimethyl xanthine) is a natural alkaloid found in tea leaves, cocoa beans, coffee beans, cola nuts and other plants. It is a psychostimulant chemical which is mostly consumed in the world. According to Reyes and Cornelis (2018) rigorous reviews of caffeine

toxicity conclude that consumption of up to 400 mg caffeine/day in healthy adults is not associated with adverse effects. According to Canada (2008), the amount of caffeine in alcoholic drink is 0 mg/L. But the standard level of caffeine in some refreshing beverages like coke is 26 mg/250 ml. The balanced consumption of caffeine is very beneficial to health like improving memory and speed up reaction times as studied by radiological society of North America in 2005. But if it is consumed more than the limit it creates nervousness, irritability , insomnia to sensory disturbances, diuresis, gastrointestinal disturbances, elevated respiration and dysfunctions of liver and renal system as well (Nawrot *et al.*, 2003). Some Lature has mentioned that combining alcohol and caffeine may mask some of the alcohol's depressant effects.

VII Higher alcohols

(IARC, 1988) found higher alcohols and fusel alcohols (1-propanol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol and phenylethyl alcohol) are formed in biochemical reactions by yeast on amino acids and carbohydrates.

VIII Trace elements

Trace elements from grapes are transferred during crushing into the must and eventually into wine (Eschnauer, 1982). The total concentration of mineral constituents in wine may be as high as 1000 mg/L and more . In most wines, the iron content varies from 1 to 5 mg/L and copper from 0.1 to 1 mg/L (IARC, 1988). The natural lead content of German wines has been reported to be 0.01–0.03 mg/L, and the average chromium content is 0.065 mg/L (IARC, 1988).

2.4 Coffee Cherry

Coffee is a plant belonging to the family *Rubiaceae*, genus *coffee.* Its seeds are called coffee beans and can be processed into drinks. Although coffee lifespan may extend up to 100 years, its most productive years are from 5 to 25 and can equally grow from 5 to 25 m tall National Coffee Association (USDA, 2018).

Coffee originated from Ethiopia and then spread across to Egypt, Yemen and Italy and all over Europe. Coffee has continuously gained a place in the world. Globally, coffee is the

Sond largest most traded commodity after petroleum. It is mostly produced in the tropics and consumed in the temperate region. There are two commercially explored species of coffee plants, including *Coffee arabica* (Arabica) and Coffee canephora (Robusta), which account for 75% and 25% respectively, of the world's coffee production (Mussatto *et al.*, 2011). But according to Von Enden *et al.* (2002), three species of coffee dominate the international market thus; Arabica (*Coffee arabica*) 70% has the highest quality in terms of taste and aroma, Robusta (*Coffee canephora*) 28% has the highest caffeine content and *Coffee liberica* 2%.

2.4.1 Anatomy of coffee cherry

Fig. 2.3 shows the transverse and longitudinal Sections of Coffee berry (fruit)

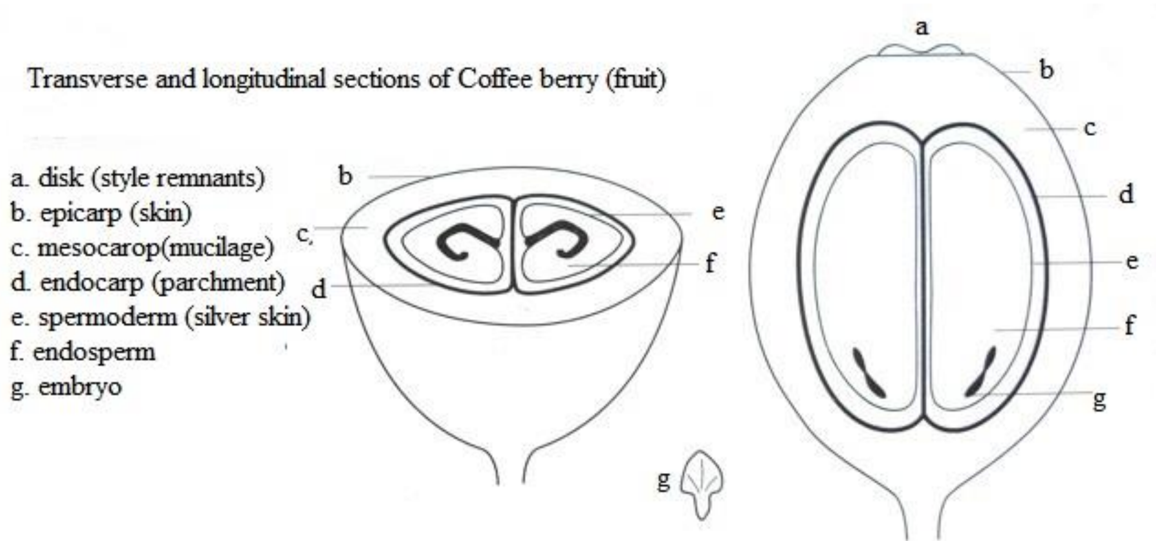


Fig. 2.3 Transverse and longitudinal sections of coffee berry (fruit) (Wintgens, 2009).

The coffee fruit can be divided into two main parts, the pericarp and the seed.

I. Pericarp

The pericarp is the outer three layers of the fruit: the pulp (skin), mesocarp (mucilage), and endocarp (parchment).

II. Pulp (Skin)

The pulp, also referred to as the peel, skin, or epicarp, is the outermost layer of the coffee fruit. It is formed by a single layer of compact parenchyma cells (cells with thin primary

walls that contain chloroplasts and are capable of absorbing water). The color of the pulp at the beginning of fruit development is green due to the presence of chloroplasts which then disappear as the fruit matures (Castro and Marraccini, Jan- Mar 2006). Color upon maturation depends upon coffee variety, but is most commonly red or yellow. Red skin color comes from anthocyanin pigments, while yellow skin color is attributed to luteolin (Borem, 2008).

Coffee pulp is the first product obtained during processing, and it represents around 43 % of the weight of the coffee fruit on a fresh weight basis, or about 29% of the weight of the whole berry (Braham and Bressani, 1979). The amount of coffee pulp and husk produced for a single ton of fresh coffee is 0.5 and 0.18 ton respectively (Roussos *et al.*, 1995). The dried material has about l0% crude protein, 21% crude fiber, 8% ash, and 44% nitrogen-free extract (Braham and Bressani, 1979). Pulp and husk are rich in carbohydrates, mineral and proteins, however they also contain organic compounds such as tannins, chlorogenic acid and caffeine (Fan *et al.*, 2003). The astringent aftertaste giving compound is tannin (Ashok and Upadhyaya, 2012).

It is also interesting to note that the chemical composition of the fermented and dehydrated coffee pulp is very similar to dehydrated and nonfermented coffee pulp. Other investigators Aguirre (1966) has reported similar values for the protein content of dehydrated coffee pulp, although variations from 9.2 to 11.2% have also been reported (Aguirre 1966, Bressani *et al* .1972). Variations in the carbohydrate fraction are also found in the Lature; for example, an average value of 46% was given by Jaffe and Ortiz (1952) whereas Aguirre (1966) suggested values of 57.8-66.1% on dry weight basis. Fat content seems to be less variable (Jaffe and Ortiz, 1952) with values ranging from 2.3 to 2.5% on a dry-weight basis. According to Setyobudi *et al.* (2018), fat content was 1.8% in coffee pulp and its pH was 4.25 (Ameca *et al.*, 2018). It should be expected, however, that these values will change according to coffee variety, location, and agricultural practices. Caffeine has been reported by Jaffe and Ortiz (1952) to be 0.51% on a dry weight basis; whereas, others have given values of 1.3% (Molina *et al.*, 1974), also on a dry-weight basis. Regarding tannin content, the following levels have been found: 4.5% (Aguirre, 1966);1.44% (Jaffe and Ortiz, 1952) and 2.4% (Molina *et al.*, 1974) .Chlorogenic and caffeic acids have been reported to be 2. 7 I and 0.31% and (Molina *et al.*, 1974) gave values of 2.6 and 1.6% for the same components.

According to Sera *et al.* (2013) coffee fragrance and flavor is related to chlorogenic acids. The main constituents of the carbohydrate fractions on a dry-weight basis have been reported to be: cellulose 27 .65%; reducing sugars as glucose 12.40%; nonreducing sugars 2.02%; and total pectic substances 6.52% (Wilbaux, 1956). Fractionation of the cellular wall and structural polysaccharides of coffee pulp by the Van Soest method indicates a cellular content of 63%, suggesting that the material has a relatively high level of nutrients. Coffee-pulp protein has similar or higher levels of amino acids than other products such as cottonseed and soybean flours. Likewise, coffee pulp generally has higher concentrations of amino acids than corn (Braham and Bressani, 1979). The composition of coffee pulp is shown in the Table 2.1

Table 2.1 Composition of coffee pulp

Parameters (%)	Values
Moisture	76.7%
Crude Fiber	3.4%
Crude Protein	2.1%
Ash	1.5%
Nitrogen free extract	15.8%
Tannins	7.8% dry wt.
Pectic substances	6.5% dry wt.
Non reducing sugars	2.0% dry wt.
Reducing sugars	12.4% dry wt.
Chlorogenic acids	2.6% dry wt.
Caffeine	1.3% dry wt.
Total Caffeic acid	1.6% dry wt.

Source: Braham and Bressani (1979)

III. Mucilage

Mucilage, which is located between the coffee pulp and the coffee hulls and represents about 5% of the dry weight of the coffee berries (Bressani *et al.*, 1972). Mucilage constitutes a layer approximately 0.5-2 mm thick that is strongly attached to the coffee hulls. From the physical point of view, it is a colloidal liquid system, and being a hydrogel, it is lyophilic. Chemically the mucilage contains water, pectins, sugars, and organic acids. During maturation of the coffee berry, calcium pectate located in the middle lamella and protopectin

from the cellular wall are converted into pectins. This transformation or hydrolysis of the protopectins results in a disintegration of the cellular wall, leaving the cellular plasma free. Besides pectins, this plasma contains sugars and organic acids derived from the metabolism and conversion of starches into sugars (Carbonell and Vilanova, 1974). Studies have shown that the mucilage/water ratio of the mesocarp increases as growing altitude increases (Borem, 2008). Total pectic substances run as high as 39% with an average value of 35.8%. Most of the total sugar content is in the reducing form . The chemical composition of the mucilage has also been reported as being: 84.2% water; 8.9% protein; 4.1% sugar; 0. 91% pectic acid; and 0.7% ash (Belitz, 2009). This fraction apparently has neither tannins nor caffeine, but contains pectin-degrading enzymes that so far have not been well identified (Aguirre, 1966). The composition of mucilage is shown in Table 2.2.

Table 2.2 Composition of mucilage

Parameters (%)	Values
Water	84.2%
Protein	8.9%
Glucose (reducing sugar)	2.5%
Sucrose (non-reducing sugar)	1.6%
Pectin	1.0%
Ash	0.7%

Source: Clifford and Wilson (1985)

IV. Endocarp (Coffee hull)

The endocarp, or parchment, is the innermost layer of the pericarp and is the hull that envelops the coffee bean. It is formed of three to seven layers of sclerenchyma cells (fibrous cells that serve as the principal support cells in plants). The cells of the endocarp harden during coffee fruit maturation, thus limiting the final size of the coffee seed, or bean. In arabica coffee, the average weight of the parchment with 11% moisture content is around 3.8% of total coffee fruit weight (Wilbaux, 1961, as cited in Borém, 2008. Coffee hulls

enclose the coffee beans and represent about 12% of the berry on a dry-weight basis . Crude fiber is significantly higher in coffee hulls (Braham and Bressani, 1979).

V. Seed

The coffee seed, or bean, comprises a silver skin, an endosperm, and an embryo. Coffee seed (bean) sizes vary; however, they average 10 mm long and 6 mm wide.

VI. Silver skin

The silver skin, also called the perisperm or spermoderm, is the outermost layer that wraps the seed. It is formed from the nucellus, or central portion, of the ovule. Generally, some remnants of the silver skin remain on the bean pre-roast, and come off during coffee roasting as chaff. The silver skin may be polished off of the bean; however, it is generally accepted that this diminishes coffee flavor. It has also been proposed that the presence of a large amount of silver skin on milled coffee is a sign of coffee picked before its ideal ripeness. In some regions the silver skin may take on a darker hue, in which case the beans are called fox beans. Fox beans are not considered to be a defect.

VII. Endosperm

The endosperm is the principal reserve tissue of the seed, and is composed of only one tissue, though the cells in the exterior and interior portion of the endosperm vary in oil content and cell wall thickness. The chemical content of the endosperm is of utmost importance since it is the precursor to the flavor and aroma of roasted coffee. The chemical compounds found in the endosperm can be classified as soluble or insoluble in water. The water-soluble compounds are caffeine, trigonelline, nicotinic acid (niacin), at least 18 chlorogenic acids, mono-, di-, and oligosaccharides, some proteins and minerals, and carboxylic acids. Components insoluble in water include cellulose, polysaccharides, lignin, and hemicellulose, as well as some proteins, minerals, and lipids (Borem, 2008).

VIII. Embryo

The embryo is composed of a hypocotyl (embryo axis) and two cotyledons and is 3-4 mm long (Wintgens, 2009). Coffee seeds germinate via epigeal germination, in which the hypocotyl elongates and pushes the seed upward above ground. The original cotyledons stay underground; however, new cotyledons will form.

2.4.2 Important chemical components in Coffee cherry for alcoholic beverages

I. Caffeine

Caffeine is a stimulant alkaloid found in aerial parts of many hot beverages, including coffee and tea. Due to its health impact, quantification of caffeine level in coffee is of paramount importance for consumers and traders, as well (Asfew and Dekebo, 2019). According to (Asfew and Dekebo, 2019), the percentage masses of caffeine (w/w %) in the original coffee samples were 1.30 ± 0.11% for beans, 0.90 ± 0.11% for pulp and 0.65 ± 0.10% for leaves. The melting point of caffeine extracted from coffee beans and tea leaves was found to be 238°C (Pradeep *et al.*, 2015).

II. Polyphenols

Polyphenols are Secondary metabolites that are produced by plants to protect themselves against plant diseases , but they play important roles in human health in protecting against a number of diseases related to oxidative stress and free radical-induced damage (Vladimir Knezevic *et al.*, 2012). According to Geremu *et al.* (2016), the amount of total polyphenols recovered from different coffee pulp extracts varied from 1809.9 to 489.5 mg GAE/g. Coffee pulp from Ababuna exhibited the highest polyphenol (1809.9 mg GAE/g) content when its cherry pulp was extracted with 80% methanol. However, the lowest (489.5 mg GAE/g) value was obtained from a cherry pulp of 741 extracted with 80% ethanol.

The coffee cherry has also a good antioxidant property. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity for cell damage (Geremu *et al.*, 2016). These anti-oxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell *et al.*, 1995). The methanol extract from the hybrid of Ababuna showed the highest antioxidant activity (70.2 %), which is in line with its high polyphenol content. This shows the potency of the extract from the hybrid for use as a good source of antioxidants.

Thus, Coffee cherry pulp can be used as an alternative source to extract polyphenols and antioxidant compounds for the benefit of human health (Geremu *et al.*, 2016).

III. Anthocyanins

Coffee pulp is the waste produced during the pulping operation of the coffee berries. Coffee pulp is reported as a good candidate of material for food colorant production. The food pigment found in coffee pulp is anthocyanin. The anthocyanin content in coffee pulp is reported up to 25 mg of monomeric anthocyanins/100 g of fresh pulp on a dry weight basis (Hartati *et al.*, 2012)

2.5 Coffee processing

The coffee process begins with removal of the external components of the coffee cherry, either through dry or wet methods, leaving only green coffee beans. Coffee cherry consists of smooth tough outer skin or pulp; soft yellowish pulp or outer mesocarp; and greyish-green fibrous endocarp (parchment) surrounding seeds (Purseglove, 1974). Pulping removes the pulp and the fleshy outer mesocarp leaving a thin viscous, highly hydrated layer, the inner mesocarp, also called mucilage. In order to facilitate drying and hulling, beans must be fermented naturally in tanks for the mucilage to be removed. There are still conflicting views as assumptions to whether the mucilage is degraded by the natural microflora and/or by endogenous enzymes. Conversely, there is general agreement that cell-wall pectic polysaccharides are degraded during the fermentation. In order to better control the wet fermentation process, a thorough knowledge of potential substrates for microbial and plant enzymes, in particular the polysaccharides, is required. There is presence of pectins in coffee mucilage (Coleman *et al.*, 1955). Furthermore, the reported degrees of methyl esterification of mucilage pectins seem to be very low (Garcia *et al.*, 1991).

After harvesting, the coffee fruits are separated from the pulp, which is carried out by dry or wet processing (Clarke and Macrae, 1987; Illy and Viani, 1995) .The dry process is simple and inexpensive. The whole cherries are dried under the sun in open air, followed by the separation of the hull (dried pulp and parchment) for getting the green beans. On the contrary, the wet process requires more care and investment, but results in a superior coffee quality. In the wet process, the pulp of the coffee cherries, which is made up of pulp and mesocarp, is removed mechanically, but the parchment remains attached to the beans. After drying either under the sun or in a dryer, the parchment is removed to produce the green coffee beans (Ghosh and Venkatachalapathy, 2014)

2.5.1 Dry processing

According to Ghosh and Venkatachalapathy (2014), this is the simpler of the two methods and is popular in Brazil to process Robusta coffee and in Sri Lanka to process Arabica coffee. In case of dry processing coffee cherries are dried as such in the open yard until the moisture content reduce up-to 12% (wt.). In this case one of the most important processing is racking. The greater number of racking gives a uniform and better quality product. The coffee cherries are dried immediately after harvest. This is usually sun drying on a clean dry floor or on mats. The bed depth should be less than 40 mm and the cherries should be raked frequently to prevent fermentation or discoloration. However, there are problems associated with this method. The most serious problem is dust and dirt blown onto the produce. Another problem is rainstorms often appear (even in the dry season) with very little warning, this can soak the produce very quickly. Finally, laborer has to be employed to prevent damage or theft. Sun drying is therefore not recommended.

I. Hulling

The dried cherry is then hulled to remove the pericarp. This can be done by hand using a pestle and mortar or in a mechanical huller. The mechanical hullers usually consist of a steel screw, the pitch of which increases as it approaches the outlet so removing the pericarp (Ghosh and Venkatachalapathy, 2014)

II. Cleaning

The hulled coffee is cleaned by winnowing. Defective beans are also removed. Sorting takes place both in the producing and manufacturing countries to achieve high quality coffee beans, and is carried out by either mechanical or optical means. In the mechanical method, defective beans are hand-picked and fed into air classifiers (catadors) where they enter an adjustable rising current of air (Ghosh and Venkatachalapathy, 2014).

2.5.2 Wet processing

In this method the cherry is squeezed in a pulping machine or pestle and mortar which removes the outer fleshy material (mesocarp and pulp) leaving bean covered in mucilage. This mucilage is fermented and dispersed. The bean is washed and dried. Pulping involves

the removal of the outer red skin (pulp) and the white fleshy pulp (mesocarp) and the separation of the pulp and beans. Immature cherries are hard and green and very difficult to pulp. If the coffee is to be wet processed, correct harvesting is essential. For small-scale units, the cherries can be pulped in a pestle and mortar, this is very labor intensive. The two most common pulpers and most suitable for small-scale units are the drum and the disc pulpers.

I. Drum pulpers

This involves a rotating drum with a punched sheet surface and adjustable breast plate between which the coffee cherries are pulped, the pulp and the beans separated. The distance between the drum and the breast plate has to be adjusted so that the pulp is removed without the beans being damaged. These can be manually operated or attached to a treadle or bicycle. For larger scale units, motorized drum pulpers are available (Ghosh and Venkatachalapathy, 2014)

II. Disc pulpers

The same concept is involved with the disc pulper. The only difference is that rather than the cherries being squeezed between a breast plate and a drum, a disc with a roughened surface is used (Ghosh and Venkatachalapathy, 2014).

2.5.2.1 Mucilage removal

Fermentation of the mucilage takes place in large tanks over 24 to 40 h. However, the fermentation procedure which results in the best quality, as well as allowing a reasonably convenient and rapid factory routine is the two-stage 'dry' fermentation process (Wootton, 1971)

During the first stage the mucilage is degraded and during the Sond stage it is soaked in water for 24 to 48 h. The amorphous gel of mucilage around the bean consists of hemicelluloses, pectic substances and sugars and is insoluble in water. This can be removed by chemical methods, warm water or by an 'aqua pulper'. However, for small-scale units the only feasible method is fermentation. Fermentation involves the beans being placed in plastic buckets or tanks and left until the mucilage has been broken down. Natural enzymes in the mucilage and feasts; bacteria in the environment work together to break down the mucilage.

The coffee should be stirred occasionally and a handful of beans should be tested by washing them in water.

If the mucilage can be washed off and the beans feel gritty rather than slippery, the beans are ready. The beans should then be washed immediately as 'off' flavors develop quickly. After fermentation, the coffee is known as 'parchment coffee', since the seed retains its endocarp layer. It must be dried to about 10-12% (wt.) moisture content to ensure stability.

2.5.2.2 Drying

To prevent cracking the coffee beans should be dried slowly to 10% moisture content (wet basis). Drying should take place immediately after to prevent 'off' flavors developing. The same drying methods can be used for this as for the dry processed coffee (Ghosh and Venkatachalapathy, 2014)

2.5.2.3 Hulling

After drying the coffee should be rested for 8 h in a well-ventilated place. The thin parchment around the coffee is removed either by hand, in a pestle and mortar or in a small huller.

2.5.2.4 Cleaning

The hulled coffee is cleaned by winnowing.

2.6 Coffee processing in Nepal

According to Tiwari (2010) In Nepal generally two methods of processing are practiced namely, dry and wet processing. However presently, most of coffee is wet processed in which farmers harvest ripe fresh cherries and sell to the pulping centers and then the cherries are pulped, fermented, washed and dried to produce dry parchment at the pulping center. Dry parchment is collected by processor/traders and hulled at the central processing unit to produce green beans (Shrestha *et al.*, 2009) and the green beans are exported. But the quality of coffee in wet processing rely on operational processes in the pulping center and their management quality of available water used in pulping center, typed of pulping machine, fermentation duration, facilities available for drying, washing process, storage etc. (CoPP, 2008)

2.7 Coffee production in Nepal.

Coffee is one of the important cash crops in mid hills of Nepal (Aoki and Suvedi, 2012; Chaudhary *et al.*, 2008; Poudel *et al.*, 2009; Tiwari, 2010). It is reported that the coffee farming in Nepal was first introduced into Aanpchaur of Gulmi District in Nepal (Paudyal, 2012). The Nepalese coffee has also been able to create market demand abroad (Aoki and Suvedi, 2012; Dahal and Dhakal, 2009; Khanna, 2016) as well in the country probably because of aroma, taste, flavor and healthy and suitable environmental condition where these coffees are produce from hilly slopes. The productivity of Nepalese coffee has been estimated only to be 800 kg/ha. Currently coffee is cultivated in around 40 districts but commercial cultivation is done in around 20-22 hilly districts covering of Mid, Western and Eastern Development Regions of Nepal (MoAD, 2014).

In Kavre, the mean yield of fresh cherry is 1027.20 kg/ha, while it is 1849.36 kg/ha in lalitpur (Karki,2018). In Nepal, mostly the coffee producers grow the coffee in small scale with 100-150 plants (Tiwari, 2010). Productivity is comparatively low then other countries because in Nepal most of the farmers don't use chemical inputs while cultivating the coffee (Tiwari, 2010). Though organic coffee has higher demand in the international market. But from government side established mechanism has not developed yet for promoting the organic certification (Tiwari, 2010). Only small fraction of coffee is sold as certified organic coffee in the international market.

2.8 Marketing of coffee in Nepal

Among the different agricultural products produced and exported from Nepal, coffee is growing as a competitive one with 7.3% share of country's total of 15% agricultural export share (FAOSTAT/World Bank, 2006). Quality obligatory for agricultural products set by these countries are very high that Nepal in many situations may fails to meet these criteria (Tiwari, 2010). In the world market, the demand of organic and highland coffee is high. Due to climatic peculiarity of mid hills of Nepal and thus the coffee produced in these regions have great scope in international markets.

At present, more than 65% of Nepalese coffee is exported especially to Japan, Europe and USA in the form of parchment by the coffee mills and 35% of the total product is

processed and supplied in the domestic market. Nepal exports only super quality green bean to overseas markets. Medium and low-quality green beans are roasted, grinded and sold in the domestic markets. Coffee contributes about 0.04% to GDP of Nepal (Anon., 2004). It provides 5 times more yield than that of maize and millets and 2-3 times more yield than that of any other cash crops (NTCDB, 2075) .

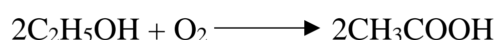
2.9 Coffee waste water

The environmental impact of wet and semi-wet processing is considerable. Problems occur through large amounts of effluents disposed into watercourses heavily loaded with organic matter rather than its inherent toxicity. Providing the self-purification of the watercourse is exceeded, the microbial degradation reduces the level of oxygen to anaerobic conditions under which no higher aquatic life is possible (Von Enden *et al.*, 2002). Waste water from mechanical mucilage removers (following the pulping) contains a certain amount of sugars (disaccharide carbohydrates), but its apparent gel like texture comes from the segments of undigested mucilage and pectic substances which have been removed from the parchment by mechanical means. In order to be biodegraded, the solid materials have to be fermented, acidified and hydrolyzed by natural fermentation in a later stage (Von Enden *et al.*, 2002). During fermentation and acidification of sugars in the waste water, pectin oligo-saccharides get out of solution and float on the surface of the waste water. The remaining highly resistant materials left in the effluent water are acids and flavonoid color compounds from coffee cherries. At around pH 7 and over, flavonoids turn waste water into dark green to black color staining rivers downstream from coffee factories. However, flavonoids do not do any harm to the environment nor add significantly to the BOD or COD (Von Enden *et al.*, 2002).Values for BOD indicating the amount of oxygen needed to break down organic matter are high in coffee waste. Resistant organic materials which can only be broken down by chemical means indicated by the COD make up around 80% of the pollution load. The material making up the high COD can be taken out of the water as precipitated mucilage solids. Other substances to be found in small amounts in coffee waste water are toxic chemicals like tannins, alkaloids (caffeine) and polyphenolics. However, these toxic substances mainly staying the disposed solids of the coffee pulp (Von Enden *et al.*, 2002).

During the fermentation process in the effluents from pulpers, fermentation tanks and mechanical mucilage removers, sugars will ferment in the presence of yeasts to alcohol and CO_2. However, in this situation the alcohol is quickly converted to vinegar or acetic acid in the fermented pulping water. The simplified chemical formula for biological fermentation of 6 carbon sugars by yeasts to ethanol is typified by the fructose to ethanol reaction (Padmapriya, 2013).

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Ethanol is quickly broken down by bacteria into acetic acids. This complex enzymatic catalyzed reaction is simplified as (CRUZ, 2014).



$$2C_2H_5OH + O_2 \longrightarrow 2CH_3COOH$$

The acidification of sugars will drop the pH to around 4, and the digested mucilage will be precipitated out of solution and will build a thick crust on the surface of the waste water, black on top and slimy orange/brown in color underneath. If not separated from the waste water, this crust will quickly clog up waterways and further contribute to anaerobic conditions in the waterways (Von Enden *et al.*, 2002). Coffee waste waters are high in organic loadings and exhibit a high acidity. When washed or semi washed coffee is processed in large quantities, untreated effluents greatly exceed the self-purification capacity of natural waterways. The crude fiber content was found higher than industrial waste pulp in Kenya (Gautho *et al.*, 1991) but was less than pulp obtained by semi-washed process in Brazil. In order to overcome the pollution potential of processing waste waters, a clear understanding of waste water constitution in inevitable to design a feasible treatment system. Especially when expanding wet coffee processing or setting up new large scale processing operations, treatment of waste waters needs to be considered (Von Enden *et al.*, 2002).

2.10 Coffee as a nutrient source for yeast

These days the coffee pulps are being used for making a coffee wine by fermenting the coffee pulp (Haile and Kang, 2019). Since the coffee pulp and mucilage contains the nutrients which is used as a food by *Saccharomyces* cerevisiae. The pulp contains reducing sugars as glucose 12.40%; non-reducing sugars 2.02% as dry basis as mentioned above. Similarly, it has high concentration of amino acid than in corn. This makes pulp as an additional source

of nutrient for yeast. Similarly, the mucilage contains the 8.9% protein, 4.1% sugar on wet basis. So, this also makes mucilage as a suitable source of nutrient for yeasts. The main constituents of the carbohydrate fractions on a dry-weight basis have been reported to be: cellulose 27 .65%; reducing sugars as glucose 12.40%; non-reducing sugars 2.02%; and total pectic substances 6.52% (Wilbaux, 1956).

2.11 Health aspects of caffeine and alcohol

Numerous brands of alcohol/caffeine combination drinks have been produced, which contain high levels of caffeine and alcohol. In 2010, the US FDA issued warnings to several manufacturers of combination drinks identifying caffeine as an 'unsafe food additive' and stated that their sale violated federal law. There are also restrictions on the production and sale of caffeinated alcohol beverages in some countries, including Canada, where caffeine can only be mixed with alcohol if it comes from a natural source. Current European legislation (European Directive 2002/67/EC on the labeling of foodstuffs containing caffeine) rules that beverages containing upward of 150 mg/L (other than tea or coffee) must be marked as high caffeine content (Attwood, 2012). When caffeine or caffeine-containing energy drinks are mixed with alcohol, locomotor activity is increased more than alcohol alone. Alcohol mixed with energy drinks (or alcohol and caffeine) results in enhanced stimulation (in humans and animals), decreased perceived intoxication (in humans), and increaed desire to drink or actual drinking (in humans and animals) when compared to alcohol alone. These observations suggest that the combination of alcohol and energy drinks is riskier than alcohol alone. Caffeine was an unsafe food additive when combined with alcohol (Marczinski and Fillmore, 2014). Similarly, Caffeine and alcohol consumption both increase the risk of osteoporotic fractures in middle-aged women (Avila *et al.*, 1991). Despite the large range of caffeine doses (2.0–5.5 mg/kg resulting in absolute levels of 46–383 mg) and alcohol levels 0.29–1.068 g/kg (resulting in blood alcohol concentration (BAC) from 0.032 to 0.12%) investigated, caffeine had no effect on the judgement of subjective intoxication (Benson *et al.*, 2014).

Part III

Materials and methods

3.1 Raw materials collection

Ripe coffee cherries (*Coffee arabica* L) were bought from the Bhirgaun, Dhankuta, Nepal (27º01'12.8"N 87º21'48.3"E, elevation of 1269.0 masl). Sugar and wine yeast *Saccharomyces cereviseae* was obtained from the lab of Central Campus of Technology, Dharan, Nepal.

3.1.2 Chemicals required

AR grade chemicals and distilled water were used throughout the work for the experiments. The chemicals used are Sodium Hydroxide (HIMEDIA- GRM1183, Assay 97.00-103.50%), Oxalic acid (Qualigens, Assay 99.5%), Carbon tetrachloride, Folin- Ciocalteau, Potassium permanganate, Methanol, Ethanol, Sodium bisulphite, Gallic acid, Iodine, Oxalic acid, Sulphuric acid, sodium thiosulphate, Carbon tetrachloride, Caffeine standard, Chromotropic acid, Hydrochloric acid.

3.1.3 Equipments required

The euipments used in the experiment are spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO- 291), Weighing balance (AMPUT Electronic Balance Model No-457, Sensitivity±0.01 g), Oven, Incubator, Kjeldahl digestion set, Conical flask, pH meter, Separating funnel, Test tubes, Pycnometer, Bottles, Water bath (Intake Serological Water Bath), Thermometer, Refractometer, Fermentation glass jar, Cork.

3.2 Methodology

The total work was based on the preparation of alcoholic beverages of three different byproducts of coffee cherry i.e. Pulp, mucilage and mucilage with seeds. The different fermented product coffee pulp and mucilage were coded: A for mucilage with seed, D for pulp and E for mucilage extract and extract of raw pulp is coded as R. Similarly, commercial wines were used for comparing the prepared beverages, Red wine and White wine and coded as B and C respectively.

3.2.1 Preparation of must

3.2.1.1 Pulp

Ten kg of cherries were weighed by the weighing balance situated in wet lab of Central Campus of Technology. The cherries were cleaned and washed properly. Degraded coffee was rejected manually by floatation technique as it was the quick and effective method. Generally, the coffee cherries are pulped by the demucilager machine and several such machines have been developed. But the machines were unavailable in the Central Campus of Technology. So, all the cherries were pulped manually by hand in hygienic condition. The weight of the pulp obtained from the cherries was 4.3 kg. It was kept inside the fermenting glass jar. Little amount of juicy part was attached to the pulp. The TSS of the pulp was found to be 16 ºBx. The distilled water was added to it till the water nearly covers all the pulp so that mashup can be easier where TSS decreased to 4 ºBx. The TSS was increased to 18 ºBx. by adding sugar. The addition of sugar is known as chaptalization. (Kumoro *et al.*, 2012) have also explained about the addition of sugar in the fruit juice to acquire the desire TSS in the preparation of fruit wines. The calculation was done according to Rai and Subba (2016). The must was stirred properly the pH of the must was measured by using the pH meter.

3.2.1.2 Mucilage extract

The mucilage with the beans after removing pulp was taken in the hygienic buckets. The weight of the beans with mucilage taken was 2.85 kg. The TSS of the mucilage attached with the beans was measured with the hand refractometer. It was also found to be 16 ºBx. Distilled water at 20ºC was added until TSS reaches 4 °Bx. It was rapidly rubbed with the hand by wearing rubber gloves for 5 mins allowing the juice to get extracted from the beans. The beans were separated from the juice by muslin cloth. The TSS after the mucilage extraction was measured. It was found to be 4 ºBx. The sugar was added to increase the TSS to 18 ºBx.

3.2.1.3 Mucilage with seeds

About 2.85 kg of beans with mucilage was taken in the clean buckets. Now Distilled water at 20 ºC was added to the beans until TSS reaches 4 ºBx. It was also mixed properly by

agitating with the sterilized glass rod. The TSS was found to be 4 ºBx. The sugar was added to the mix to raise the TSS to 16 ºBx.

3.2.2 Pasteurization of juice

All the three different types of must were pasteurized at 70 to 72ºC for 15 min and bought to room temperature by cooling in tap water.

3.2.3 Starter preparation and fermentation

Pitch of sufficient quantity was developed before preparation of mash in the medium having low concentration. Pitching was done when the culture of the pitch was at its optimum stage of growth. The prepared yeast was inoculated with the wine's yeast at the rate of 0.25 gram dry yeast per L of must (Rai and Subba, 2016) by activating in the must at 37 warm condition and was filled into sterile translucent jar up to $2/3^{rd}$ of their capacity. The bottles were then plugged by cotton, agitated and kept for fermentation at incubator until the TSS reach 5 ºBx.

3.2.4 Fermentation

After two days of pitching, when the vigorous evolution of CO_2 ceased, the necks of jars were closed tightly with corks. It was necessary to create a strict anaerobic condition inside the bottles during fermentation for improving the quality of product. The progress of the fermentation for each sample were analyzed by monitoring the drop in TSS. The TSS of the fermented must were observed every two successive days from the initial day of pitching. After the TSS reached 5 ºBx, the fermentation was ceased for each of the sample. Similarly, Change in pH pattern was also studied during fermentation.

3.2.5 Addition of sucrose

Again, Table sugar was added to each sample as a flavoring agent. The sugar was added to make the TSS to 10 ºBx for each sample (Rai and Subba, 2016)

3.2.6 Racking, chaptalization and bottling

3.2.6.1 Pulp

The fermented juice was first separated from the solid residue of pulp with the help of muslin cloth in a separate clean vessel. Sugar was added as a flavoring agent. The sugar was added to make the TSS to 10 ºBx. The fermented juice was allowed to settle for 2 h in the vessel. The clear fermented juice was siphoned from the lees in clean and sterilized wine bottles using a sterilized polyethylene pipe.

3.2.6.2 Mucilage extract

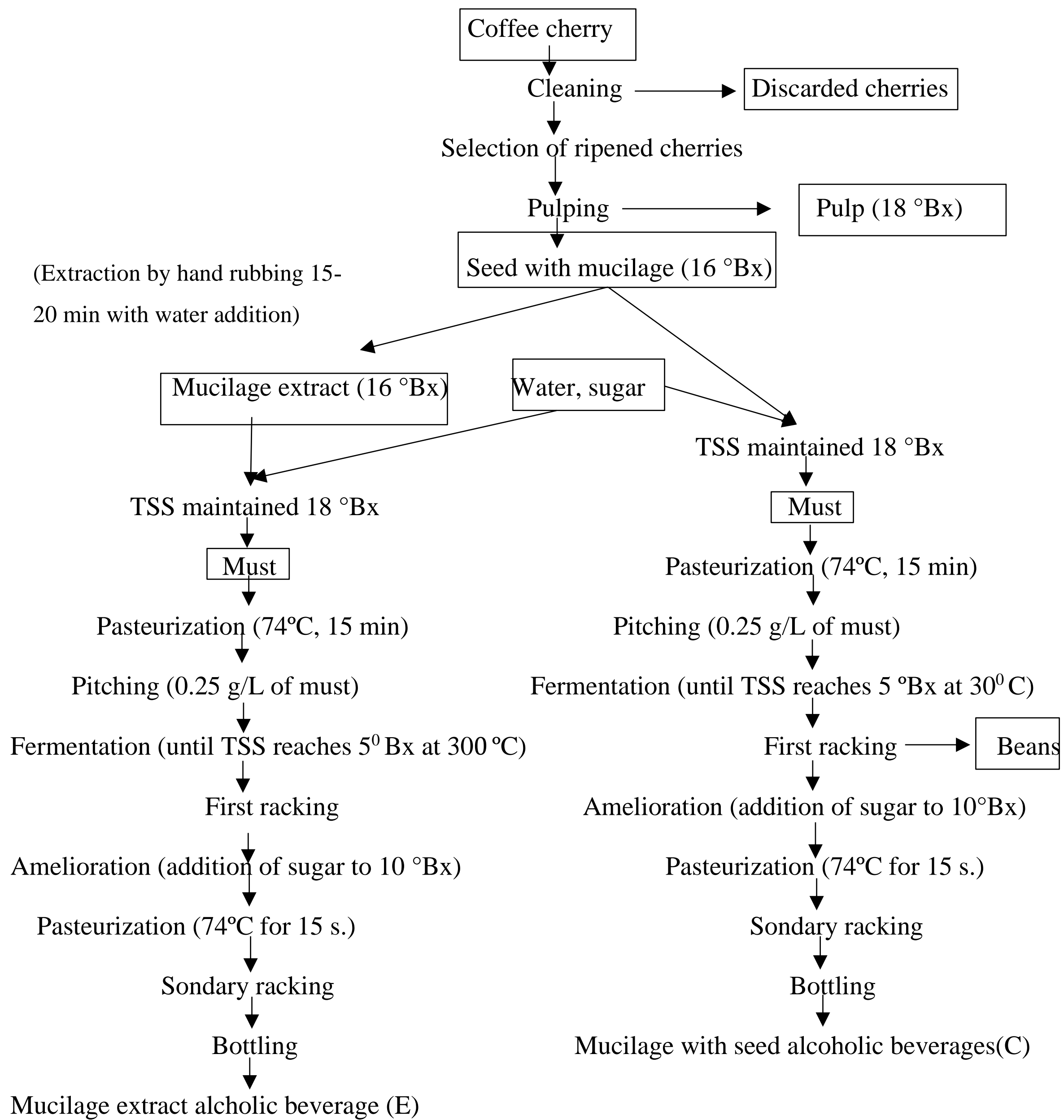
Table sugar was added to fermented alcoholic beverage make the TSS to 10 ºBx for adding the sweet flavor to the product. The beverage was allowed to settle for about 2 h. Now it was also siphoned to clean and sterilized bottles by using sterilized polyethylene pipe.

3.2.6.3 Mucilage with seeds

At first the beans were separated from the fermented mass by using the muslin cloth to a clean vessel. Table sugar was added to it to make the TSS to 10 ºBx. for adding flavor to the product. The beverage was allowed to settle for about 2 h. Now it was also siphoned to clean and sterilized bottles by using polyethylene pipe.

3.2.7 Pasteurization

Pasteurization was done in bottle at 74℃ for 15 s of holding time (Anon., 2016). Fig. 2.4 shows the general flow chart for the alcoholic beverages of coffee cherries


Coffee cherry
Cleaning
Discarded cherries
Selection of ripened cherries
Pulping
Pulp (18 °Bx)
Seed with mucilage (16 °Bx)
(Extraction by hand rubbing 15-20 min with water addition)
Mucilage extract (16 °Bx)
Water, sugar
TSS maintained 18 °Bx
Must
Pasteurization (74ºC, 15 min)
Pitching (0.25 g/L of must)
Fermentation (until TSS reaches 5⁰ Bx at 300 ºC)
First racking
Amelioration (addition of sugar to 10 °Bx)
Pasteurization (74ºC for 15 s.)
Sondary racking
Bottling
Mucilage extract alcholic beverage (E)
TSS maintained 18 °Bx
Must
Pasteurization (74ºC, 15 min)
Pitching (0.25 g/L of must)
Fermentation (until TSS reaches 5 ºBx at 30⁰ C)
First racking
Beans
Amelioration (addition of sugar to 10°Bx)
Pasteurization (74ºC for 15 s.)
Sondary racking
Bottling
Mucilage with seed alcoholic beverages(C)

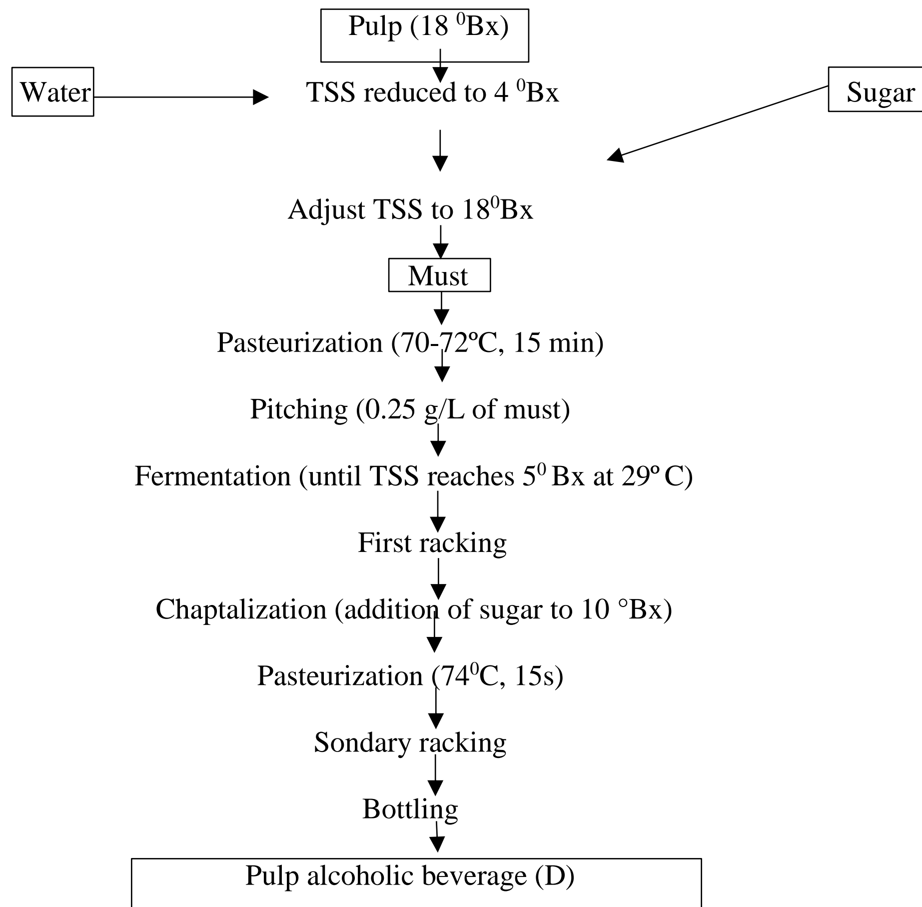


Fig. 2.4 General flow chart for the alcoholic beverages of coffee cherries

3.3 Analytical methods

The quality of the pulp and alcoholic beverages of the pulp, mucilage and mucilage with extract were analyzed for chemical composition and properties (TSS and alcohol content) and sensory analysis (appearance, aroma, taste, aftertaste, color and overall acceptance).The different parameters viz., alcohol by volume (%), aldehydes, esters, acidity, volatile acidity, methanol, caffeine, polyphenols of different alcoholic beverages were analyzed.

3.3.1 Analysis of raw materials

3.3.1.1 Determinations of total soluble solid (TSS) and pH

The TSS of the raw material i.e. coffee cherry was analyzed by Hand refractometer (Model WYT-32, Zhongyou Optical Instruments) and pH was measured by pH meter (Japsin Industrial Instrumentation).

3.3.1.2 Reducing sugar and total sugar

It was determined using the method of AOAC (2005).

3.3.1.3 Crude protein

The protein content in the pulp of coffee cherry was determined using the method of K.C and Rai (2007).

3.3.1.4 Ash content

It was determined using the method of K.C and Rai (2007).

3.3.1.5 Moisture content

It was determined using the method of Roussos *et al.* (1995).

3.3.1.6 Fat

It was determined using the method of K.C and Rai (2007).

3.3.1.7 Ash

It was determined using the method of K.C and Rai (2007).

3.3.1.8 Caffeine

It was determined using the method of Amos *et al.* (2014)

3.3.1.9 Moisture

It was determined using the method of K.C and Rai (2007).

3.3.1.10 Polyphenols

It was determined using the method of Geremu *et al.* (2016).

3.3.1.10.1 Experimental materials and sample preparation

For the extraction of polyphenols, 80% methanol was used. Ten gram of coffee pulp was mixed with 100 ml of the solvent and ground for 3 min in a homogenizer and transferred to conical flask. The ground samples were extracted using the maceration technique by soaking the samples in the solvents for 24 h at room temperature, followed by filtration using Whatman No. 1 filter paper. The filtered extract was used to determine the total polyphenol content. About 500 ml of sample was allowed to dry to know the dry mass.

3.3.1.10.2 Determination of total polyphenol content

Total polyphenol content (TPC) of coffee pulp extract was determined using the Folin–Ciocalteu method. The intensity of blue color reflects the quantity of polyphenol compounds, which can be measured using a spectrophotometer. Gallic acid was used as a standard and the total polyphenols were expressed as mg/g gallic acid equivalents (GAE) from the calibration curve (R^2= 0.9993) using gallic acid. Gallic acid (0.5 g) was accurately weighed into a 10 ml volumetric flask, dissolved in 10 ml absolute methanol and the solution was made up to 100 ml with 80% of the same solvent. For standard curve 0, 1, 2, 3, 4 and 5 ml of standard solutions were added into a 100 ml flask and diluted to give 0, 50, 100, 150, 400 and 500 mg/L of gallic acid. Then, 0.5 ml of each sample was introduced into test tubes and mixed with 2.5 ml of a tenfold dilute Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with aluminum foil and allowed to stand for 30 min at room temperature before the absorbance was read at 765 nm using UV/Vis spectrophotometer (T80, China). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

i. Calculations

Finally, total polyphenols were calculated using equation;

The Total Polyphenol Content (mg GAE/ g) = $C \times V/m$

Where; C= Concentration of polyphenols in mg/ml

V= Volume of sample taken for dilution in ml

m= Dry mass of sample in V ml.

3.3.2 Analysis of alcoholic beverage

3.3.2.1 TSS

The TSS of all the fermented beverages were analyzed by hand refractometer (Model WYT-32, Zhongyou Optical Instruments) and pH was measured by pH meter (digital, portable, japsin Industrial Instrumentation).

3.3.2.2 Alcohol determination

Alcohol content was determined according to FSSAI (2012). By using specific gravity chart and the values were expressed in percentage (v/v).

3.3.2.3 Methanol determination

Methanol in each sample was determined by chromotropic acid colorometric method as per (AOAC, 2005). Briefly, 2 ml of $KMnO_4$ solution (3 g $KMnO_4$ dissolved in a mixture of 15ml H_3PO_4 and 85 ml of distilled water) was pipetted into a 50 ml volumetric flask, chilled in ice bath. 1 ml of distillate sample was added to the flask and stand for 30 min in ice bath. The excess of $KMnO_4$ solution decolorized with 2% sodium sulphite solution and 1ml of chromotropic acid solution (% aqueous solution) was added. Then 15 ml of 70℃ for 15 min and cooled. The volume was made up to 50 ml, and the absorbance was read at 575 nm against a reagent blank containing 5.5% ethanol treated similarly. Standard methanol solution (0.025% by volume in 5.5% ethanol) was also treated simultaneously in the same manner, and the absorbance recorded. Methanol content in the wine was calculated as follows:

Methanol content (%, v/v) = Sample absorbance × 0.025/ Standard absorbance

3.3.2.4 Ester content

Esters in each sample was determined by titrimetric method as per method of (FSSAI, 2012). Briefly, 200 ml of wine was taken for distillation and 50 ml of distillate was collected. Then it was neutralized with 0.1 N NaOH. Further 5 ml excess 0.1 N NaOH was added and reflux for 1 h. It was cooled and back titrated the unspent alkali against 0.1 N Sulphuric acid carry out blank simultaneously taking 50 ml of distilled water. The difference in titer value in milliL of standard sulphuric acid gives equivalent ester. The values were expressed in gram per 100 L of ethyl alcohol as ethyl acetate.

Ester express as ethyl acetate= $(V\times 0.0088 \times 100 \times 1000 \times 2)/ V_1$ g/100 L of abs. alcohol

Where, V = Difference of titer value of std. H_2SO_4 used for blank and sample in ml

V_1 = Alcohol % by volume

3.3.2.5 Aldehyde content

Aldehyde was determined according to method of (FSSAI, 2012). Briefly, 50 ml of distillate (from specific gravity determination) was taken in 250 ml iodine flask 10 ml of sodium bisulphite (0.05 N) solution was taken then after flask was kept in dark place for 30 minute with occasional shaking, 25 ml of standard iodine solution (0.05 N) was added and back titrated excess iodine against standard sodium thiosulphate solution (0.05 N) using starch indicator (1 %) to light green end point. Following same procedure blank sample was carried out using 50 ml distilled water. The difference in titer value in ml of sodium thiosulphate gives equivalent aldehyde content. The values were expressed in gram per 100 L of absolute alcohol as acetaldehyde.

Aldehydes expressed acetaldehyde = $V\times 0.0011 \times 100 \times 1000 \times 2/V_1$ g/100 L of abs. alcohol

V= Difference in titer of blank and sample in ml of sodium thiosulphate solution

V_1 = Alcohol percentage by volume

3.3.2.6 Acidity determination

Total acidity was determined according to FSSAI (2012). Volatile acidity was determined according to FSSAI (2012).

3.3.2.7 Polyphenols

It was determined using the method of Geremu *et al.* (2016).

3.3.2.7.1 Experimental materials and sample preparation

Determination of total polyphenol content:

Total polyphenol content (TPC) of coffee pulp extract was determined using the Folin–Ciocalteu method. The intensity of blue color reflects the quantity of polyphenol compounds, which can be measured using a spectrophotometer. Gallic acid was used as a standard and the total polyphenols were expressed as mg/g gallic acid equivalents (GAE) from the calibration curve (R^2= 0.9993) using gallic acid. Gallic acid (0.5 g) was accurately weighed into a 10 ml volumetric flask, dissolved in 10 ml absolute methanol and the solution was made up to 100 ml with 80% of the same solvent. For standard curve 0, 1, 2, 3, 4 and 5 ml of standard solutions were added into a 100 ml flask and diluted to give 0, 50, 100, 150, 400 and 500 mg/L of gallic acid.Now, 100 ml of each sample of alcoholic beverage was taken and allowed to dry. The dry mass of sample after evaporation was taken for each sample. Then, 0.5 ml of each sample was introduced into test tubes and mixed with 2.5 ml of a ten fold dilute Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with aluminum foil and allowed to stand for 30 min at room temperature before the absorbance was read at 765 nm using UV/Vis spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO- 291). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Calculations

Finally, total polyphenols were calculated using equation;

The Total Polyphenol Content (mg GAE/ g) = $C \times V/m$

Where; C= Concentration of polyphenols in mg/ml

V= Volume of sample taken for dilution in ml

m= Dry mass of sample in V ml.

3.3.2.8 Caffeine

The caffeine content in each sample was determined by the method of Amos *et al.* (2014).

Preparation of caffeine standard solutions:

A 100 ppm stock standard of caffeine was prepared by dissolving 25 mg of caffeine in 250 ml purified carbon tetrachloride (CCl_4) in a volumetric flask (250 ml). Working standards were prepared by pipetting 10, 20, 30, 40 and 50 ml, respectively aliquots of the stock standard solution into separate volumetric flasks (100 ml) and diluting to volume with purified carbon tetrachloride to produce concentrations of 10, 20, 30, 40 and 50 mg/L, respectively standard solution. The absorbance of each solution was measured at absorption maximum of 270 nm using 10 mm quartz cuvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve. Caffeine extraction procedure: An aliquot (5 ml) of the drink sample was drawn with a 10 ml pipette and placed into a 125 ml separating funnel followed by the addition of distilled water (10 ml), then 20% aqueous Na_2CO_3 solution (1 ml) and analytical grade CCl_4 (20 ml). The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous CCl_4 layer was removed to a clean 50 ml volumetric flask. Another 20 ml portion of CCl_4 was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the CCl_4 solvent layers combined. This volume was made up to 50 ml with the solvent. This procedure was repeated for all the drink samples. The absorbance of the resulting solutions was then measured on UV/Vis spectrophotometer at 270 nm using 10 mm quartz cuvette.

Quantitative caffeine determination:

Quantitative analysis of caffeine was performed by Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO- 291). The λ max was determined by scanning the standard solution from 200-60 nm and the obtained results gave an absorption spectrum, which was characterized by a single intensive absorption band located in the UV range at λ max = 270 nm. Standard linear calibration curve was run to obtain the linear range of sample

analysis, correlation factor was with accepted value = (0.996) and the standard calibration curve was linear over the range (10-60) ppm caffeine with equation ($y = 0.035x + 0.100$). The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve.

3.3.2.9 Total flavonoids content (TFC)

Total flavonoids content were determined using a modified aluminium chloride ($AlCl_3$) assay method (Lusia Barek *et al.*, 2015). Briefly, 2 ml of extract solution was taken in a test tube. Then, 0.2 ml of 5% $NaNO_3$ was added and allowed to stand for 5 min. Later, 0.2 ml of 10% $AlCl_3$ was added and mixed properly and allowed to stand for 5 min. After this, 2 ml of 1 N sodium hydroxide (NaOH) was added in the tube and the final volume was adjusted to 5 ml by adding distilled water. The absorbance was measured after 15 min at 510 nm. The test result was correlated with standard curve of quercetin (20, 40, 60, 80, 100 µg/ml) and the total flavonoids contents were expressed as mg of the quercetin equivalent per gram (mg QE/g) of dry matter in extract.

3.3.2.10 Color determination

It was determined using (Kathryn *et al.*, 2015). All 5 samples were taken in cuvette. Each samples absorbance was recorded in three different wavelengths i.e. 420, 520, 620 nm. The triplicate reading for each sample were taken and its wine color intensity and wine hue was calculated by using the following equations.

Wine Color Intensity = $A_{420} + A_{520} + A_{620}$

Wine Hue = A_{420} / A_{520}

3.4. Sensory analysis

Sensory analysis was evaluated with reference to wine (Fessler, 1988) by twelve panelists and converting scores of quality parameters in percentage for total quality score of 100. Sensory parameters were analyzed with quality score of 15%, 30%, 30%, 15% and 10% for appearance, aroma, taste, aftertaste and overall acceptability respectively. A 2 h training session was conducted for 4 days to familiarize panel members with sensory attributes.

3.5 Statistical analysis

The data were analyzed for ANOVA by using GenStat program (GenStat Discovery Edition 3 2011) and paired t test was performed at 5% level of significance. The means were separated using Tukey's HSD post hoc test ($P < 0.05$).

Part IV

Results and discussion

Coffee pulp, mucilage and mucilage with beans of ripe coffee cherries were subjected to alcoholic fermentation and coded as D, E and A respectively. Commercial red and white wines were used to compare the sensory attributes and coded as B and C for red and white wine respectively.

4.1 Chemical composition of coffee pulp and mucilage

Chemical composition of the coffee pulp was analyzed (Table 4.1). Protein content for coffee pulp was similar to findings of but slightly different in caffeine content and reducing sugar. The caffeine content was found to be less which might be due to variation of caffeine extracting solvent. Similarly, reducing sugar was slightly different which might be due to the difference in harvesting time and geography. In contrary, ash content is slightly less in which might be due to the difference in geography, harvesting time and variation of processing technology (Haile and Kang, 2019) . The crude fiber content was found higher than industrial waste pulp in Kenya (Gautho *et al.*, 1991) but was less than pulp obtained by semi-washed process in Brazil . The fat content was similar to the findings of Setyobudi *et al.* (2018) and similar pH value was obtained in the study in Mexico (Ameca *et al.*, 2018). Crude fat, crude fiber caffeine content and TSS of the mucilage were found to be 0.7%, 1.5%, 1.05% and 15°Bx respectively. The data of Belitz *et al.* (2009) were similar to our findings (Table 1). The result of analysis of coffee pulp and mucilage is shown in Table 4.1

Table 4.1 shows the result of analysis of coffee pulp and mucilage

Particulars	Coffee pulp	Mucilage
Moisture (%)	75.7±0.2	85.3 ± 0.6
Dry matter (%)	24.3 ± 0.2	14.7 ± 0.6
Crude Protein (%)	8.1±0.36	7.2 ± 0.3
Fat (%)	1.53±0.05	0.7 ±0.00
Ash (%)	6.4±0.05	1.1 ± 0.1
Crude fiber (%)	6.3 ± 0.2	1.5 ± 0.22
Total sugar (%)	12.06±0.41	4.3 ± 0.4
Reducing sugar (%)	10.9±0.36	-
Caffeine (%)	1.11±0.11	1.05 ± 0.05
TSS (ºBx)	18±0.5	15 ± 0.5
pH	4.3±0.15	3.7± 0.1
Polyphenols (mg GAE/g)	1205.2 ± 4.42	618.32 ± 3.2
Flavonoid (mg QE/g)	697.3 ± 2.1	531.54 ± 2.7

Values are means of triplicate ± standard deviations.

4.2 Changes in pH during fermentation

Fig. 4.1 shows the change in pH during fermentation

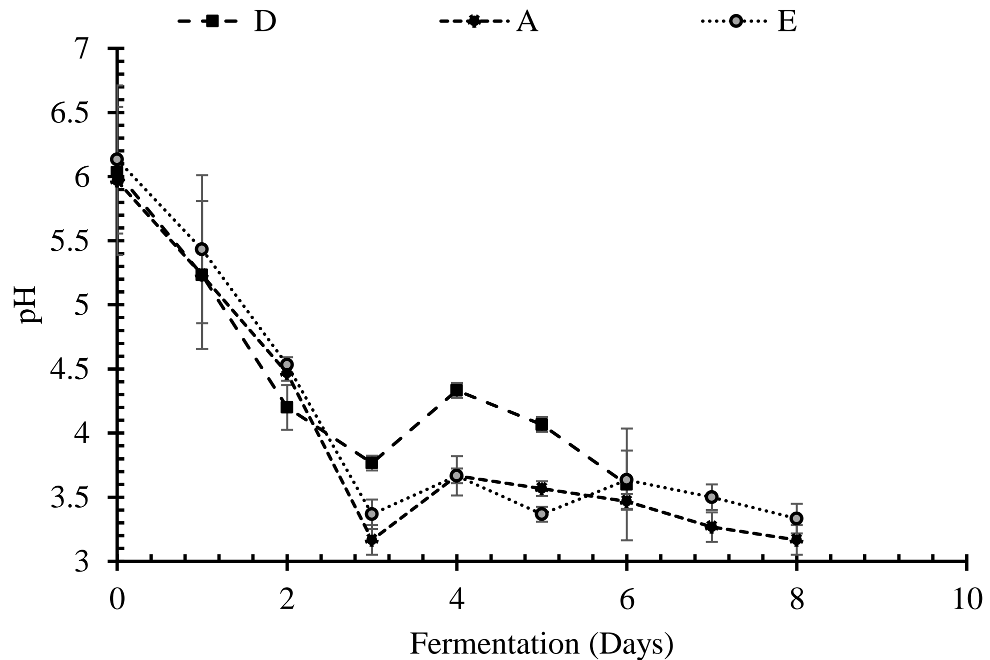


Fig. 4.1 Change in pH during fermentation

Vertical error bars represent ± standard deviations.

During the course of fermentation, pH was statistically analyzed. From appendix B.1, it was seen that, there was significant difference (P<0.05) in pH initially between the samples. It was also seen that there was no significant difference in pH between the sample E and A at days 3 and 4. There was significant difference in pH between samples D with others two samples in each day. But it was seen that at day 6, there was no significant difference between the samples. The fall of pH up to day 2 was seen maximum in sample D than other two sample. This shows faster conversion of sugar into carboxylic acids in pulp.

Similarly, ANOVA result (Appendix B.1) shows that there was significant difference (P<0.05) in pH value among fermentation days. As describes in beer fermentation, pH value is decreased up to two days of fermentation and then it remains constant until the complete fermentation (Guymon *et al.*, 1961). Hence it can be concluded that the first phase of graph is similar with that beer fermentation. The reason to decrease of the pH was that there was

always decrease in pH at sugar metabolism except in alkaline fermentation where protein metabolism occurs. Likewise, increase in pH at second phase of the graph was due to utilization of acid compounds by yeast on fermentation process.

4.3 Changes in TSS during fermentation

Fig. 4.2 shows the change on TSS during fermentation.

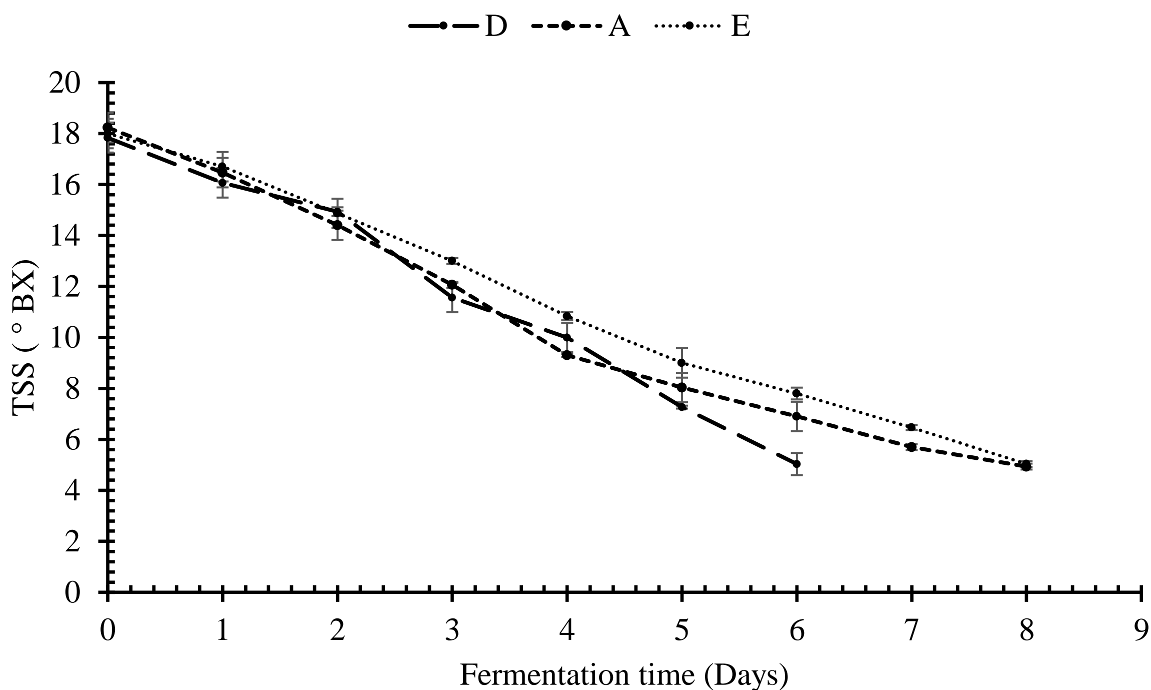


Fig. 4.2 Change in TSS during fermentation

Vertical error bars represent ± standard deviations.

During the course of fermentation, the TSS of the products was statistically analyzed. The statistical analysis showed that there was significant difference (P<0.05) between the TSS of each product i.e. D, E and A. From the Appendix B.2, it was observed that there was no significant difference between E and A in first, Sond and eight days of fermentation. Similarly, there was significant difference in TSS among fermentation days. The TSS was decreasing gradually up to 8 days like that in cherry wine (Jia *et al.*, 2019). The change in TSS signifies that alcoholic fermentation is continuously occurring as the day progress. Because the decrease in the TSS is due to the conversion of sugars into alcohol (Kasture and

Kadam, 2018). There was rapid decrease of TSS of pulp than mucilage and mucilage with seeds from Sond days to third days of fermentation. Similarly, the desired value of TSS i.e. 5 was achieved in six days which is early than the other two products. This concludes that there was faster rate of sugar utilization in pulp than the other two products. From the Appendix B.2, it was seen that the rate of decrease of TSS was similar up to 2 days between the E and A but significantly different with the D. This is because the mucilage content in both samples. After 2 days, the decrease of TSS was significantly different with one another.

4.4 Sensory analysis

Sensory analysis was performed with the aid of 12 panelists evaluating appearance, aroma, taste, aftertaste and overall acceptability of different samples. The different samples were, alcoholic beverages of pulp (D), mucilage extract (E), mucilage with seed (A), red wine (B) and white wine (C). The alcoholic beverage of pulp was subjected for paired T test and LSD tests at 5% level of significance with the red wine of the market. Similarly, the other two alcoholic beverage of mucilage extract and mucilage with seeds were subjected for one-way ANOVA and LSD tests at 5% level of significance with the white wine of the market. The results of the statistical analysis showed that there was significant difference between the samples on the basis of appearance, aroma, test, aftertaste and overall acceptability. The maximum sensory scores that a panelist can give for the appearance, aroma, taste, aftertaste and overall acceptability were 15, 30, 30, 15, and 10 respectively.

4.4.1 Appearance

There was a significant difference between the samples A, C and E based on the appearance. The sensory data were subjected for LSD at 5% level of significance. Fig. 4.3 shows the mean sensory scores for appearance of samples A,C and E

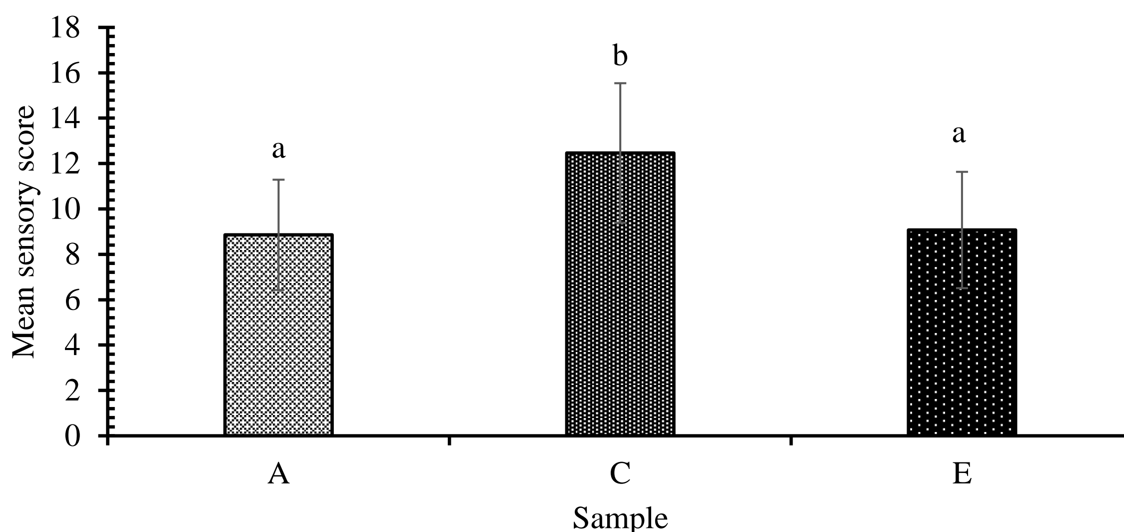


Fig. 4.3 The mean sensory scores for appearance of samples A, C and E

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

From Fig. 4.3, it was seen that there was a significant difference between the samples A and C and between samples C and E. But there was no significant difference between the samples A and E. From the above table, we can see that among the three products, sample C got the highest mean score based on the appearance. This was due to sthe haziness of samples A and E. The samples were not clarified properly where sample C is market white wine which was clarified.

Paired t test was carried out between sample B and D. There was significant difference between the samples B and D based on appearance. The sensory data were subjected for LSD at 5% level of significance. We can see that the highest mean score is of the sample B. This was due to sample B i.e., market red wine was subjected to clarification but that sample D i.e., pulp alcoholic beverage was not clarified properly. So, clarification had affected on its appearance. Fig. 4.4 shows mean sensory scores for appearance of sample B and D

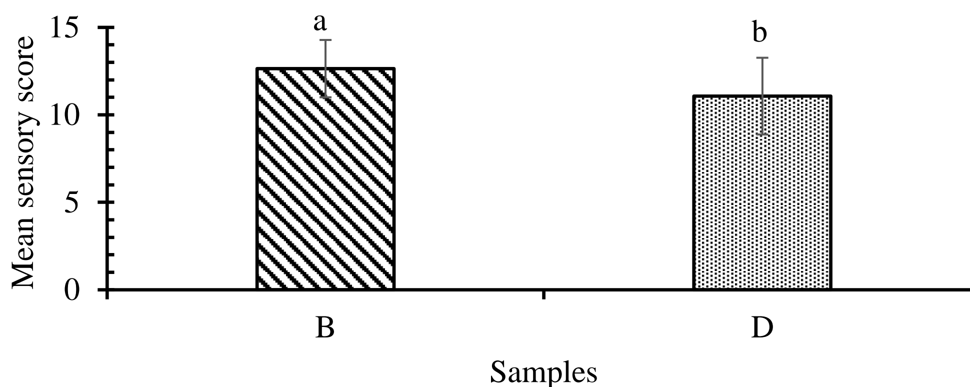


Fig. 4.4 Mean sensory scores for appearance of sample B and D

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

4.4.2 Aroma

ANOVA test was carried out between samples A, C and E. There was significant difference between the samples A, C and E. The sensory data were subjected for LSD at 5% level of significance. Fig. 4.5 shows the mean sensory scores for aroma of samples A, C and E

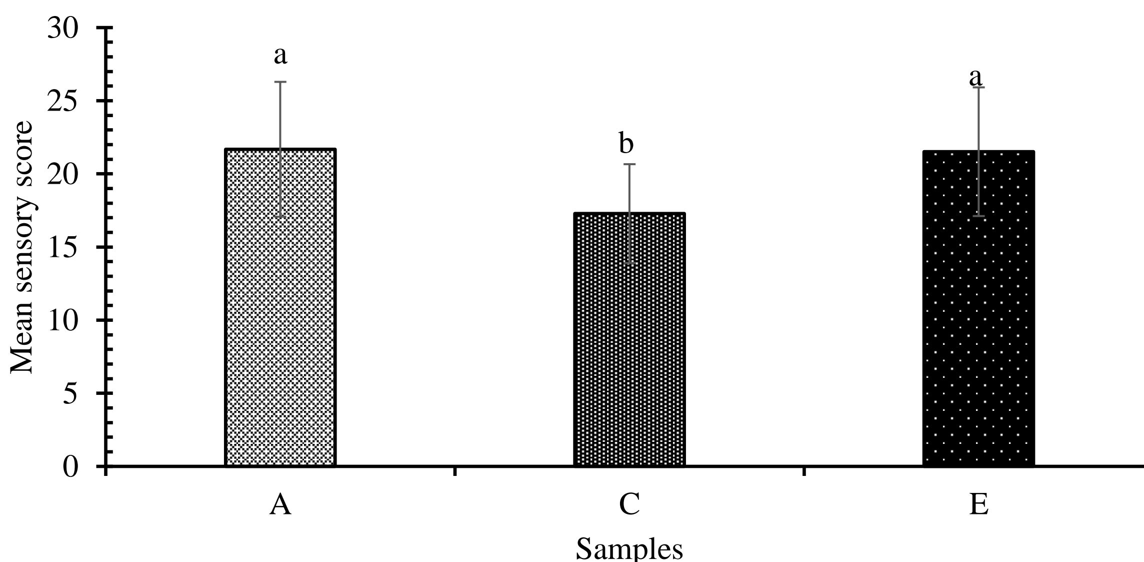


Fig. 4.5 Mean sensory scores for aroma of samples A, C and E

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

In Fig. 4.5, we can see that there was no significant difference between samples A and E based on aroma. It may be due to that both are the product of mucilage. But among these two samples A and E, sample A got higher score. Similarly, there was significant difference between the samples A and C and samples C and E based on aroma. This may be due to the addition of different aroma giving compounds added to the white wine. But this was undesirable to the panelist due to its synthetic type aroma. The highest mean score for the alcoholic beverages of coffee mucilage were may be due to the natural volatile components present in the coffee mucilage. The natural volatile components were mainly acetic acids and propionic acids. According to Bressani *et al.* (2018) acetic and propionic acids increased throughout fermentation and acetic acid was the main acid detected. According to Bressani *et al.* (2018) chlorogenic compounds also increased at the end of fermentation. And the chlorogenic compounds is the ester compound. Since ester are one of the fruity aroma giving compounds. Similarly, Haile and Kang (2019) have mentioned that microorganisms in coffee fermentation contribute to the production of ethanol and lactic, butyric, acetic, and other higher carboxylic acids during the fermentation of pectinaceous sugars. According to Bressani *et al.* (2018) there is modification of compounds such as proteins, carbohydrates, chlorogenic acids in green coffee beans. Due to this unique aroma was developed at the end of fermentation which is responsible for high sensory score in sample A and E.

Paired t test was carried out between sample B and D. There was significant difference between the samples B and D based on aroma. The sensory data were subjected for LSD at 5% level of significance. The mean score for the sample B and D were 24.5 and 19.03571 respectively. Fig. 4.6 shows the mean sensory scores for aroma of samples B and D.

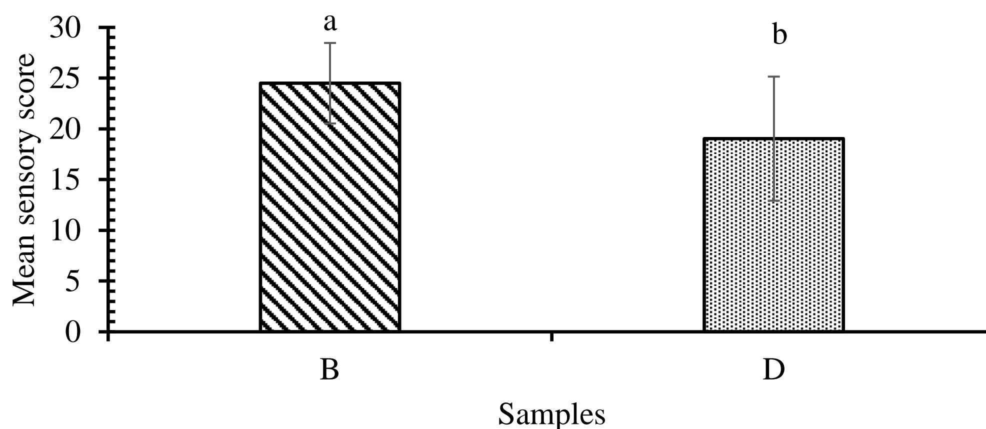


Fig. 4.6 Mean sensory scores for aroma of samples B and D.

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

The highest mean score was observed in sample B. As commercial wine was treated with different spices but that sample D i.e., pulp alcoholic beverage was not treated with spices. So, spices had affected on aroma. Here in red wines, spices enhanced the natural aroma giving compounds. The low sensory value in terms of aroma for the pulp alcoholic beverage is due to more extraction of chlorogenic acids in the beverages that enhanced off raw coffee bean like aroma. Since, methanol content was high in the alcoholic beverage of pulp which extracted the more volatile compounds than required that resulted in more raw coffee bean like aroma development in the drink. Because Molina *et al.* (1974) mentioned that methanol could extract chlorogenic acid in the fermented beverage.

4.4.3 Taste

ANOVA test was carried out between samples A, C and E. There was significant difference between the samples A, C and E based on taste. The sensory data were subjected for LSD at 5% level of significance. Fig. 4.7 shows the mean sensory scores for taste of samples A, C and E.

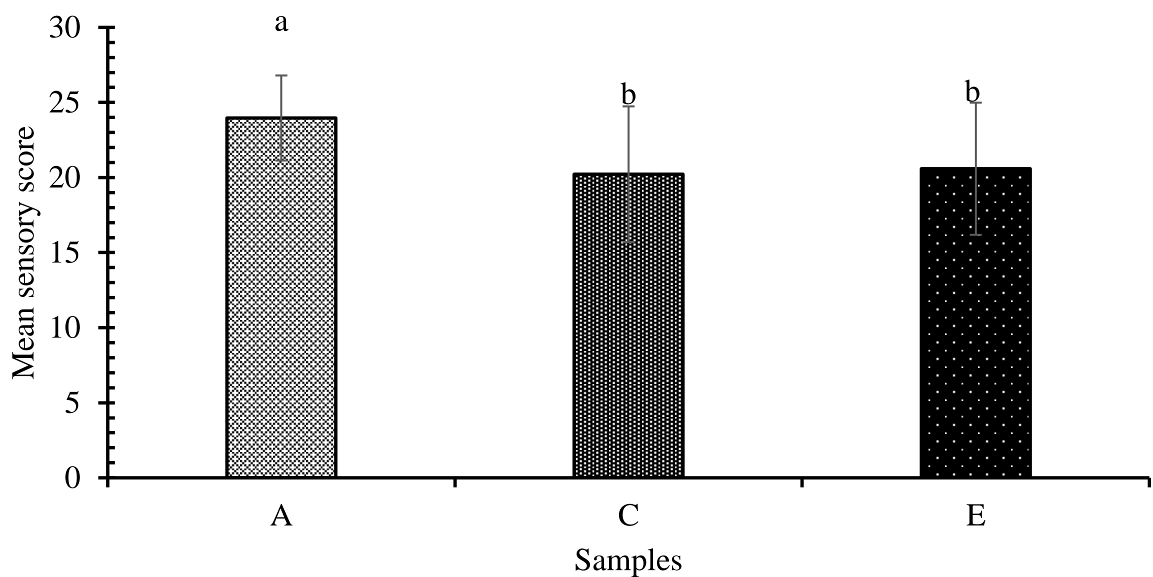


Fig. 4.7 Mean sensory scores for taste of samples A, C and E

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

As in the above bar graph, there was no significant difference between the samples C and E. But there was significant difference between the samples A and C and samples A and E based on taste. The highest mean score was of the sample A and the least mean score was of the sample C. The unique and natural taste of alcoholic beverage of mucilage defined by panelists may be due to chlorogenic acid, epicatechin, is chlorogenic acid that got extracted mucilage. According to Sera *et al.* (2013) coffee fragrance and flavor is related to chlorogenic acids. The low sensory score in taste in sample C may be due to the synthetic like flavor, lacking of such chlorogenic acids in the market white wine.

Paired t test was carried out between sample B and D. The mean score for the sample B and D were 21.03 and 24.14 respectively. There was significant difference (P<0.05) between the samples B and D based on taste. Based on taste, the highest mean score was of the alcoholic beverage of coffee pulp due to its natural taste. The natural taste may be provided by the chlorogenic acids, isochlorogenic acids, epicatechin. The lowest mean score for the sample B may be due to its synthetic taste or absent of chlorogenic acids. Fig. 4.8 shows the mean sensory scores for taste of samples B and D

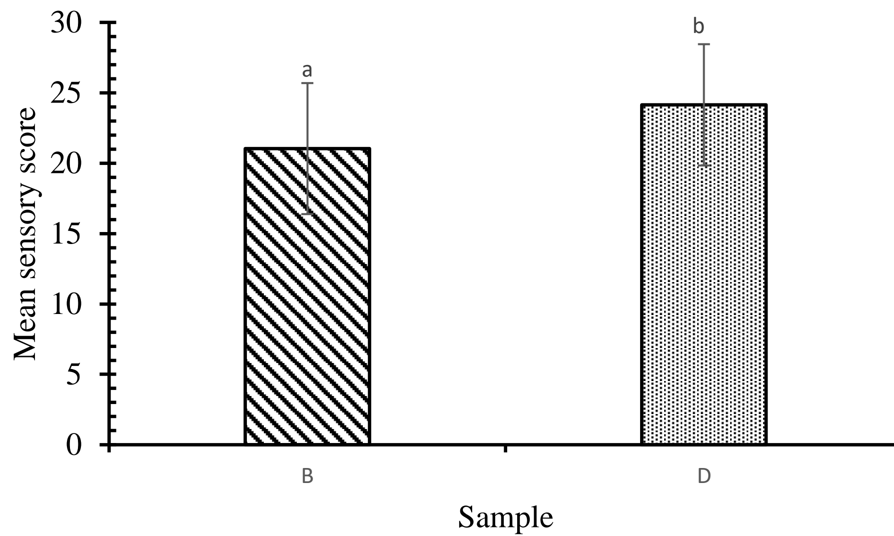


Fig. 4.8 Mean sensory scores for taste of samples B and D

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

4.4.4 Aftertaste

ANOVA test was carried out between samples A, C and E. There was significant difference between the samples A, C, and E based on aftertaste. The sensory data were subjected for LSD at 5% level of significance. Fig. 4.9 shows the mean sensory scores for afertaste of samples A, C and E

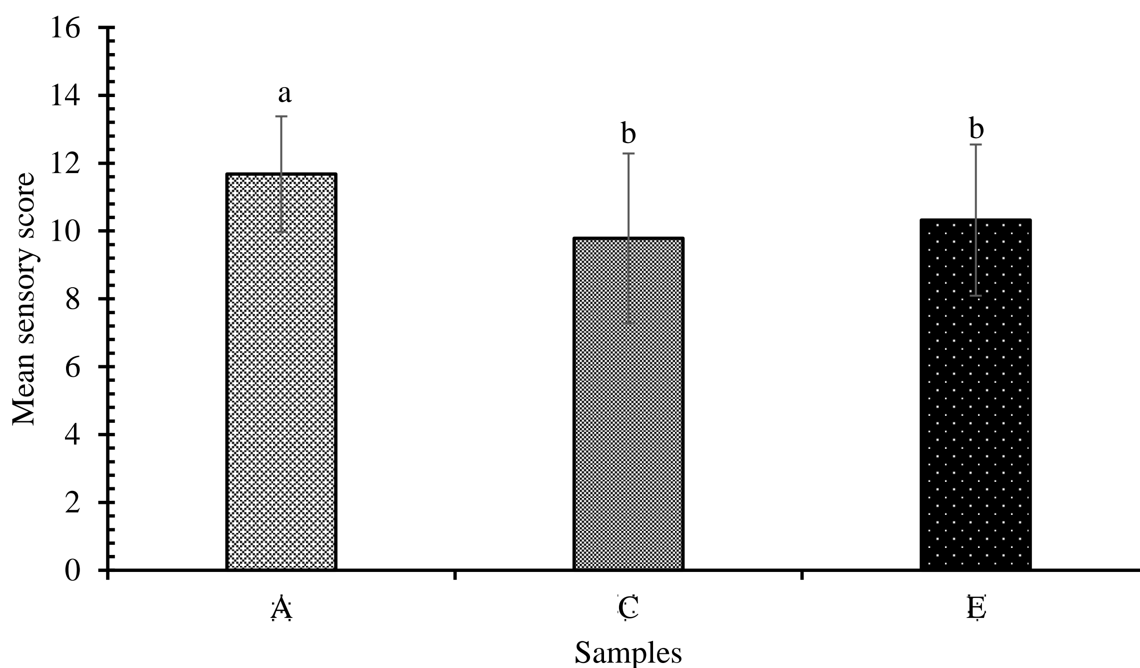


Fig. 4.9 Mean sensory scores for afertaste of samples A, C and E

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

There was no significant difference between the sample C and E based on aftertaste. But there was significant difference between the samples A and C and samples A and E. The highest mean score for the sample A may be due to its natural and astringent taste of coffee. The astringent aftertaste was given by tannin compound that was isolated from coffee mucilage. This sensation is felt in the mouth after consumption of some wines, strong tea or unripened fruit (Ashok and Upadhyaya, 2012). This tannin may be the compound that have given natural aftertaste in sample A. The least mean score for the sample C may be due to the synthetic type aftertaste.

Paired t test was carried out between sample B and D. The mean score for the samples B and D were 10.892 and 12.214 respectively based on aftertaste. There was significant difference (P<0.05) between them. Based on aftertaste, the highest mean score of alcoholic beverages of pulp was due to its natural aftertaste. The natural aftertaste in alcoholic beverage of coffee pulp may be due to higher tannin compound in coffee pulp than in market

wine. The astringent aftertaste giving compound is tannin (Ashok and Upadhyaya, 2012). The least mean score for sample B may be due to the synthetic type aftertaste. Fig. 4.10 shows the mean sensory scores for aftertaste of samples B and D

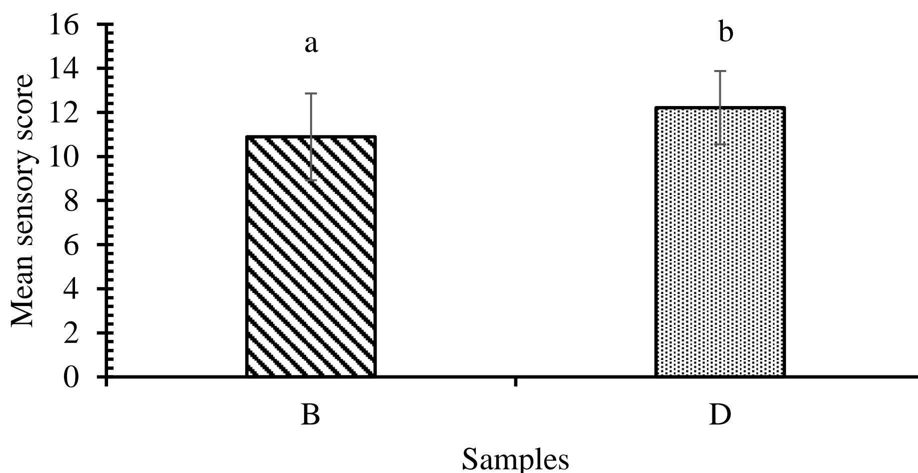


Fig. 4.10 Mean sensory scores for aftertaste of samples B and D

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

4.4.5 Overall acceptability

ANOVA test was carried out between samples A, C and E. Fig. 4.11 shows the mean sensory scores for overall acceptability of samples A, C and E

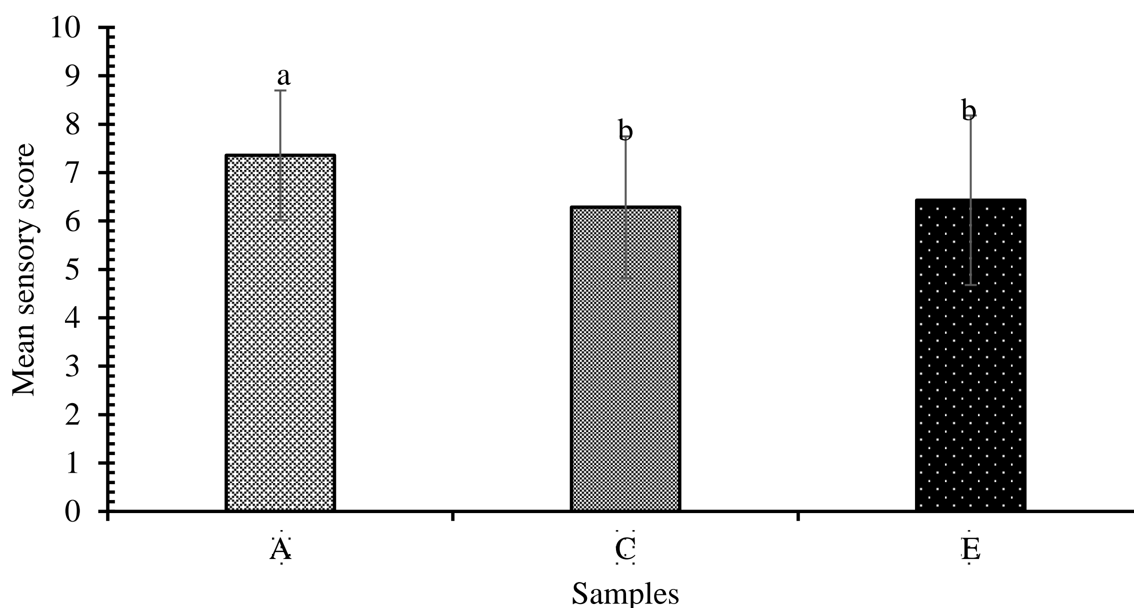


Fig. 4.11 Mean sensory scores for overall acceptability of samples A, C and E

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

There was significant difference between samples A, C and E based on overall acceptability. The sensory data were subjected for LSD at 5% level of significance.

There was no significant difference between the samples C and E. But there was significant difference between the samples A and C and samples C and E. The highest mean score for the sample A was due to its natural flavor. The least score for the sample C was due to its synthetic flavor. Paired t test carried out between samples B and D.

Paired t test was carried out between sample B and D. The mean score for the sample B and D were 6.7 and 8.2 respectively based on overall acceptability. There was significant difference (Appendix C.10) between the samples B and D in terms of overall acceptability. The mean score for the sample D was found to be higher. This concludes that if optimization is done to alcoholic beverage of coffee pulp, then coffee pulp can be utilized as a useful byproduct as an alcoholic beverage. Fig. 4.12 shows the mean sensory scores for overall acceptability of samples B and D.

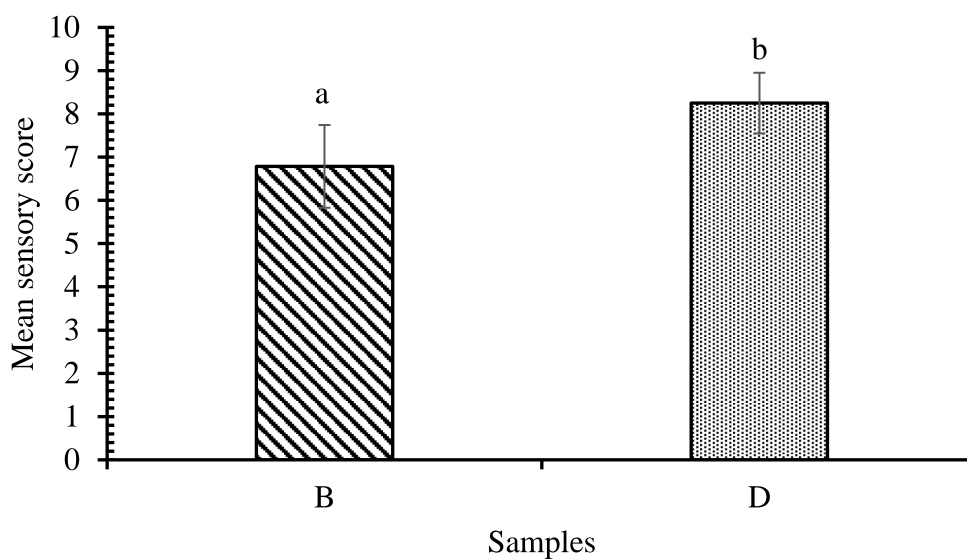


Fig. 4.12 Mean sensory scores for overall acceptability of samples B and D

Vertical error bars represent ± standard deviation of scores given by 12 panelists. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

4.5 Methanol content

Based on methanol content, there was no significant difference between the samples A and E. But there was significant difference between the samples A and D and between samples D and E. The data were subjected for LSD at 5% level of significance. Fig. 4.13 shows the methanol content of samples D, E and A

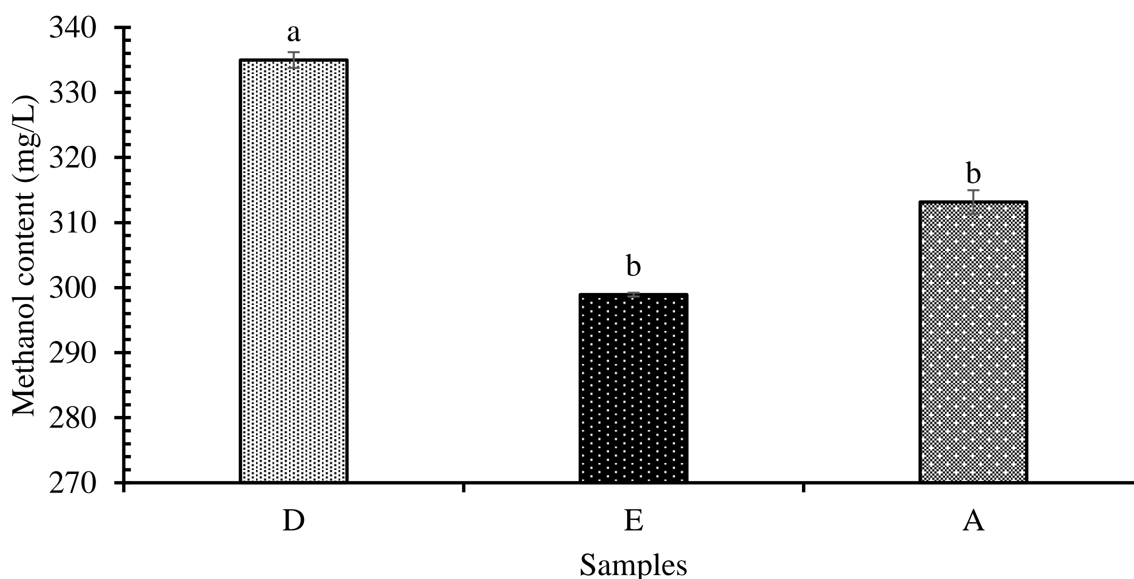


Fig. 4.13 Methanol content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

In Fig. 4.13, among the three fermented beverages, the sample D contained the highest level of methanol content i.e. 334.88 mg/L. Similarly, the E contained 298.67 mg/L level of methanol. Coffee pulp is about 1.9 times richer in pectin than coffee mucilage (Garcia *et al.*, 1991) . So, the highest level of the methanol content in the pulp beverage is due to the methylated pectin that gets transfer in the beverage during fermentation. The methanol level in red wine that can be accepted by human body is 400 mg/L (Hodson *et al.*, 2017). This conclude that the pulp alcoholic beverage is acceptable to drink in terms of methanol acceptability. Similarly, the mesocarp alcoholic beverage is also in the range which is drinkable.

4.6 Esters

There was no significant difference between the samples A and E. But there was significant difference between the samples D and E and samples A and D. The data were subjected for LSD at 5% level of significance. Fig. 4.14 shows the esters content of samples D, E and A

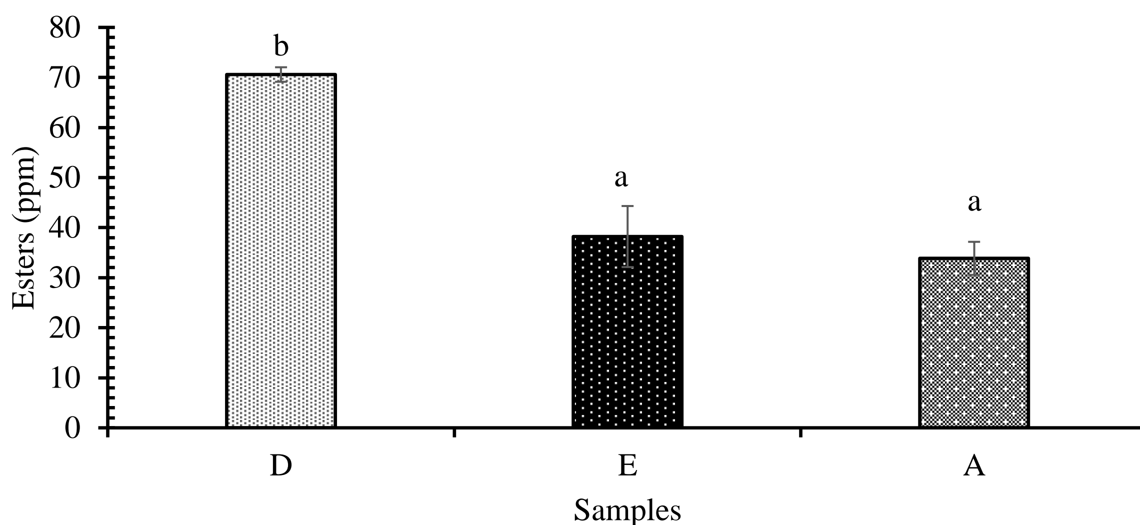


Fig. 4.14 Esters content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

The concentration of esters in sample D, E and A were 70.58 ppm, 38.21 ppm and 33.86 ppm respectively. Since, the alcohol content in the pulp was higher than others and due to the maximum alcohol production. Because esters are formed due to the reaction between the fatty acids and alcohol (Saerens *et al.*, 2007). The difference in the esters content may be also due to the difference in the carbon, nitrogen content between the samples (Saerens *et al.*, 2007). Postel *et al.* (1972) found 44–122 mg/L ethyl acetate in white wine. Similarly, Martinez *et al.* (1998) found 23-27 mg/ L of esters in red wine.

4.7 Alcohol content

There was no significant difference between the samples D and E. But there was significant difference between the sample A and E and samples between samples A and D. The data were subjected for LSD at 5% level of significance. Fig. 4.15 shows the ethanol content of samples D, E and A

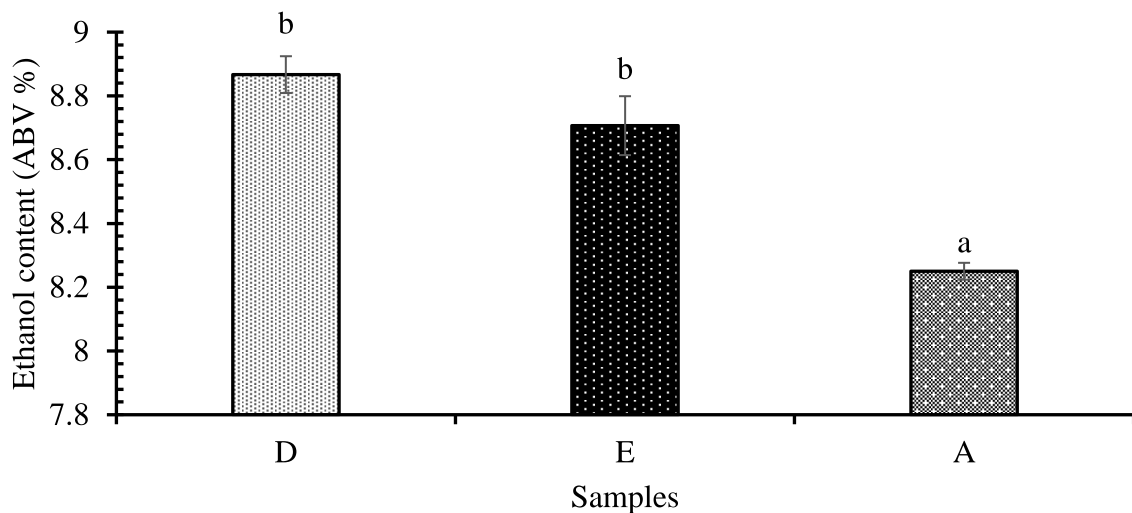


Fig. 4.15 Ethanol content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

The content of alcohol in samples D, E and A were 8.9%, 8.76% and 8.27% respectively. There was maximum utilization of sugar in the sample pulp resulting in the more alcohol. The difference in the content of ethanol may be due to the difference in the must formation, and also may be due to the difference in the chemical constituents between pulp and mucilage. The alcohol content in wine was found to be 10.6- 10.8% v/v (Martinez *et al.*, 1998)

4.8 Aldehyde content

There was significant difference between the samples D, E and A. The data were subjected for LSD at 5% level of significance. Fig. 4.16 shows the aldehyde content of samples D, E and A

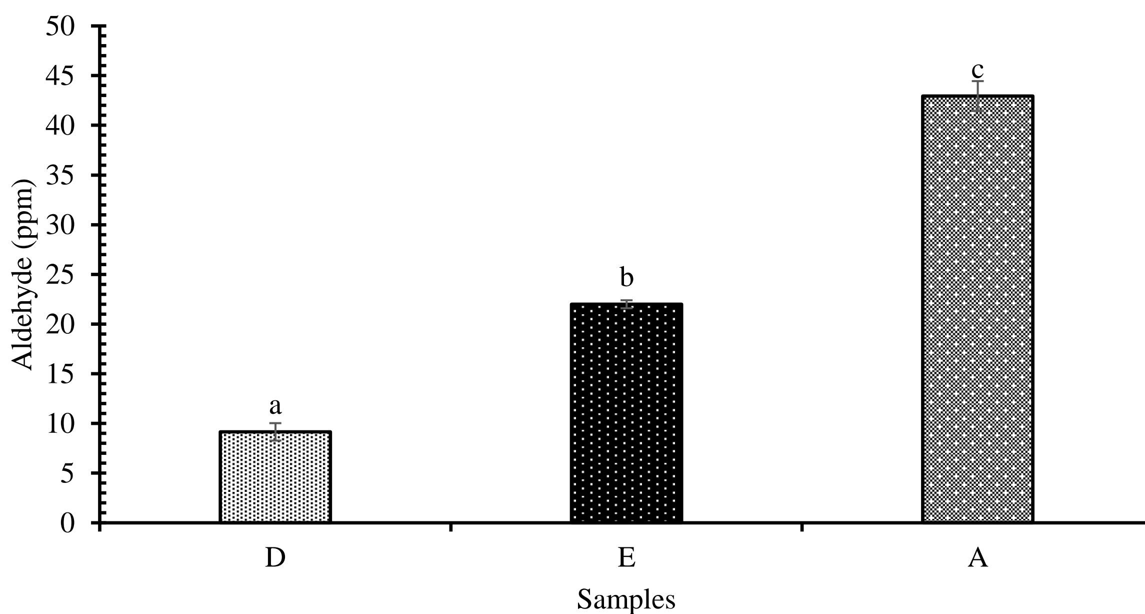


Fig. 4.16 Aldehyde content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

The aldehyde content in samples D, E and A were 9.15 ppm, 22.00 ppm and 42.94 ppm of absolute alcohol respectively. According to Osborne *et al.* (2006) aldehyde enhances the color of red wine by assisting the polymerization of anthocyanins and phenolics. This may be the reason of minimization of aldehyde content in the pulp. Here high aldehydes in sample E may be due to the lack of anthocyanins and oxidation of some ethanol. The lower chance of oxidation in other beverages may be due to the pulp and seed that is fermenting along with it. Similarly, the maximum value of aldehyde in sample A may be due to more solid content. As Martinez *et al.* (1998) have mentioned the high level of acetaldehyde (163 ppm) in must with high solid content.

4.9 Total acidity

There was significant difference between the samples D, E and A based on total acidity. The data were subjected for LSD at 5% level of significance. Fig. 4.17 shows the total acidity of samples D, E and A

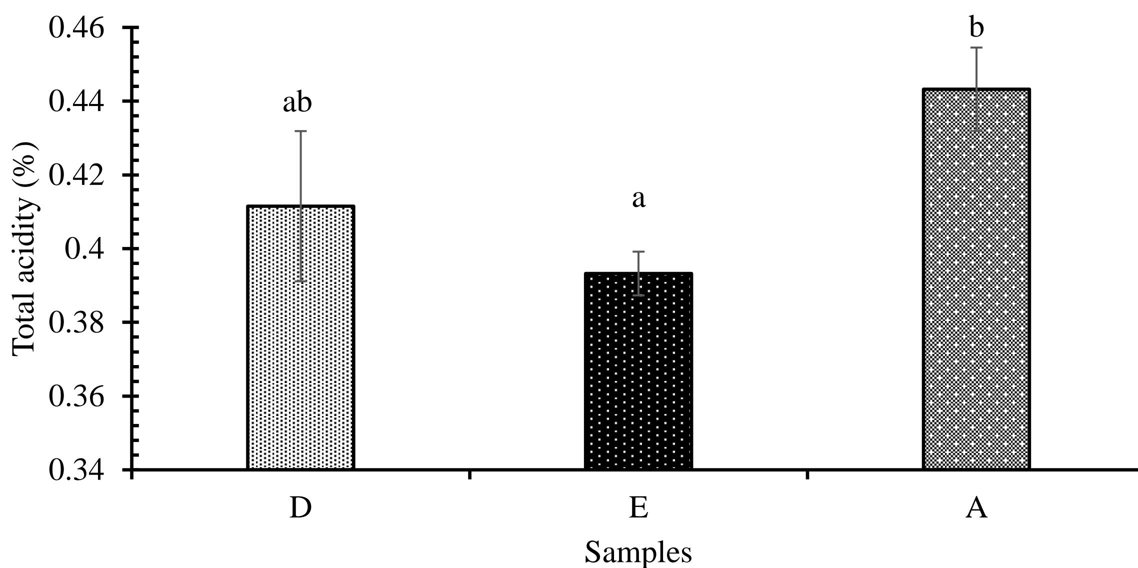


Fig. 4.17 Total acidity of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

There was no significant difference between the samples D and E and between samples A and D. The mean value of the total acidity in the samples pulp, mucilage and mucilage with seeds were 0.41%, 0.39% and 0.44% respectively. The maximum value for the mucilage with seeds may be unconsumed fatty acids for the production of esters. And the minimum values for other samples may be due to the utilization of carboxylic acids in the production of esters.

4.10 Volatile acidity

There was significant difference between the samples D, E and A based on total acidity. The data were subjected for LSD at 5% level of significance. Fig. 4.18 shows the volatile acidity of samples D, E and A

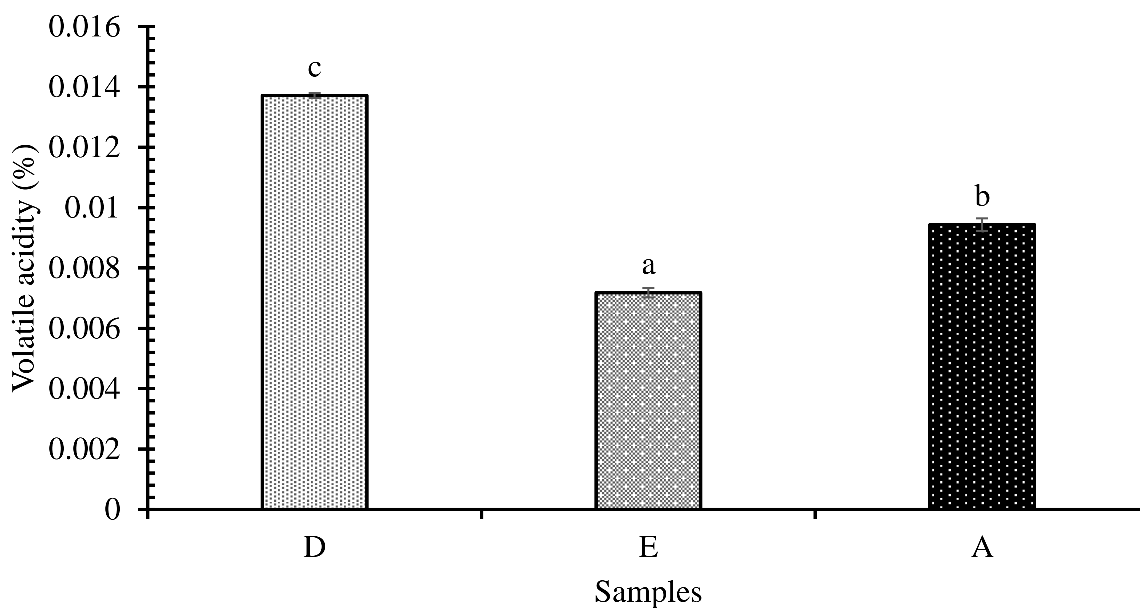


Fig. 4.18 Volatile acidity of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

Comparatively all the sample showed lesser value than the value of volatile acidity that contains in wine (Zoecklein *et al.*, 1995). As shown in above bar diagram, the sample D contained the maximum value of volatile acidity. This may be due to the difference in the chemical composition between the raw materials. The level of volatile acidity in wine is 0.19 g/L to 0.41 g/L (Martinez *et al.*, 1998).

4.11 Color

For sample white wine, mucilage and mucilage with seeds. Fig. 4.19 shows the color intensity of samples E, A and C

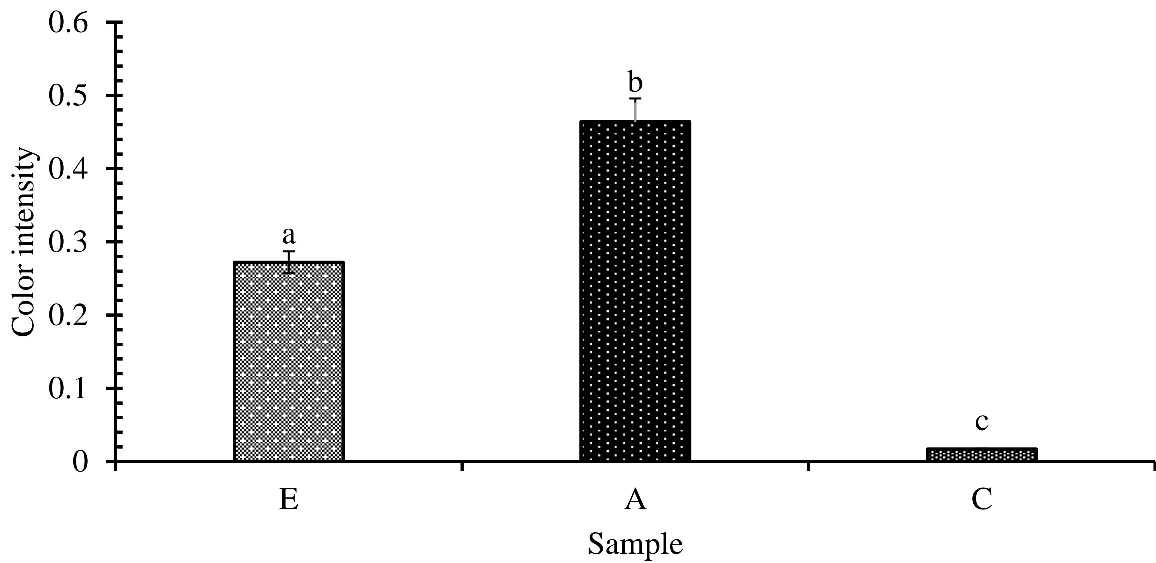


Fig. 4.19 Color intensity of samples E, A and C

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

Based on color intensity, there was a significant difference (P<0.05) between the all three samples. Mucilage with seeds had the highest color intensity and white wine got the least value of color intensity. Fig. 4.20 shows the hue measurement of samples E, A and C

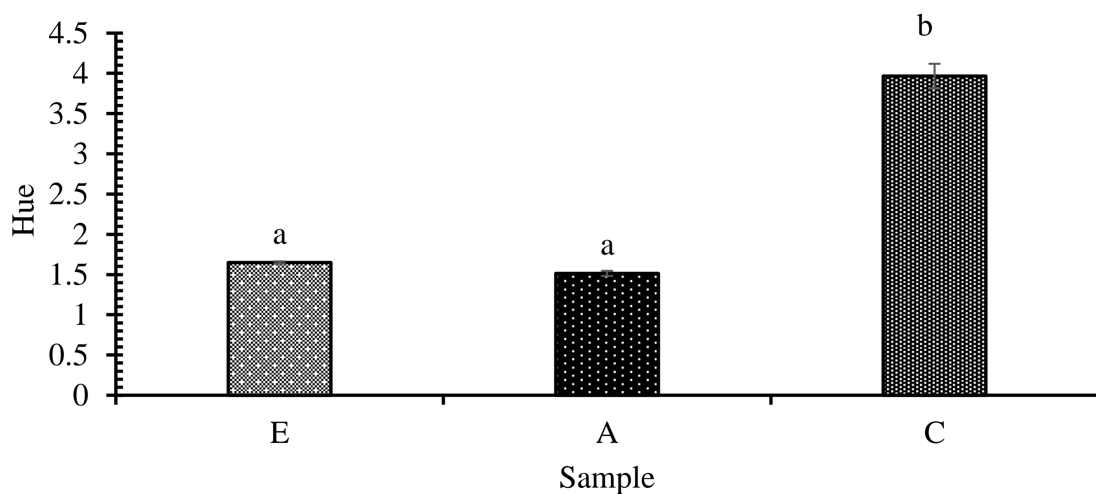


Fig. 4.20 Hue measurement of samples E, A and C

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

Based on hue, there was no significant difference between sample A and E. But there was a significant difference ($P<0.05$) between the samples C and E and between samples A and C.

For the sample B and D. Fig. 4.21 shows the color intensity of samples B and D

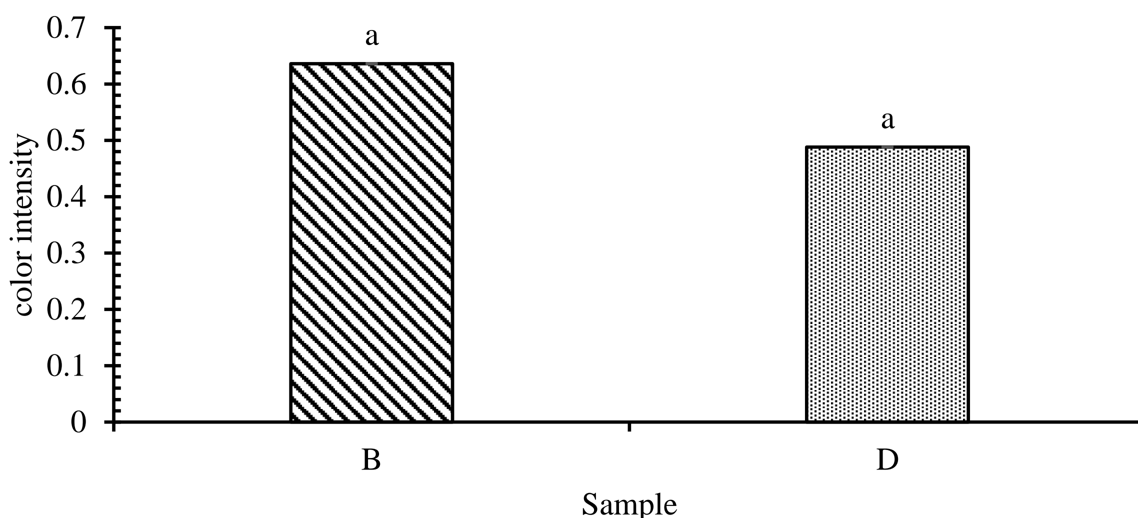


Fig. 4.21 Color intensity of samples B and D.

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

Based on color intensity there was no significant difference ($P<0.05$) between the samples B and D. The red color is due to anthocyanin compound present in the pulp of coffee cherry. According to Borem (2008) it has been reported that red skin color comes from anthocyanin pigments, while yellow skin color is attributed to luteolin. Fig. 4.22 shows the color hue of samples B and D.

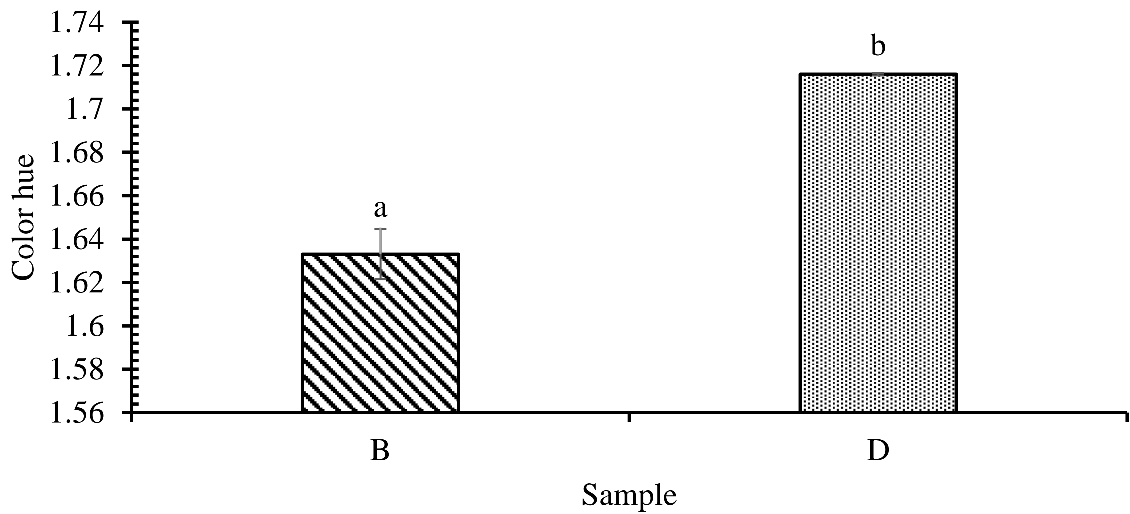


Fig. 4.22 Color hue of samples B and D

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

Based on color hue, there was significant difference (P<0.05) between samples B and D. The mean score for hue was 1.716 which was higher than red wine (1.633). The higher value of hue score in alcoholic beverage of pulp was that it was not subjected to clarification process but that market red wine was subjected to clarification process.

4.12 Caffeine

Fig. 4.23 shows the caffeine content of samples D, E and A

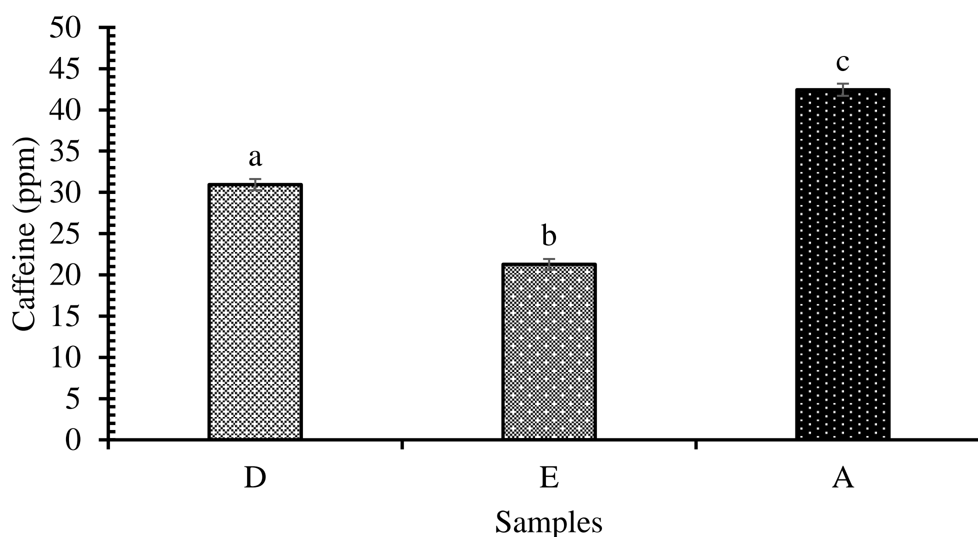


Fig. 4.23 Caffeine content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

There was significant difference (P<0.05) between all three samples based on caffeine content. The maximum value of caffeine content was seen in sample A than in sample D. The minimum value of caffeine content was seen in alcoholic beverage of mucilage. This is due to that; the coffee beans contain the maximum value of caffeine content. According to Asfew and Dekebo (2019), caffeine content of coffee beans ranged from 1.21 ± 0.14% to 1.43 ± 0.19%, caffeine content of coffee pulp was within range of 0.78 ± 0.11%-0.97 ± 0.16% and that of coffee leaves was within range of 0.57 ± 0.03%-0.76 ± 0.05%.

The value of caffeine content in the alcoholic product is very less as compared to that in raw pulp, beans. The low values can be attributed to the fact that caffeine molecules in coffee beans are complexed with chlorogenic acids (Horman and Viani, 1972), and the hydrogen bonds between caffeine and chlorogenic acid molecules have to be broken during fermentation.

Similarly, caffeine is less soluble in ethanol. The solubility in ethanol is 1 g in 66 ml (approx. 15 mg/ml) . This may be the reason for less value of caffeine in sample D.

4.13 Polyphenols

Fig. 4.24 shows the polyphenols content of samples R, D, E, and A

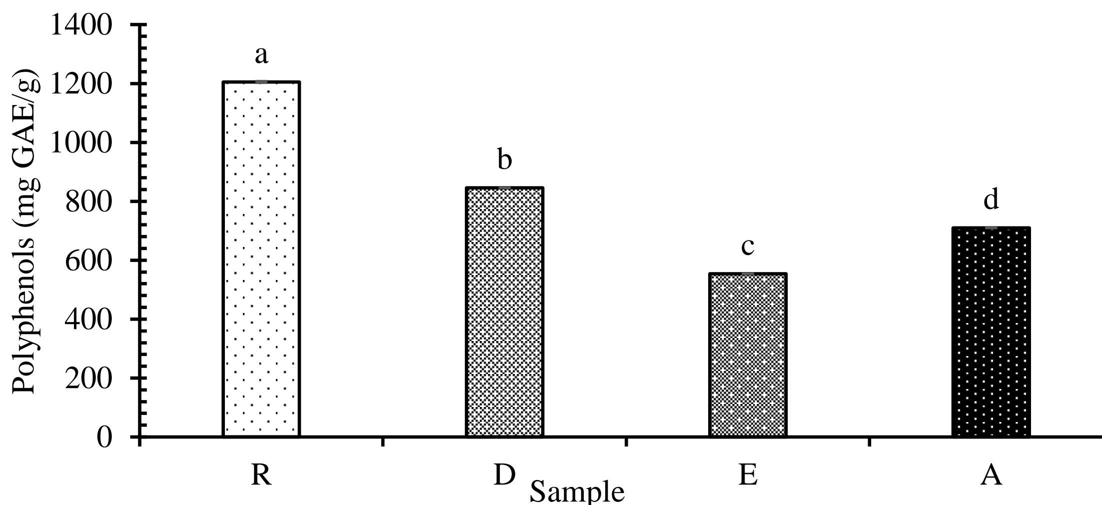


Fig. 4.24 Polyphenols content of samples R, D, E, and A.

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at p>0.05.

There was significant difference (P<0.05) between all the above samples based on polyphenols. Raw pulp contained 1205.2 mg GAE/g of extract which was between the range 1809.9 to 489.5 mg GAE/g (Geremu *et al.*, 2016). Among the three samples of alcoholic beverages, sample D contained the maximum value of polyphenols i.e. 845.7 mg GAE / g. This maximum value was due that pulp contained the highest value of polyphenols than other parts of pericarp (Geremu *et al.*, 2016). Mucilage contained the minimum value of polyphenols i.e. 554 mg GAE/ g. This was due to minimum value of polyphenols in mucilage.

4.14 Total flavonoids contents *(TFC)*

Fig. 4.25 shows the flavonoid content of samples D, E and A

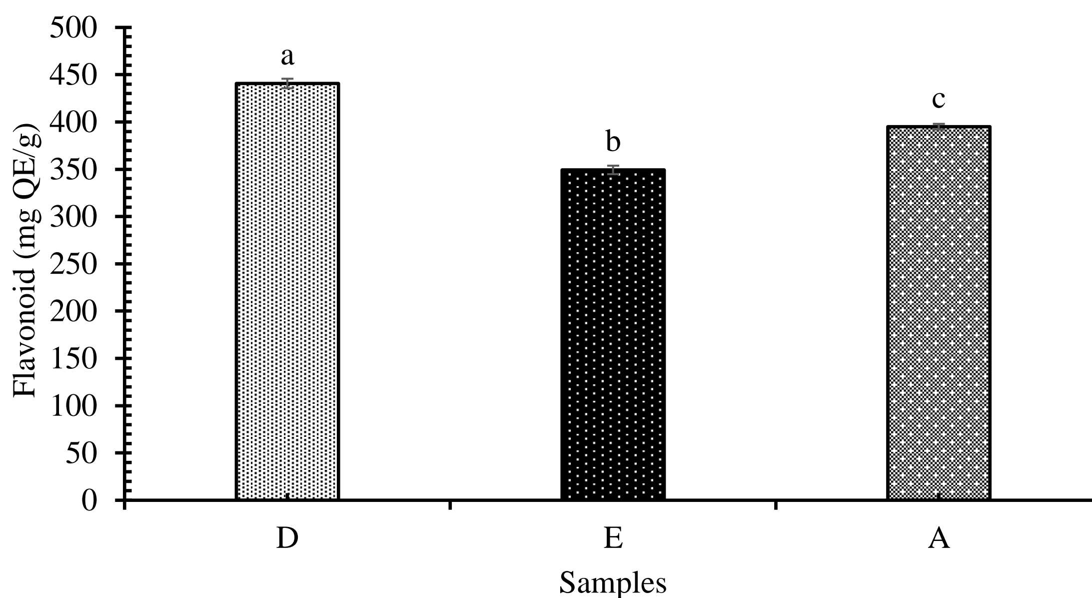


Fig. 4.25 Flavonoid content of samples D, E and A

Vertical error bars represent ± standard deviation. Bars with same letters for any sensory parameter are not significantly different at $p>0.05$.

The content of flavonoid was also similar to that of phenolic content i.e., maximum in D. There was no significant difference ($P<0.05$) between the samples. The polymeric anthocyanins color (%) of the coffee pulp was higher (Murthy and Naidu, 2012) which might be the reason for more flavonoids in alcoholic beverage of pulp.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results and discussion, the following conclusions were drawn:

1. Alcoholic beverages can be prepared by utilizing the byproducts of coffee cherry.
2. The alcoholic beverages made from mucilage with seeds has possibility of superiority to commercial white wine if it is subjected for clarification.
3. Under the same fermentation condition, ethanol content in the pulp beverages can be made higher than the beverage made from other two parts i.e. mucilage extract and mucilage with seeds.
4. The alcoholic beverage of the pulp can be prepared which is similar to red wine.

5.2 Recommendations

Based on the present study, the following recommendations have been made:

1. Pulp of coffee can be utilized as alcoholic beverages with varying TSS, temperature and pH.
2. Study on the quality of pulp alcoholic beverage using different yeast can be carried out.
3. Study on the physiochemical properties of the alcoholic beverages of pulp, mucilage can be studied by adding spices and clarification treatment.
4. Study on the quality of coffee beans after the fermentation of yeast in the alcoholic beverage of mucilage with seeds can be carried out.
5. Study on effect of pulping methods on the utilized alcoholic beverage of pulp and mucilage can be carried out.

Part VI

Summary

In this study, coffee cherry was taken from Dhankuta, which is one of the districts for commercial cultivation of coffee in Nepal. And other essential materials (sugars and yeast) and other essential apparatus were obtained from local market of Dharan and campus laboratory. First of all, cherry was subjected to preliminary operation like cleaning, sorting and washing with plenty of water. After this coffee cherries were pulped with hand in a hygienic condition. The mucilage with beans and the pulp were separated. The pulp and the mucilage initially showed 16 °BX and 6.1 pH. Half of the part of mucilage with beans were separated to extract mucilage with distilled water separately. Calculated amount of sugar and water were added in all three parts to make juice of TSS 18 °BX. The samples were subjected for pasteurization. All the pasteurized samples were subjected for alcoholic fermentation keeping 18 °BX without varying other parameters until TSS reaches 5 °BX. After that additional amount of sugar is added to make TSS 10 °BX for flavor. Now the all the fermented samples were pasteurized again.

Five different samples A, B, C, D and E were subjected to sensory analysis. The data obtained from this were analyzed statistically. Data of samples B and D were subjected to t-test. Similarly, data of samples A, C and E were subjected to one-way ANOVA at 5% level of significance to study the difference among the all types. There was significant difference between the sample B and D in case of appearance, color, aroma, taste, aftertaste and overall acceptability. But sample D was best in case of taste, aftertaste and overall acceptability. Similarly, there was significant difference between samples A, C and E. But sample A was best in case of taste, aftertaste, and overall acceptability.

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Appendices

Appendix A

Specimen card of sensory evaluation

Sensory evaluation of alcoholic beverages of Pulp, Mucilage with seeds, Red wine and White wine

Name of the panelist: ………………………. Date: ………………….

Name of the product: Alcoholic beverage of coffee cherries

Please taste each of the samples of alcoholic beverages in the order listed below. The range of score to be provided for each parameter are included in the table. Assign your score for each parameter to all the samples.

Parameters(scores)	Samples				
	A	B	C	D	E
Appearance (1-15)					
Aroma (1-30)					
Taste (1-30)					
Aftertaste (1-15)					
Overall acceptance (1-10)					

Comments………………………………………………………………………………….

Signature

Appendix B

ANOVA result for the effect of fermentation days on the Ph and TSS of the different samples of alcoholic beverage

Table B.1 Two-way ANOVA between the fermentation period and sample variety on pH

Fermentation period	Variety		
	Pulp	Mucilage	Mucilage with seed
0	6.0333 ± 0.5774^{axy}	6.1333 ± 0.0577^{ax}	5.9000 ± 0.1000^{ay}
1	5.2333 ± 0.0577^{by}	5.4333 ± 0.0577^{bx}	5.2333 ± 0.0577^{by}
2	4.2000 ± 0.1732^{cdy}	4.5333 ± 0.0577^{cx}	4.4667 ± 0.0577^{cxy}
3	3.7667 ± 0.0577^{dex}	3.3667 ± 0.1154^{dy}	3.1667 ± 0.1154^{fy}
4	4.3333 ± 0.0577^{cx}	3.6333 ± 0.1527^{dy}	3.6667 ± 0.0577^{dy}
5	4.0667 ± 0.0577^{cdex}	3.3667 ± 0.0577^{dz}	3.5667 ± 0.0577^{dy}
6	3.6000 ± 0.4358^{ex}	3.6333 ± 0.2309^{dx}	3.4667 ± 0.0577^{dex}
7		3.5000 ± 0.1000^{dx}	3.2667 ± 0.1154^{efy}
8		3.3333 ± 0.1154^{dx}	3.1667 ± 0.1154^{fx}

Values represent means± standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-f) represent difference in values between the same sample in different day . Alphabets (x-z) represent difference between the samples in the same day.

Table B.2 Two-way ANOVA between the fermentation period and sample variety on TSS

Fermentation period	Variety		
	Pulp	mesocarp	Mesocarp with seed
0	17.833±0.288^{ap}	18.2333±0.251^{ap}	18.000±0.1000^{ap}
1	16.066±0.057^{bp}	16.4667±0.577^{bq}	16.700±0.1732^{bq}
2	14.933±0.115^{cp}	14.4000±0.173^{cq}	14.866±0.0577^{cq}
3	11.5667±0.05^{dp}	12.0667±0.115^{dq}	13.000±0.0000^{dr}
4	10.00±0.1000^{ep}	9.3000±0.2645^{eq}	10.833±0.2886^{er}
5	7.2667±0.251^{fp}	8.0333±0.0577^{fq}	9.0000±0.0000^{fr}
6	5.033±0.5774^{gp}	6.9000±0.1000^{gq}	7.8000±0.2645^{gr}
7	-	5.7000±0.1732^{hq}	6.4667±0.0577^{hr}
8	-	4.9333±0.0577^{iq}	5.0333±0.0577^{iq}

Values represent means± standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-i) represent difference in values between the same sample in different day . Alphabets (p-r) represent difference between the samples in the same day.

Appendix C

ANOVA result for sensory analysis of alcoholic beverages of Pulp, Mucilage with seeds, Red wine and White wine

Table C.1 One-way ANOVA for the appearance between the samples A, C and E.

Tests of Between-Subjects Effects

Dependent Variable: Scores

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	229.310[a]	2	114.655	15.119	.000
Intercept	8621.440	1	8621.440	1136.893	.000
Samples	229.310	2	114.655	15.119	.000
Error	614.250	81	7.583		
Total	9465.000	84			
Corrected Total	843.560	83			

a. R Squared = .272 (Adjusted R Squared = .254)

Table C.2 T-Test: Two-Sample B and D Assuming Unequal Variances

	Sample B	Sample D
Mean	12.64286	11.07142857
Variance	2.68254	4.80952381
Observations	28	28
Hypothesized Mean Difference	0	
Df	50	
t Stat	3.037896	
P(T<=t) one-tail	0.00189	
t Critical one-tail	1.675905	
P(T<=t) two-tail	0.003781	
t Critical two-tail	2.008559	

Table C.3 One-way ANOVA for the aroma between the samples A, C and E.

Tests of Between-Subjects Effects

Dependent Variable: Scores

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	334.500[a]	2	167.250	10.488	.000
Intercept	34323.857	1	34323.857	2152.478	.000
Samples	334.500	2	167.250	10.488	.000
Error	1291.643	81	15.946		
Total	35950.000	84			
Corrected Total	1626.143	83			

a. R Squared = .206 (Adjusted R Squared = .186)

Table C.4 T-Test: Two-Sample B and D Assuming Unequal Variances

t-Test: Two-Sample Assuming Unequal Variances

	Sample B	Sample D
Mean	24.5	19.03571
Variance	15.74074	37.36905
Observations	28	28
Hypothesized Mean Difference	0	
Df	46	
t Stat	3.967575	
P(T<=t) one-tail	0.000126	
t Critical one-tail	1.67866	
P(T<=t) two-tail	0.000252	
t Critical two-tail	2.012896	

Table B.5 One-way ANOVA for the taste between the samples A, C and E.

Tests of Between-Subjects Effects

Dependent Variable: Scores

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	279.167[a]	2	139.583	8.547	.000
Intercept	39520.048	1	39520.048	2419.987	.000
Samples	279.167	2	139.583	8.547	.000
Error	1322.786	81	16.331		
Total	41122.000	84			
Corrected Total	1601.952	83			

a. R Squared = .174 (Adjusted R Squared = .154)

Table C.6 Paired T test carried out between samples B and D

t-Test: Two-Sample Assuming Unequal Variances

	Sample B	Sample D
Mean	24.14286	21.03571
Variance	18.57143	21.66534
Observations	28	28
Hypothesized Mean Difference	0	
Df	54	
t Stat	2.591962	
P(T<=t) one-tail	0.006125	
t Critical one-tail	1.673565	
P(T<=t) two-tail	0.01225	
t Critical two-tail	2.004879	

Table C.7 One-way ANOVA for the After taste between the samples A, C and E.

Tests of Between-Subjects Effects

Dependent Variable: Scores

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	53.310[a]	2	26.655	5.668	.005
Intercept	9429.762	1	9429.762	2005.128	.000
Samples	53.310	2	26.655	5.668	.005
Error	380.929	81	4.703		
Total	9864.000	84			
Corrected Total	434.238	83			

a. R Squared = .123 (Adjusted R Squared = .101)

Table C.8 Paired T test carried out between samples B and D

t-Test: Two-Sample Assuming Unequal Variances

	Sample B	Sample D
Mean	10.89286	12.21429
Variance	3.876984	2.767196
Observations	28	28
Hypothesized Mean Difference	0	
Df	53	
t Stat	-2.7127	
P(T<=t) one-tail	0.00449	
t Critical one-tail	1.674116	
P(T<=t) two-tail	0.00898	
t Critical two-tail	2.005746	

Table C.9 One-way ANOVA for the Overall acceptance between the samples A, C and E.

Tests of Between-Subjects Effects

Dependent Variable: Scores

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18.952[a]	2	9.476	4.061	.021
Intercept	3760.048	1	3760.048	1611.449	.000
Samples	18.952	2	9.476	4.061	.021
Error	189.000	81	2.333		
Total	3968.000	84			
Corrected Total	207.952	83			

a. R Squared = .091 (Adjusted R Squared = .069)

Table C.10 Paired T test carried out between samples B and D

t-Test: Two-Sample Assuming Unequal Variances

	Sample B	Sample D
Mean	6.785714	8.25
Variance	0.915344	0.490741
Observations	28	28
Hypothesized Mean Difference	0	
Df	49	
t Stat	-6.5343	
P(T<=t) one-tail	1.75E-08	
t Critical one-tail	1.676551	
P(T<=t) two-tail	3.49E-08	
t Critical two-tail	2.009575	

Appendix D

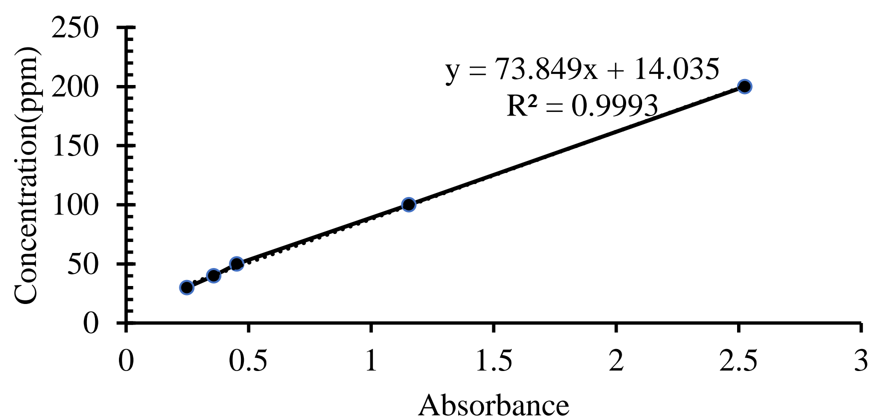


Fig. D.1 Calibration curve for Polyphenol content

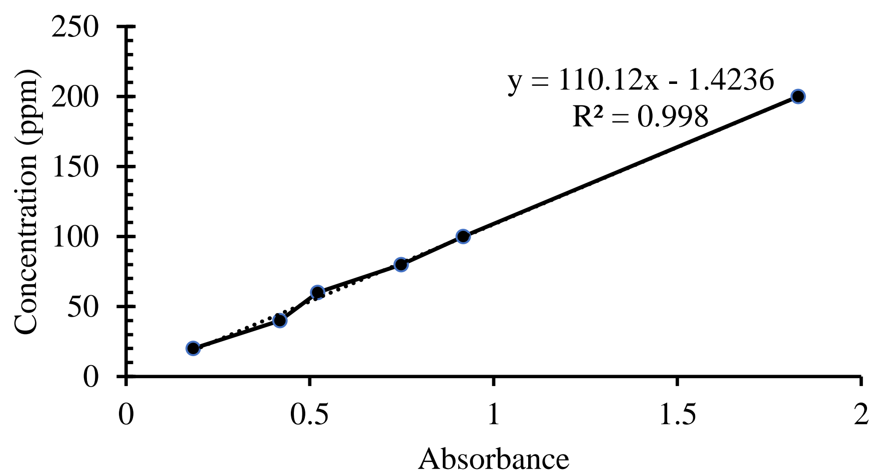


Fig. D.2 Calibration curve for methanol content

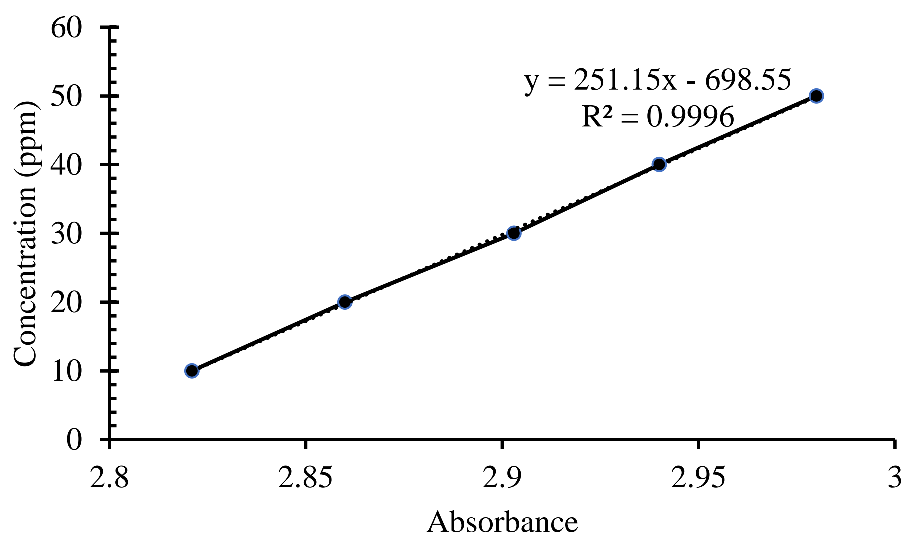
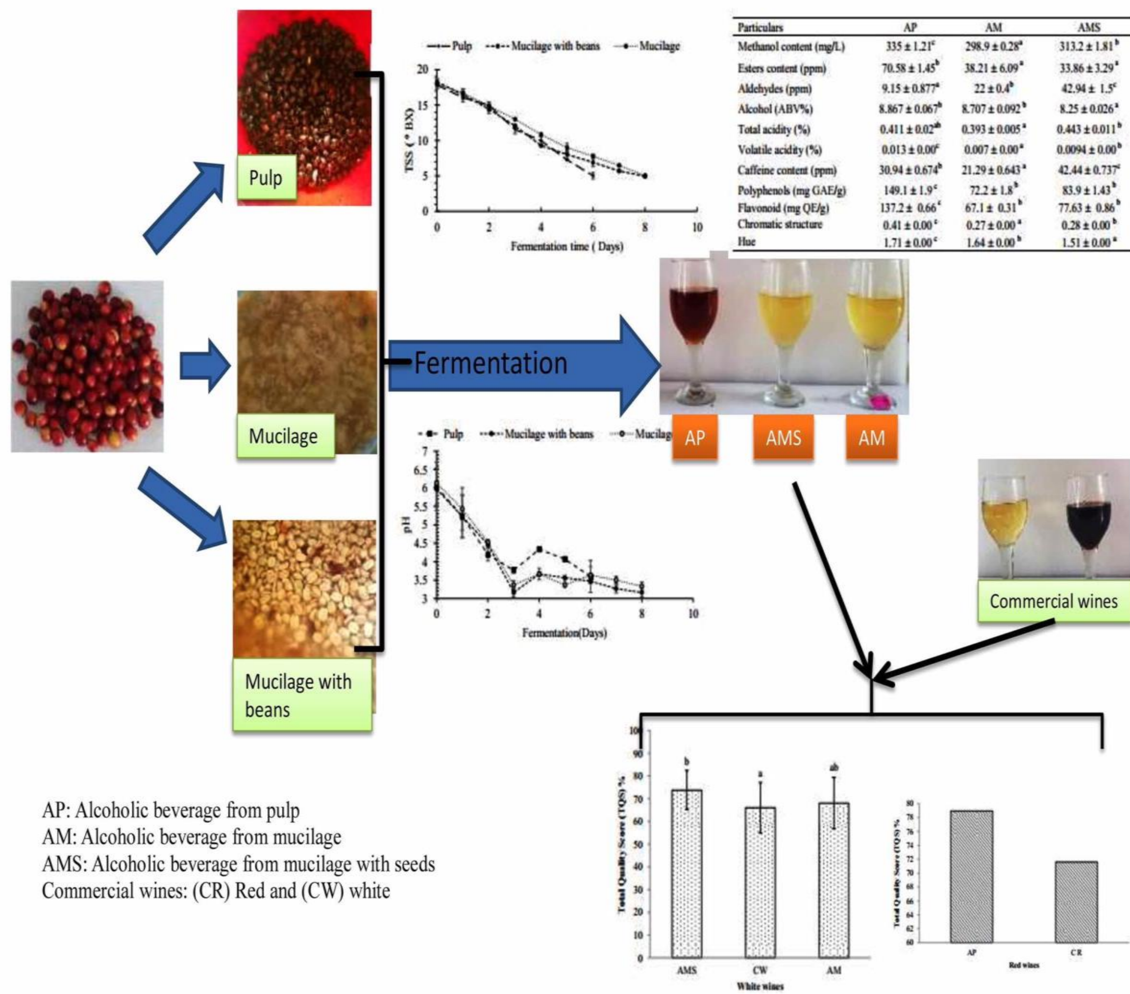


Fig. D.3 Calibration curve for caffeine content



Particulars	AP	AM	AMS
Methanol content (mg/L)	335 ± 1.21^{c}	298.9 ± 0.28^{a}	313.2 ± 1.81^{b}
Esters content (ppm)	70.58 ± 1.45^{b}	38.21 ± 6.09^{a}	33.86 ± 3.29^{a}
Aldehydes (ppm)	9.15 ± 0.877^{a}	22 ± 0.4^{b}	42.94 ± 1.5^{c}
Alcohol (ABV%)	8.867 ± 0.067^{b}	8.707 ± 0.092^{b}	8.25 ± 0.026^{a}
Total acidity (%)	0.411 ± 0.02^{ab}	0.393 ± 0.005^{a}	0.443 ± 0.011^{b}
Volatile acidity (%)	0.013 ± 0.00^{c}	0.007 ± 0.00^{a}	0.0094 ± 0.00^{b}
Caffeine content (ppm)	30.94 ± 0.674^{b}	21.29 ± 0.643^{a}	42.44 ± 0.737^{c}
Polyphenols (mg GAE/g)	149.1 ± 1.9^{c}	72.2 ± 1.8^{b}	83.9 ± 1.43^{b}
Flavonoid (mg QE/g)	137.2 ± 0.66^{c}	67.1 ± 0.31^{b}	77.63 ± 0.86^{b}
Chromatic structure	0.41 ± 0.00^{c}	0.27 ± 0.00^{a}	0.28 ± 0.00^{b}
Hue	1.71 ± 0.00^{c}	1.64 ± 0.00^{b}	1.51 ± 0.00^{a}

Fig. D.4 Graphical abstract of the study

Color plates

Plate. P.1 Pulping of coffee cherry

Plate. P.2 Weighing pulp

Plate. P.3 Weighing mucilage with seeds

Plate. P.4 Fermentation

Plate. P.5 Measurement of pH and TSS of samples

Plate. P.6 Pasteurization of samples

Plate. P.7 Distillation of samples

Plate. P.8 Samples for methanol
determination

P.9 Spectrophotometric analysis

P.10 Sensory analysis

P.11 Samples in wine glass

P.12 Samples in commercial bottles