STUDY OF PHYSICOCHEMICAL PROPERTIES OF COFFEE BEANS FROM DIFFERENT PROCESSING METHODS

by

Bipin Aryal

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2022

Study of physicochemical properties of coffee beans from different processing methods

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

By

Bipin Aryal

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

March 9, 2022

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This dissertation entitled *Study of Physicochemical properties of coffee beans from different processing methods by* Bipin Aryal has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

Dissertation Committee

1. **Head of the Department** _____

   (Mr. Navin Gautam, Asst. Prof.)

2. **External Examiner** _____

   (Mr. Anup Halwai, FTQCDO, Head, Dhankuta)

3. **Supervisor** _____

   (Mr. Yadav K. C., Asst. Prof.)

4. **Internal Examiner** _____

   (Mr. Kabindra Bhattarai, Asst. Prof.)

5. **RMC Member** _____

   (Mr. Basanta Kumar Rai, Prof.)

March 9, 2022

Acknowledgements

I would like to express deep gratitude to my respected supervisor Mr. Yadav K.C, Assistant professor, Central Campus of Technology for his precious advice, valuable suggestions, guidance, encouragement, motivation and constructive comments, which enabled me to carry out this thesis successfully.

I am also indebted to Assoc. Prof. Dr. Dil Kumar Limbu, Campus Chief, Central Campus of Technology, Hattisar, Dharan, for his kind co-operation and suggestion in providing an opportunity and facilities to perform this work successfully. I am also equally thankful to Asst.Prof. Navin Gautam, Head of Food Technology department CCT and Prof. Basanta Kumar Rai, Head of central department of technology for their generosity and co-operation.

I would like to express many thanks and gratitude to all the teachers and staff members, librarian for their support. My special thanks go to Mr. Prajwal Bhandari, Head Technical Assistant, CCT, Hattisar, Dharan and Mr. Mahesh Basnet for making my entire study in an enthusiastic and passionate environment. Also, many thanks go to my senior Mr. Nabindra Shrestha, my classmates Ashish chhetri, Shiva Kumar Shrestha, Upendra Pokharel, Pratik Rai, Naresh Shrestha, Chiranjivi Belbase, Sanjog Kharel and my juniors Mr. Ngahendra Adhikari, Abhishek Gajurel, Santosh Regmi and Manzil Shrestha for helping with great effort.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of the day.

Date of submission: March 9, 2022

Bipin Aryal

Abstract

Fully ripened coffee beans of species *Coffea Arabica* from Kakakelia farm of Dadabazar-3 Dhankuta, Nepal, were subjected to different processing methods with the aim of studying physicochemical properties of coffee beans with 6 different processing method which were wet fermentation, semi-dry fermentation, and fermentation with yeast strain, 0.25%, 0.5% and 1% concentration of Polygalacturonase (PG) enzymes. Different physical parameters (length, breadth, l/b ratio, bulk density) and chemical (caffeine, total phenolic content, DPPH, flavonoids and tannin) parameters were studied.

Physical properties of samples with different methods of processing had no significant difference. Caffeine, antioxidants, flavonoids, total polyphenols of samples were measured and found high in samples treated with 0.25% PG. There is no significant difference in tannin and caffeine content among different processing methods, but DPPH, total phenolic content, flavonoids were found significantly different ($p<0.05$) between samples. From Principal Component Analysis (PCA), it shows that 0.25% PG, 0.5% PG and yeast samples were best in terms of chemical properties and correlation plot shows that strong positive correlation between Total Phenolic Content (TPC) and DPPH, DPPH and flavonoids. This findings indicates that it is possible to improve quality of green beans by optimizing the enzyme concentration and methods of processing.

Table of Contents

List of tables

List of Figures

List of Abbreviations

Abbreviations	Full form
PG	Polygalacturonase
T.F.	Theaflavin
T.R.	Thearubigin
ºC	Degree Centigrade
KG	Kilogram
TSS	Total soluble solids
M.	Meter
CGA	Chlorogenic acid
GAE	Gallic acid equivalent
QE	Quercetin
wb	Wet Basis

List of Diagram

Part I

Introduction

1.1 General introduction

Coffee is the second largest traded commodity in the world after petroleum (Beniteza *et al.*, 2019). It is a genus of flowering plants in the Rubiaceae. Coffee species are shrubs or small trees native to tropical and southern Africa and tropical Asia. Two species of coffee plant Coffee Arabica and coffee canephora. The flatter and more-elongated Arabica beans are more widespread than Robusta but more delicate and vulnerable. Robusta coffee is cheaper to produce has twice the caffeine contain than Arabica and typically the choice for inexpensive coffee drinks (Myhrvold). Coffee is a high value cash crop which is 3 times higher than other cash crops. Coffee produced in Nepal is all Arabica variety of bourbon or mixed type and Nepali coffee is considered as organic coffee because of its aroma and body. In Nepal, coffee is produced above 1000-1600 m from sea level and over 40 districts of Nepal are considered best for coffee production (Acharya and Dhakal, 2014).

Coffee berry shows four anatomical fractions: the coffee bean proper or endosperm, the hull or endocarp, a layer of mucilage or mesocarp, and the pulp or exocarp. Coffee beans have a flat surface, and in the fruit, these flat surfaces face each other. Each bean is enveloped by a delicate spermoderm tissue known as the silver skin and is held in place by the membranous endocarp, also known as the parchment or coffee hull, that surrounds the individual beans and is brittle when dried. The hull is surrounded by a layer of mucilage 0.5-2 mm thick, that it is enclosed by a thick layer of spongy cells called the pulp. This characteristic has been used to advantage in the process that has been practiced for a long time to separate the beans from the rest of the fruit's structural components (Braham and Bressani 1979).

Processing method of coffee has a significant role for the quality of final coffee. However, presently, most of coffee is wet processed in which farmers harvest ripe fresh cherries and sell to the pulping center 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 and then the green beans are exported (Shrestha *et al.*, 2008). Quality of coffee in wet processing relies on operational processes in the pulping center and their management, quality of water used, pulping machine types, fermentation time, drying facilities, washing process, storage (Tiwari, 2010).

In Nepal, about 70% of the coffee produced is processed by the wet method, but farmers in rural areas are still practicing dry method because of transportation problem to sell their fresh cherry to the pulping centers. Wet method requires more care than dry one, which enhances the bean appearances thus rendering the batches more valuable. The pulp containing water and sugar, the moist parchment skin and beans all would ferment rapidly, rot if transported or stored as fresh. The entire coating i.e. covering of pulp, mucilage, parchment and the silver skin of the actual seed of the coffee fruit must be removed and the beans dried and cleaned to make it ready for the final consumption (Subedi, 2010).

In dry method, coffee beans are sorted first and then cherry is dried and when this is finished, the parchment and pulp are removed in one single operation, producing beans that are characteristically lower in acidity, sweet, smooth and more complex in flavor (Tesfa, 2019).

In wet method, the pulp is separated from the parchment. In this way slippery mucilage is exposed which is commonly removed by a process commonly called fermentation. This is followed by drying and washing of the beans in the parchment. (Subedi, 2010).

1.2 Statement of the problem

Coffee is one of most consumed beverage in the world (Blinova *et al.*, 2017). Green coffee is produced either by dry processing or by wet processing. The dry process is simple and inexpensive (Ghosh and Venkatachalapathy, 2014). In the present context, coffee processed by traditional method gives lower quality beans in terms of bioactive components.

In Nepal farmers generally use dry processing of coffee beans due to lack of knowledge in processing methods, non-availability of enough water for processing, since large amount of water is required for wet processing method, transportation problem, due to unavailability of demuciliger (Subedi, 2010). Accelerated fermentation methods lowers the processing time of coffee beans and quality in final product is superior to dry and wet processed coffee (Haile and Kang, 2019a). There are very few works on research of the coffee processing

methods in Nepal. So, this study focus on different processing methods of coffee beans and their effect on final quality.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was ′effect of different processing methods on quality of green coffee beans.′

1.3.2 Specific objectives

The specific objective of this study was

1. To study time required for fermentation by different processing method.
2. To study different physical, physiochemical properties of coffee beans.
3. To study the effect of different processing method on quality and bioactive attributes of coffee beans.

1.4 Significance of study

The proposed work was focused on different coffee processing methods for demucilation such as Semi-dry, wet, enzyme added and natural fermentation methods and hence time required for different processing methods and their effect on final quality of coffee beans which is useful for many researcher and industrial sector. This study can be helpful to reduce the time of processing without affecting the final quality of coffee beans and also, reduction of waste. In Nepal, the production of the coffee is increasing and people are attracted to this business day to day. Among the various cash crops for commercialization, coffee is income generation opportunities in the mid hills of Nepal (Chaudhary *et al.*, 2008). Some Districts like Gulmi, Palpa, Argakhanchi, Lalitpur, Tanahu, Kavre, Sindhupalchowk, Lamjung, Kaski, Gorkha, Syangja, Parbat, and Baglung are successfully growing and producing coffee beans. So if farmers and producers know about best way of processing of depulped beans for mucilage removal which is simple without compromise in quality will help to grow our coffee production and consumption nationally and we can export our coffee to foreign market as well.

1.5 Limitation of the study

- Coffee beans are carried for over 6 hours after harvesting for removal of exocarp.

1.6 Delimitation of the study

1. Roasting and sensory analysis of green beans was not done.
2. Only 1 variety of coffee sample was used.
3. Not all physicochemical analysis of beans were done.

Part I

Literature review

2.1 Coffee production and consumption

Coffee is relatively new crop to Nepal and grown by small farmers however it holds enormous market potential. Study of Agriculture Development reported that 229 ton coffee was produced in Nepal from 1911 ha with more than 70% of this exported in the year 2013/14 with involvement of 27000 small farmers. However, 67 tons coffee was imported in the same year. There is huge international and internal demand of Nepali coffee and also enormous potentiality of area expansion. Currently, coffee is grown in 43 districts of Nepal out of which 23 districts are commercially producing coffee beans. Nepalese coffee enters into international market as specialty coffee and there are 11 registered processing and marketing facilities operated by co-operatives and independent merchants. In Nepal coffee is produced in steep, Marginal and shady land since the production cost is less and income per tree is 1 to 6 dollar per annum and considered as attractive cash crop (Acharya and Pun, 2016).

Coffee is one of the high value cash crops grown in Nepal with potential high quality for domestic as well as international niche market. 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 (Nepal, 2007).

In Nepal, coffee was initially known as the drink of the foreigners, tourists and expatriate, but nowadays, it has become popular among the Nepalese and therefore has received numbers of domestic consumers. In terms of area coverage and production, Nepalese coffee has tiny presence in comparison with the world production and area. However, Nepalese highland and organic coffee is known in the international markets owing to its high quality cupping and sound aroma (Poudel *et al.*, 2009). Nepalese coffee has high demand in Japan, America, South Korea, Germany and the Netherlands. However, in comparison with demand Nepalese coffee has low production and below the standard quality specified by the developed countries. Most of the coffee grown in Nepal is considered organic as coffee is grown in the natural condition and most of the farmers do not use chemical fertilizers and

pesticides during cultivation and processing. There has been growing interests from both government and non-government sectors for promoting organic coffee and farmers are also motivated to produce coffee owing to higher demand in the international market. Considering the importance of high value crops including coffee and with the view of expanding the production and productivity, government has promulgated several agricultural policies, strategies and guidelines for the promotion of production, processing and marketing of high value crops. For instance, the Coffee Policy 2004 was promulgated with an aim of fostering production and marketing of coffee. In this context of emerging coffee as a valuable commodity, it is worthwhile to evaluate the focus and implication status of agricultural policies for promoting the production, processing and marketing of coffee in the country (Tiwari, 2010).

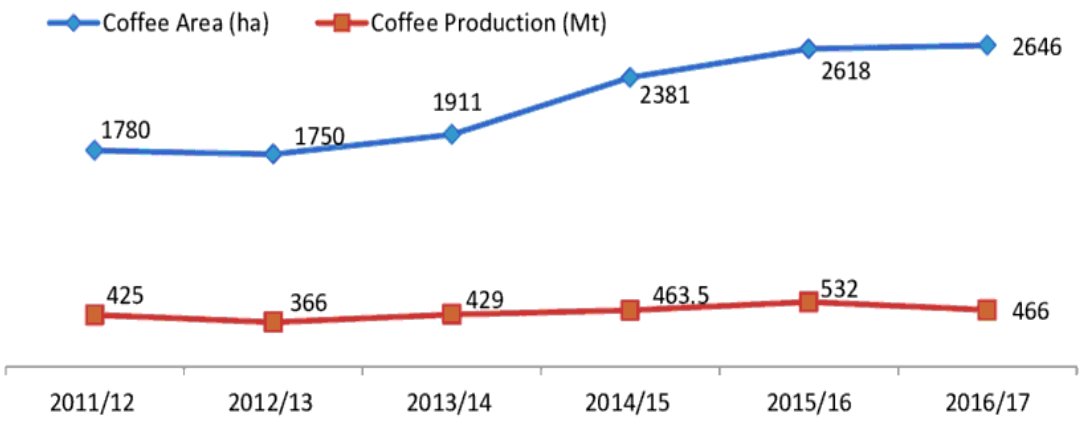


Fig. 2.1 Area and production of coffee in Nepal from 2011 to 2017

Source: (Acharya, 2019)

Coffee is the most popular beverage of world after water and it is the second most traded Commodity after raw oil. The vast majority of world coffee is the Arabica species. In February 2021, the Information Commissioner's Office composite indicator continued its upward trend, averaging 119.35 US cents/lb. as prices for all groups. This is the highest monthly average since October 2017 when the ICO composite indicator reached 120.01 US cents/lb. Global exports in January 2021 totaled 10.21 million bags, compared with 10.59 million bags in January 2020, and shipments in the first four months of coffee year 2020/21 increased by 3.7% to 41.88 million bags. Exports from the world's largest coffee-producing

region, South America, increased by 15.5% to 23.26 million bags as shipments from Brazil grew by 24.3% to 16.77 million bags. However, exports from the other three regions declined in October 2020 to January 2021. Shipments from Asia &Oceania decreased by 3.9% to 12.19 million bags. Africa's exports decreased 13% to 3.81 million bags as shipments from three of the region's five largest producers declined. Shipments from Central America & Mexico fell by 17.5% to 2.62 million bags as parts of the region were severely affected by hurricanes Iota and Eta (Ico, 2020).

2.2 Coffee Consumption

Coffee consumption by people is increased day by day and they prefer coffee over tea. There are several issue regarding the less consumption of nepali coffee some of which are high price of Nepali coffee, less awareness on Nepalese coffee since production of coffee in Nepal is new and number of consumers are unaware about Nepalese coffee followed by lack of marketing, unavailability of Nepalese coffee in market and tendency of export oriented market of Nepalese coffee (Karki and Regmi, 2016).

2.3 Different variety of coffee

Coffee belongs to the genus Coffea which in turn is a member of the family Rubiaceae, which is one of the largest flowering plant families containing some 500 genera and 6000 species. The number of varieties discovered by different author's ranges from 26 to 100. Some varieties of coffee are (Teketay, 1999).

- Arabica coffee *(Coffea Arabica)*
- Robusta coffee *(Coffea canephora)*
- Liberica coffee *(Coffea liberica)*

Coffea Arabica was first described by Linnaeus in 1753. Its fruits are round, smooth, slightly bitter, and chocolaty in color, with a smooth crust and an intense aroma. Brazil is the leading producer and exporter of *Coffea Arabica (Oliveira et al., 2011)* and the second largest consumer. There are currently 11 varieties frequently planted in producing countries. These varieties are the result of breeding projects intended to achieve early maturity, a size suitable for mechanical harvesting, rust resistance, high-density planting, and improved beverage quality. The Coffea canephora species, also known as Robust or Conillon coffee, is stronger and more productive than Arabica coffee, but its flavor is less popular with consumers, and

it is mainly used in the formulation of blends and espressos. Indonesia is the largest producer of this coffee, followed by Brazil, Vietnam, the Ivory Coast, and Uganda. The two species Coffea *Arabica* and *C. canephora* feature differing levels of caffeine, chlorogenic acids, sucrose, and amines (Schwan *et al.*, 2012b).

Table 2.1 Classification of coffee

Classification of coffee	
Kingdom	vegetable
Sub-kingdom	Angiosperm
Class	Dicotylendon
Sub-class	Sympetalae
Order	Rubiales
Family	Rubiaceae
Genus	Coffea
Sub-genus	Eucoffea
Species	Canephora
	Liberica

Source: (Author and Vogt, 2019)

2.4 Composition of coffee beans

Table 2.2 Composition of coffee beans

Chemical composition (dry weight)	Coffea Arabica	Coffea canephora
Sucrose (%)	9.3	5.45
Putrescine-free(μg/g)	47.9	11.1
Linoleic acid (%)	6.1	3.7
Oleic acid (%)	8.3	12.3
Chlorogenic acid (%)	4.1	11.3
Caffeine (%)	1-2	>3
β-Tocopherol (μg/g)	58.46	13.1

Source: (Schwan *et al.*, 2012a)

2.5 Anatomical structure of coffee

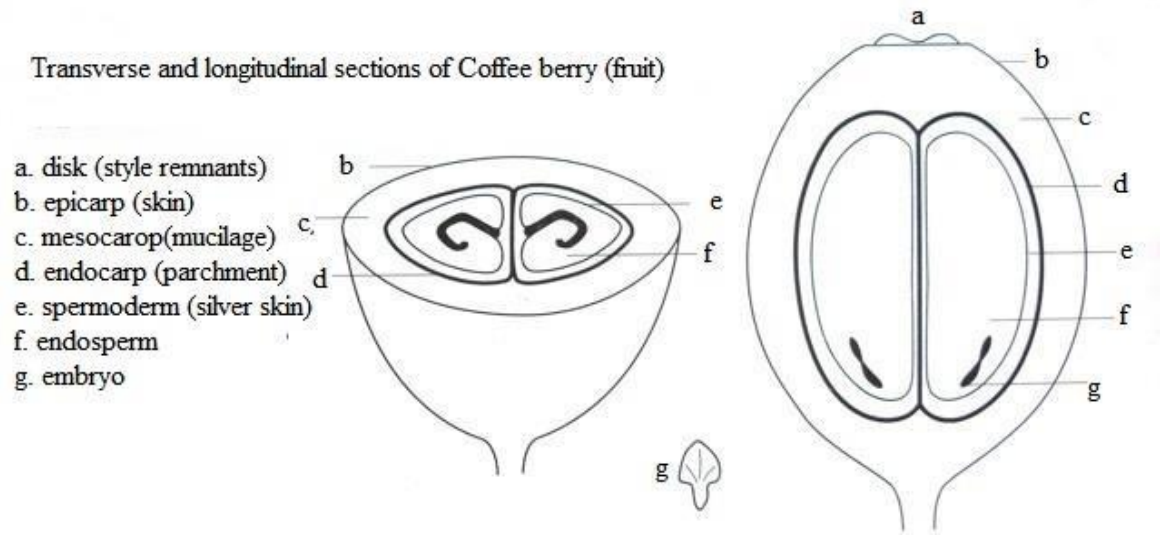


Fig 2.4 Anatomical structure of coffee

Source: (Wintgens, 2009)

2.5.1 Pericarp

The pericarp is the external 3 layers of the fruit: the exocarp (skin), mesocarp (mucilage), and endocarp (parchment).

2.5.2 Exocarp

The exocarp, likewise described 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 main walls which contain chloroplasts and can take in water). The color of the exocarp at the start of fruit advancement is green due to the presence of chloroplasts which then vanish as the fruit develops. Color upon maturation relies on the coffee range, however, is most typically red or yellow (Borem, 2008).

2.5.3 Mesocarp

The mesocarp, likewise described as the mucilage, is the flesh of the coffee fruit. While "pulp" can often describe exclusively the mesocarp, the term normally describes a mix of the exocarp and part of the mesocarp gotten rid of throughout pulping. In unripe coffee fruit, the tissue is stiff. With maturation, pectolytic enzymes break down pectic chains, leading to an insoluble hydrogel that is rich in sugars and pectins. Research studies have actually revealed that the mucilage/water ratio of the mesocarp enhances as growing altitude boosts. In the damp processing technique, this mucilage layer is eliminated through managed fermentation. In the dry technique, the mucilage, together with the exocarp and endocarp, is left undamaged throughout drying (Subba, 2021).

2.5.4 Endocarp

The endocarp, or parchment, is the innermost layer of the pericarp and is the hull that covers the coffee beans. It is formed of 3 to 7 layers of sclerenchyma cells (fibrous cells that act as the primary assistance cells in plants). The cells of the endocarp harden throughout coffee fruit maturation, hence restricting the last size of the coffee seed, or bean.

The silver skin, likewise called the perisperm or spermoderm, is the outermost layer that covers the seed. It is formed from the nucellus, or main part, of the ovule. Typically some residues of the silver skin continue to be on the bean pre-roast and come off throughout coffee roasting as chaff. The silver skin might be polished off of the bean; nevertheless, it is

typically accepted that this lessens coffee taste. It has actually likewise been proposed that the presence of a huge quantity of silver skin on milled coffee suggests coffee chose prior to its perfect ripeness. In some areas, the silver skin might handle a darker tone, in which case the beans are called fox beans. Fox beans are ruled out to be a flaw (Husniati and Oktiani, 2019)

2.5.5 Endosperm

The endosperm is the primary reserve tissue of the seed and is composed of only one tissue though the cells in the outside and indoor part of the endosperm differ in oil material and cell wall density. The chemical material of the endosperm is of utmost value because it is the precursor to the taste and fragrance of roasted coffee. The chemical substances discovered in the endosperm can be classified as soluble or insoluble in water. The water-soluble substances are caffeine, trigonelline, nicotinic acid (niacin), a minimum of 18 chlorogenic acids, mono-, di-, and oligosaccharides, some proteins and minerals, and carboxylic acids. Parts insoluble in water consist of cellulose, polysaccharides, lignin, and hemicellulose, in addition to some proteins, minerals, and lipids (Borem, 2008).

2.5.6 Embryo

The embryo is composed of a hypocotyl (embryo axis) and 2 cotyledons and is 3-4 mm long. Coffee seeds sprout through epigeal germination, where the hypocotyl lengthens and presses the seed up above ground. The initial cotyledons remain underground; nevertheless, brand-new cotyledons will form (Husniati and Oktiani, 2019).

2.6 Physical properties of coffee beans

2.6.1 l/b ratio

The l/b ratio is defined as the ratio of length to breadth of the grain. It is used to determine the shape of the individual grain. The value of l/b ratio above 3 is generally considered as slender and below 3 is generally considered as bold (Rather *et al.*, 2016). l/b ratio of coffee beans is in the range of 0.4 -0.7 (Severa *et al.*, 2012b).

2.6.2 Bulk density

Bulk density is defined as the weight per standard volume measured in a standard manner. It is also known as ‘test weight’, ‘bushel weight’ or ‘specific weight’. The factor that affects the bulk density are insect infestation, excessive foreign matter and moisture content. Bulk density is required for the design of storage, transport and separation systems. It has also been used to determine the dielectric properties (Ashwin Kumar *et al.*, 2017).

According to the study of (Nakilcioğlu-Taş and Ötleş, 2019) bulk density of green coffee beans is 0.43 ± 0.015.

2.6.3 Particle density

Particle density, previously termed ‘relative density’ or ‘specific gravity’, is sometimes used as the basis of the broad classification of aggregates into normal, lightweight or heavy varieties. The particle density of aggregates is the ratio of the mass of a given volume of material to the mass of the same volume of water, but different values are obtained in determination depending upon the allowance made for pores (Haan *et al.*, 1994).

According to the study of (Nakilcioğlu-Taş and Ötleş, 2019) particle density of green coffee beans is 0.92 ± 0.009 .

Particle density and bulk density is affected by pore space, texture and organic matter content so different coffee variety may have different physical properties.

2.6.4 Porosity

Bulk porosity is a value calculated by dividing the volume of the voids in the coffee by total coffee volume. This property is related to the particle density and bulk density of coffee (Ortega-Rivas, 2009).The porosity development in coffee samples significantly increased with increasing the degree of roasting process in the range of 43.68 ± 0.52 to 62.06 ± 1.15%. In roasting process, since the simultaneous weight loss and volume increase, the density of coffee decreases and porous structure develops. This development is highly dependent on the roasting conditions. The differences between particle density and bulk density of coffee samples in this study were an indicator of coffee samples which had comparably high internal/external (or both) porosity. Roasting-induced changes which occur in pore structure have a significant effect on the final product quality. The loss of flavor compounds and the

following change in flavor profile during storage are related to the pore structure of coffee due to which porosity in coffee is one of the major physical characteristics. Porosity of coffee is increased owing to both the degradation of the intercellular matrix and the destruction of the cells during roasting process. After roasting, the similar change in porosity of coffee samples has been also observed into the results (Nakilcioğlu-Taş and Ötleş, 2019).

2.7 Moisture content

The amount of moisture in coffee parchment and beans is important for two reasons, coffee that is too high or too low in moisture will not maintain high cupping quality, and customer trade coffee, not water. Green coffee that is high in moisture (greater than 12 percent w.b.) can deteriorate due to bacteria, mold or yeast, especially if the seed is killed. If the seed remains alive, enzymatic activity will cause the cupping quality to change. In any case, the parchment coffee moisture level should be lowered to below 12 percent soon after harvest. When the ambient relative humidity is about 70 percent, coffee beans will gradually equilibrate to about 12 percent moisture. Thus green coffee beans is generally dried to 12 percent moisture, and bought and sold at this moisture percentage. If the bean dries to below 9 percent moisture, it will shrink enough to become distorted, which will give the appearance of low-quality coffee. Coffee of consistent quality commands a higher price. The range for maintaining quality is quite broad, making it easier to know the coffee is at the correct moisture. Precisely knowing the coffee is at 12 percent moisture is not necessary except in the market. Knowing the coffee is between 12 and 9 percent moisture at all times after it is dried is important to ensuring that your coffee has the best quality at the time of sale (Gautz *et al.*, 2008).

2.8 Processing of coffee beans

Processing method of coffee has great role for the quality. However, presently, most of coffee is wet processed in which farmers harvest ripe fresh cherries and sell to the pulping center 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 and then the green beans are exported (Shrestha *et al.*, 2008). quality of coffee in wet processing rely on operational processes in the pulping center and their management, quality of available water used, pulping machine types, fermentation time, drying facilities, washing process, storage (Tiwari, 2010).

In Nepal, about 70% of the coffee produced is processed by wet method, but farmers in rural areas are still practicing dry method because of transportation problem to sell their fresh cherry to the pulping centers. Wet method requires more care than dry one which enhances the bean appearances thus rendering the batches more valuable. The pulp containing water and sugar, the moist parchment skin and beans all would ferment rapidly, rot if transported or stored as fresh. The entire coating i.e. covering of pulp, mucilage, parchment and the silver skin of the actual seed of the coffee fruit must be removed and the beans dried and cleaned to make it ready for the final consumption (Subedi, 2010).

2.8.1 Dry processing

In dry method, coffee beans are sorted first and then cherry is dried and when this is finished, the parchment and pulp are removed in one single operation. This is simple method with less labor cost. The cherries are either sun-dried or machine dried with the outer fruit intact until the fruit gets moisture content of 12%. After drying they are de-hulled mechanically, producing beans that are characteristically lower in acidity, sweet, smooth and more complex in flavor than wet processed coffee beans (Tesfa, 2019).

2.8.2 Wet processing of coffee

In this wet method, the pulp is separated from the parchment. In this way slippery mucilage is exposed which is commonly removed by a process commonly called fermentation. This is followed by drying and washing of the beans in the parchment. Removal of parchment by hulling gives the clean coffee. Many steps in the wet method of coffee processing make it rather expensive but, if properly carried out, it gives a very high quality coffee (Subedi, 2010).

In Nepal, wet processing technology has been introduced recently for quality export of green beans. Several wet processing plants are established in the major coffee producing areas of the country.

2.9 Harvesting

Coffee is generally harvested when berries turn dark red color which is about 8-9 months after flowering has taken place. The harvest generally starts from October and continues up

to March. Ripe fruit can be plucked by hand, or picked with small rakes, or else with poles depending up on the availability of the labor (Subedi, 2010).

2.10 Cherry sorting and grading

The cherry is sorting before pulping which helps to eliminate the immature, pest damaged and dry cherries as well as the leaves and other foreign materials present. After sorting coffee beans are pulped to remove outer layer (Mutua, 2000).

Grading of fresh harvested coffee is based on ripeness. Ripe and unripe cherries are separated to facilitate processing and for product quality. Sorting and grading in wet processing can be done in washing vats. Foreign matters as well as cherry of different ripeness and dryness are separated in a washing vat due to their density difference. Stones and heavy impurities are removed from the bottom, hard, partially dried cherries float and are discarded from the top (Willson, 1999).

2.11 Pulping

It is the process of mechanical removal of the pulp from the fresh cherry to have parchment coffee. The flesh and skin of the fruits are left on one side and the beans, enclosed in their parchment covering, on the other side. The lighter immature beans are then separated from the heavier, mature beans through specially designed washing channels or by shaking the beans through a strainer into a tank of water (Hicks, 2002).

2.12 Fermentation

It involves the process that allows the mucilage layer on the parchment to be washed off easily. The beans are stored in fermentation tanks for 2-3 days depending up on the weather condition during which time, the slimy layer is separate from its parchment like covering, by natural enzymes. Completion of fermentation is determined by washing a bit of the parchment with clean water and then feeling the coffee with the hand. A gritty feel is an indication of the completion of fermentation. Different chemicals like lime, alkaline carbonates, yeast, Enzymes added fermentation can also be used for removal of the mucilage which precipitates the pectin in the form of soluble pectates, which are then easily removed by washing (Mutua, 2000).

Coffee fermentation is critical for removing mucilage from parchment coffee. Coffee mucilage contains polysaccharides (pectin), cellulose, and starch. The mucilage can prolong the time needed to dry the coffee beans and, in some cases, also lead to mold development, which reduces the final quality of the coffee. The fermentation process is facilitated by enzymes that naturally occur in the coffee fruit and microflora acquired from the environment. Microorganisms (yeast, bacteria, and fungi) play a major role in degrading mucilage by producing various enzymes, alcohols, and acids during the fermentation process. There are several commercially available enzymes for coffee fermentation. Current research indicates that coffee fermentation is becoming more popular for producing specialty coffee it reported that various physiological changes occur in grains during fermentation, such as decreases in water content, simple sugars and the development of aroma and flavor precursor. This research will provide a clear understanding of coffee fermentation and its impact on major coffee quality attributes, the role of microorganisms in coffee fermentation and can direct future research into commercializing potential starter cultures (Vaast *et al.*).

2.12.1 Wet fermentation

In this method coffee pulp is removed by pulping by different processes and beans with mucilage is are placed with vats having water where processing takes place. Water should be removed at fixed interval so it required substantial amount of water (Braham and Bressani, 1979).

2.12.2 Natural fermentation

Natural fermentation is considered as best processing method because it improves both raw and roasting quality but it is more time consuming, costly and laborious and more weight loss than demuciliger. However natural fermentation required strict supervision on temperature and longtime of fermentation and good equipment and sustainable amount of water is required (Gure *et al.*, 2014).

Natural fermentation required 48 to 72h for mucilage removal and there is quality defects due to long fermentation and sometimes stinker beans are produced due to uncontrolled fermentation.

2.12.3 Different enzymes added fermentation

If coffee beans are fermented for long time there is quality defects in coffee beans due to uncontrolled fermentation and also economic losses are occurred. To accelerate the digestion of mucilage different pectin enzymes such as pectinases, pectase, polygaluctoronase etc. are added externally, pulped coffee beans is soaked with enzyme solution (1:20 w/v) .The pulped beans are soaked with enzymes in static condition until mucilage was removed and samples are taken out in interval of 30 minutes to check the completion of fermentation which is done by traditional hand feel method (Murthy and Naidu, 2011a).

Improvements were achieved by the addition of crude enzyme extract containing Pectinases. Organic acids, CGAs and CGLs in coffee brew were reduced after enzyme treatment. Demucilage of coffee beans by enzyme treatment could save much process time which is an important cost of processing. Besides, shorten the fermentation time by enzyme treatment could control the growth of microorganisms during fermentation. This treatment is also eco-friendly because of exclusion of usage of strong base for acceleration of mucilage degradation. In summary, the crude enzyme extract from *A. tubingensis* was useful for eliminating coffee mucilage and reducing beverage acidity and bitterness. This extract could be used to control the quality of coffee beverages and promote coffee processing (Tai *et al.*, 2014).

2.12.4 Fermentation using alkali

For alkali treatment we use 5 or 10 percent NaOH for 48h hours and after the fermentation is completed it is washed with water (Ulloa Rojas *et al.*, 2002).

2.13 Yeast as starter culture in coffee fermentation

In Coffee Processing it is widely believed that coffee fermentation by inoculating different microorganisms, processed by different methods, increase the production of volatile compounds and consequently improve the flavor and aroma of the beverage (Bressani *et al.*, 2020). The different fermentation methods used in the step of mucilage removal can change the chemical composition of green coffee through the action of microbial and/or endogenous coffee enzymes (Avallone *et al.*, 2001). The mucilaginous layer is formed of polysaccharides (especially pectin). The pectolytic enzymes (pectinases) from microorganisms (bacteria or yeasts) achieve the degradation of the pulp and mucilage to form alcohols and acids (acetic,

malic, lactic, butyric and other long-chained carboxylic acids) (Silva, 2014). The most important enzymes involved in coffee fermentation are polygalacturonase (PG), pectin lyase (PL) and pectin methyl esterase (PME).

The time required for this step is a function of the processing methods. The selection of microorganism for the fermentation step is based on many criteria, such as P.L. secretion, production of volatile compounds, the physical and chemical changes and the lack of production of undesirable metabolites (Silva *et al.*, 2013). The starter cultures (selected as single or multiple strains) are used to assure better fermentation control and predictability of the final product. The yeasts used as starter cultures influence the types of the compounds produced during fermentation and after roasting, which are correlated with the sensorial characteristics perceived during the cupping test. In this regard, the starter yeasts modulate the sensory characteristics of the beverage and their utilization represents an alternative for sensorial differentiation of coffee. Different sensorial profiles of coffee increase the final value of the product (Bressani *et al.*, 2020). Yeast metabolism triggers the hydrolysis of macromolecules yielding reducing sugars, amino acids and chlorogenic acids, which are important precursors of aroma. The sensorial perceptions are correlated with chemical compounds identified and compared with the procedures without microbial starters and it was demonstrated that the starter cultures have a great impact on coffee fermentation and directly affect the quality of the final beverage, because there is a causal relationship between yeast cultures, organic acids and volatile profiles. The fact that yeasts possess both high pectinolytic activity and an important role in the demuciling of coffee was known since early studies, when it was revealed that pectinolytic yeasts such *as Saccharomyces marxianus, s. bayanus, S. cerevisiae var. ellipsoideus and Schizosaccharomyces* spp. have a role in the degradation of the mucilage layer of Robusta coffee (Agate and Bhat, 1966). In fact, it was shown that yeasts are involved from the very start of the process, as they lay on the cherry surface, taking part in the natural fermentation of coffee. Later, by adding a mixture of selected pectinolytic yeasts to the normal fermentation, the mucilage-layer degradation is accelerated. Especially, a mixture of Saccharomyces species was remarkably effective leading to the complete elimination of pectic substances within 7 to 8 h.

Fermentation of green coffee beans with yeasts has positive impacts, increasing antioxidant activity and polyphenol and flavonoid contents. It also decreases the tannin content, which is usually considered the cause of the astringent characteristics and bitterness

of coffee. It is clearly observed that fermenting coffee beans with different yeasts influence the coffee quality parameters differently. This is an indication to consider using various species and strains of yeasts will be important to select the better performing yeasts according to their potential (Haile and Kang, 2019a).

2.14 Effect of fermentation on flavor

2.14.1 The positive impact of fermentation on coffee flavor

Coffee is popular as a non-alcoholic drink because of its pleasant aroma and refreshing flavors. Coffee beans themselves contain all the precursors required to provide the standard coffee flavor and aroma during roasting. However, the fermentation process can increase the diversity of coffee aroma and flavor compounds, it is more than 700 volatile and nonvolatile compounds that contribute to coffee flavor have been identified (Farah *et al.*, 2006). The coffee species, variety, geographic origin, and level of roasting determine the constitution and quantity of the flavor resulting from these compounds. A supporting report indicates that factors during the pre-harvest and postharvest processes also critically affect the coffee aroma during coffee fermentation, microorganisms produce diverse metabolites. Microbial activity and the extent of fermentation determine the concentrations of free sugars (e.g., glucose and fructose) and free amino acids that continue to surround the bean and subsequently contribute to the production of Maillard compounds and volatiles during the roasting process. Wet-processed coffee has superior aroma qualities to dry-processed coffee because of the aromatic compounds produced during the removal of the mucilage layer in wet processing .The selection of appropriate microorganisms that have a positive impact on coffee flavor and aroma during fermentation is critical, and the fermentation process should be controlled to achieve this positive impact (Gonzalez-Rios *et al.*, 2007).

2.14.2 The negative impact of fermentation on coffee flavor

The major challenge in coffee fermentation, according to several studies, is the difficulty of controlling the process. Over fermentation is one of several dilemmas described by coffee farmers and scientists .The fermentation process must be well controlled to ensure the development of beneficial microorganisms that produce a high-quality beverage with a good aroma. When fermentation fails, it results in the development of spoilage microorganisms that adversely affect the coffee's aroma and flavor. Coffee beans resulting from such

fermentations are often referred to as “stinkers” (Frank *et al.*, 1965). Under fermented coffee beans contain residual mucilage and sugar that prevent drying and create a good environment for the development of spoilage bacteria and fungi. Over fermentation encourages the production of undesirable chemical compounds, notably propionic and butyric acids, which confer off-flavors, such as an onion taste it is report that these acids should not be present in a concentration greater than $1\,mg{\cdot}mL^{-1}$. Species of the *Bacillus* genus, especially *B. megaterium*, may be responsible for the propionic acid found in coffees processed via dry or natural processing (Silva *et al.*, 2008)

As described above propionic acid is detected in high concentrations only when the fermentation process proceeds for longer than its optimum duration. *Enterobacteriaceae* and acetic acid bacteria lead to the production of excessive acetic acid during prolonged fermentation in dry processing. Over fermentation may also produce short-chain fatty acids and their esters, such as *2-methyl butanoic acid ethyl ester, 3-methyl butanoic acid ethyl ester, and cyclohexanoic acid ethyl ester*. These can be detrimental to coffee quality if they are present at concentrations higher than 1.8, 13.9, and $14\,mg{\cdot}kg^{-1}$, respectively. Furthermore, the growth of filamentous fungi and mycotoxins, which can generate off-flavors, should be controlled by the management of the fermentation and drying processes (Bade-Wegner *et al.*, 1996).

2.15 Polygalacturonase enzyme

Polygalacturonase is a hydrolytic enzyme, which acts on polygalacturonic acid (PGA), hydrolyzing α-1, 4, glycosidic bonds of pectic acid. According to its mode of action, P.G is classified as endo-PG which result in random degradation of the pectic chain and exo-PG which result in the degradation of non-reducing free ends of the pectic chain. The exo-PG has not been found to have a great effect on the solubility of pectin and endo-PG depolymerizes pectic acid in a random way, resulting in a rapid decrease in viscosity (Yahia and Carrillo-Lopez, 2018).

Potential microbial strains like *Moniliella SB9, Penicillium spp. and Aspergillus* spp. are good sources of commercial pectinase. Pectinases are now an essential part of the fruit juice industry, as well as having various biotechnological applications in the fermentation of coffee and tea, the oil extraction processes, and the treatment of pectic waste water from the fruit juice industry. Pectinase lower down the viscosity of fruit juice during the clarification

process through the degradation of pectin substance in fruit juice and getting better pressing ability of pulp, simultaneously jelly structure are breaking down and increases the yields of fruit juice. Another significant application of pectinase enzymes in industrial processes is the refinement of vegetable fibers during the starch manufacturing process, such as the curing of coffee, cocoa and tobacco, canning of orange segments, and extracting sugar from date fruits (Singh and Kumar, 2019).

Hydrolysis of protopectin and degradation of pectin during fermentation of coffee beans using pectinase is 3 to 3.5 hours for commercial pectinases. The quality in terms of physical parameter like color, smell, defects is similar to naturally fermented beans and the organoleptic quality such as bitterness, aroma, flavor, neutrality has been associated with better quality than wet processing method and leaching of soluble component in water is also reduced (Murthy and Naidu, 2011b).

2.16 Drying of coffee beans

Drying is one of the most important steps in the coffee processing. The use of natural sun drying process of coffee in terraces is still very common among the coffee producers however it requires high labor, it is a time requiring operation and on dependency on the climatic conditions. As the coffee production increases the sun drying operation in terraces happen to be problematic in terms of coffee production operation and the mechanical drying becomes a need due to the possibility of advancing the harvesting operation, allowing to harvest better coffee in terms of quality and quantity and making possible to destine usable areas for other activities. Drying is mainly concern for the degradation of the moisture content up to a certain limit12% (w.b). Drying diminishes the respiration rate of the product and increases the storage time with the minimum possible loss. If the beans are over dried it will be brittle in nature and if moisture content is more than safe storage moisture then there is a probability of mold growth in the beans for further processing. Conventionally there are 2 types of drying techniques used in the coffee processing that is sun drying and mechanical drying. The initial moisture content of the coffee is about 55-60% and after drying the final moisture content should be around 12% (w.b.) and the drying should be even and homogenized to obtain the proper color, size and to get rid of pests for the longer storage time. Generally coffee beans can be stored almost for 8 months but the pest problem and increase in moisture content during storage period are the problem.

It has been pointed out that beverages from coffee drying processed by different methods have significant difference. Coffee produced by the wet method has less body and higher acidity; it is also more aromatic than coffee produced by the dry method, resulting in a higher acceptance by consumers. It is currently accepted that the metabolic reactions in the coffee fruits that occur during different types of processing can affect the chemical composition of beans and thereby affect beverage quality. Drying characteristics and kinetics of coffee berry under the drying temperatures of 40°, 50° and 60°C also observed the moisture sorption isotherms and isosteric heat of sorption of coffee in different processing levels (Corrêa *et al.*).Shrinkage evaluation of different coffee berries during the time of drying process concluded that moisture content in the coffee berries affects its physical properties causing significant decrease in the superficial area, volume and diameter during the drying process. Different variety of coffee has different shrinkage behavior (Afonso *et al.*, 2003).

It has been pointed out that beverages from coffee drying processed by different methods have significant difference (Correa *et al.*, 2006). Coffee produced by the wet method has less body and higher acidity, it is also more aromatic than coffee produced by the dry method, resulting in a higher acceptance by consumers.

2.16.1 Sun drying or natural drying

Fresh coffee cherry assuming with a 4 to 6 cm layer in the sun requires energy up-to the extent of about 17000 kJ/kg according to FAO research. Removal of fruit tissues in the pulping step of wet-processing reduces this energy requirement by mechanically removing water and by removing tissues that inhibit water loss. However, the decision as to which processing system to apply is much more complex than mere consideration of drying efficiency. It depends from place to place. Previously it was considered that sun dried green coffee are the best in quality but now a days increase in the production and as a seasonal crop it is very difficult to go for only sun drying method. Researches also proved that mechanically dried and the sun dried coffee both gives to some extent same quality. There are different types of sun drying method; Drying in ground in presence of sun is another mode of natural drying. Depending on climatic conditions, sun drying of coffee in patios takes from 7 to15 days for parchment and from 12 to 21 days for cherries. Parchment requires more careful handling than cherry to avoid cracking and physical damage to the beans. Raking must be gentler. In tropical areas parchment is often covered during the hottest hours

of the day to avoid cracking caused by overheating. Drying racks are used which makes the coffee clear and avoid contamination from the ground. Racks are also more exposed to wind; this helps remove saturated air which helps to shorten the drying time. Plastic sheds are light wooden or metallic structures with a plastic roof and walls. The flat floor is made of concrete or tiles like a patio. Transparent or translucid plastic creates a greenhouse effect that may raise the temperature 10-15°C. Fans may be used to help remove the saturated air. Drying procedures are similar to those in a patio except that coffee is permanently sheltered from dew and rain (Ghosh and Venkatachalapathy, 2014).

2.16.2 Mechanical driers

In mechanical drying the beans are heated by the passage of hot air which also carries the moisture away. Temperatures must be monitored during natural and artificial drying. Coffee temperature should not exceed 40°C for parchment and 45°C for cherries. It is often thought that overheating can only occur in mechanical dryers. There are mainly 2 types of dryers, static and revolving. In revolving dryers, there are tray dryers with stirrer, vertical dryers and rotary dryers, cascade driers, column driers, and flex driers. In all the cases woods, coffee husk, other solid fuel, fuel oils, diesel, gases are used as the main fuel or energy sources. In case of mechanical dryers drying time varies from 20-60 hours according to the type of driers used (Venkatachalapathy, 2014).

It can be concluded that drying air temperature of 50°C and 12 h of tempering is the best treatment for natural coffee. It results in a final moisture difference of 1.7 percentage point, almost 50% less than the difference obtained with the conventional drying method. Now a day's solar assisted dryer is also available. The combination of two driers or pre-treated sun dried sample is used for the final drying in mechanical dryers. The main disadvantage of the mechanical dryers are that the drying is not uniform (Coradi *et al.*, 2007).

2.17 Impact of drying on aroma of green beans

Drying method affects the final quality of green beans. The level of aroma constituent, volatile component, ,sugar ,Amino acid ,water activity and moisture content, chlorogenic acid ,sulphur containing compounds, sensory quality and other miscellaneous compound in sun dried or mechanically dried coffee beans is found different (FareeyaKulapichitra *et al.*, 2019).

2.18 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.

2.19 Cleaning

The hulled coffee is cleaned by winnowing.

2.20 Bioactive compound present in coffee and their importance

2.20.1 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 psych stimulant chemical which is mostly consumed in the world. According to toxicity conclude that consumption of up to 400 mg caffeine/day in healthy adults is not associated with adverse effects. 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).

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. The melting point of caffeine extracted from coffee beans and tea leaves was found to be238°C (Pradeep *et al.*, 2015).

The values obtained for the caffeine content were higher in the green coffee samples, as compared to the roasted samples; this is because the observed caffeine content can be influenced by the roasting process (Burdan, 2015).

Caffeine levels are relatively stable despite being subjected to heat during roasting, although lower values tend to be present in coffee with dark roasts than in coffee with lower roasts (Wang and Lim, 2015), as evidenced by the values of caffeine acquired from the roasted samples using the semi-dry processing method of this study. Another method utilizing yeasts as starter cultures employed inoculation by directly spraying the cherries with yeast suspensions before the drying process. The study was achieved on three different yeasts (S. cerevisiae, Candida parapsilosis and Torulospora delbrueckii) and it was found that this method influenced the concentration of caffeine, chlorogenic acid and trigonelline (Ruta and Farcasanu, 2021).

Bacteria have little effect on the change of caffeine content during the pile-fermentation and also increase of caffeine depended significantly on the growth and reproduction of microorganisms, and it had no relation to the temperature and humidity. Caffeine is increased by following reason microbial enzymes that are produced by microorganisms and then excreted to the surface of tea leaves, since these enzymes will catalyze the caffeine biosynthesis to produce caffeine in vitro, or the secondary metabolism of microorganisms, that is, the caffeine is biosynthesized in vivo by microorganisms after taking the essential components such as xanthosine. Therefore, the caffeine increase must be related to the microorganisms, which possess the genetic information needed to produce caffeine or caffeine biosynthetic enzymes, also related to the components, which are absolutely necessary to the biosynthesis of caffeine. More research on exploring the reason for caffeine increase will continue in our laboratory. Some study showed that there were indeed significant compositional differences between processed products which resulted from the differences in plant variety, growth conditions, and processing methods. Among these factors, the major factor is the processing method. For example, the enzymatic oxidation of catechin (Wang *et al.*, 2005a).

The levels of caffeine content varies from 34.1 g kg^{-1} dry mass of extract in Arabica coffees to 81.6 g kg^{-1} d.m. in Robusta coffee from Indonesia. Generally, Robusta coffee extracts contain twice as much caffeine as Arabica. The caffeine content in green bean extracts of *Coffea Arabica* was very similar 34.1–38.5 g kg^{-1}, while in *Coffea Robusta* was in the range between 3.9 (for Vietnam decaf), 68.6 (Uganda Sc) to 81.6 g kg^{-1} dry mass (Jeszka-Skowron *et al.*, 2016).

According to study of (Rodriguez *et al.*, 2020) Caffeine content of green coffee beans processed from wet processing method is 29.6 mg/gm. and that of semi-dry process is 35 mg/gm.

Different factors affects level of caffeine in green coffee beans some of which are processing methods, geographical location, different environmental factors such as height above sea levels and access to sunlight, method of analysis, coffee samples of different species , same species with different variety, grinding degree and extraction method (Olechno *et al.*, 2021).

2.20.2 Total phenolic content

Green coffee, which refers to unroasted or raw bean is a good source of compounds such as chlorogenic acids, hydroxycinnamic acids, caffeine and caffeic acid which possessing antioxidant and radical scavenging activities. However, during roasting process chlorogenic acids are particularly degraded and the content of phenolic compounds as well as antioxidant capacity in coffee decreases. As a result, green coffee beans seem to be a better source of these beneficial compounds (Valentin and Watling, 2013).

Phenols constitute probably the largest group of plant secondary metabolites, varying in size from a simple structure with an aromatic ring to complex ones such as lignin. Although many of the essential oils are terpenes, some are phenolic compounds, Many simple phenols are responsible for taste and In coffee it gives astringent principal (Aldred *et al.*, 2009).

Although the main reasons of preferring coffee consumption are due to flavor and stimulating effect of caffeine for several years, the green coffee has recently received considerable attention due to the tendency of showing antihypertensive effects, preventive effects on diabetes and to reduce visceral fat and body weight. In addition to the well-known stimulant properties of caffeine, studies have also indicated that its antioxidant potential through the in vitro inhibition of lipid peroxidation induced by free radical (Castro *et al.*, 2018). However, excessive intake of caffeine can lead to irritability, mutation effects such as inhibition of DNA, anxiety and tremors, among other side effects. Formation of new melanoidins, as well as the formation of other Maillard reaction products. The TPC and TFC of the yeast fermented coffee extract has significantly higher TPC than control. Control samples had significantly lower amounts of TPC than the yeast fermented coffee extracts.

This was due to the elution of soluble phenolic compounds in the green coffee beans during the soaking period. A similar finding was observed after soaking green coffee beans in mulberry extract. TPC of the coffee extracts decreased after 6 h of soaking. Despite the decrease in TPC due to the soaking process in this study, fermentation positively influenced TPC in the coffee extracts. During the fermentation process, strongly bound phenolic compounds in the cell wall may become weakened, making them easier to extract after roasting. Phenolic compounds in coffee are mostly in the forms of chlorogenic acids with 5-O-caffeoyl-quinic acid such as caffeic, ferulic, p-coumaric, and caffeoylquinic acid. Feruloylquinic acid, di-caffeoyl-quinic acid, and proanthocyanidins are also detected in coffee (Kwak *et al.*, 2018).

When tea leaves is fermented with externally added polygalacturonase enzyme better fermentation and better quality is also obtained when partially purified pectinases from fungi is added Theaflavin and Thearubigin content is increased by 34.8% and 30.9% respectively and from pure enzyme TF and TR is increased by 38.3% and 35% and also TF: TR ratio is found to be 1:10 (Thakur and Gupta, 2012).

It was found that the TPC of green, roasted and brewed coffee samples extract increased with increasing methanol content from 70% to 85% and then decreased with increasing methanol content. The loss of TPC can be attributed to the leaching of water soluble compounds into the cooking water as well as the breakdown of these compounds during roasting and cooking. Total phenolic content in green coffee varied from 580.9 mg GAE/100 g in 70% methanol extract to 640.3 mg GAE/100 g in 85% methanol extract and 640 mg GAE/g in 100% methanol (Asfaw and Tefera, 2020). At all solvent composition the levels of TPC in green coffee higher than roasted coffee. This is due to the fact that on roasting, phenolic compounds are partially degraded and/or bound to polymer structures depending on roasting conditions. Besides, it is known that during the roasting process, the amount of polyphenols undergoing polymerization or autoxidation reactions increases, which probably leads to the formation of less active antioxidant substances (Cammerer and Kroh, 2006).

2.20.3 Total antioxidants content

Aside from its endogenous mechanisms, an organism may also acquire antioxidant components through diet. Some of the most important antioxidants, which are found particularly in plant foods, are polyphenols. These constitute a category of products of the

plant's secondary metabolism and play an important role in a number of cellular functions. When plant foods are consumed, the absorbed polyphenols may elicit a variety of important bioactivities which have beneficial effects on human health. Such polyphenols can also be found in coffee, which is one of the most popular beverages worldwide due to its pleasant taste and aroma traditionally, the beneficial effects of coffee on human health were mainly attributed to its most-investigated ingredient, caffeine, and however, other components also contribute to its valuable properties, such as its antioxidant activity. The latter is attributable mainly to its polyphenolic content, with the most abundant polyphenols being chlorogenic acid. Several studies have been performed to investigate the quantity, as well as the antioxidant (Cammerer and Kroh, 2006).

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. The values of phenolic content may varied slightly due to the presence of different amounts of sugars, carotenoids, or the duration, geographical variation or methods of extraction, which may alter the amount of phenolics. DPPH is a stable organic free radical, which loses its absorption spectrum band at 515–528 nm when it accepts an electron or a free radical species. The DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency of plant extracts. The antioxidants are the components of the plants which are capable of enacting the visually noticeable quenching of the stable purple-coloured DPPH radical to the yellow-coloured DPPH (Aryal *et al.*, 2019).

However, although we are aware that coffee beans undergo roasting prior to consumption, little data exist on the effects of roasting on coffee composition, or on the differences in antioxidant activity between green and roasted beans (Jaiswal *et al.*, 2012).

According to the study of (Masek *et al.*, 2020) the total antioxidants content of green coffee beans is increased when concentration is increased from 1 mg/ml to 4 mg/ ml. According to this research the antioxidant capacity of green coffee is 78.5 ± 0.51% for ethanol sample to 98.5 ± 0.42 and 96.5 ± 0.48 for sample in water.

2.20.4 Tannin

Tannins are complex chemical substances derived from phenolic acids (sometimes called tannic acid). They are classified as phenolic compounds, which are found in many species of plants, from all climates and all parts of the globe. They are large molecules that bind readily with proteins, cellulose, starches, and minerals. These resulting substances are insoluble and resistant to decomposition. Tannins occur in many species of coniferous trees as well as a number of flowering plant families. These tannins can leach out of the plants (USDA).

Tannins are a complex family of plant polyphenols with considerable biological activity. Because of their ubiquitous presence in foodstuffs, animals have developed defense mechanisms to neutralize their toxicity. Salivary proline-rich proteins seem to interact with tannins to render them inactive and this biological activity of tannins towards protein has been exploited in an elegant quantitative technique in which a methanol extract containing tannins is allowed to diffuse out of a central well into agarose gel containing albumin (Savolainen, 1992).

Green coffee beans contains 6.8±0.6 mg per GAE/g which is lower than roasted coffee beans and tea (Savolainen, 1992). According to research of Haile and Kang (2019a) tannin content is decreased while fermenting with yeast strain but exact data of which is not found.

2.20.5 Total flavonoid content

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups (Aryal *et al.*, 2019).

Flavonoids are a group of more than 10,000 polyphenolic compounds that are found in foods of plant origin. Broadly they can be divided into six major classes, namely, flavanones, anthocyanidins, flavan-3-ols, flavonols, isoflavones, and flavones. The growing evidence of the versatile health benefits of flavonoids including cancer prevention has generated a tremendous interest in the research related to dietary sources of flavonoids (Tuli, 2019). Flavonoids are phenolic substances that act as an antioxidant, anti-inflammatory, anti-allergenic, antiviral and also have vasodilation actions. Green coffee bean shows a high concentration of Flavonoids in hydroethanolic extraction. As reported in the literature,

genetic diversity and biological, environmental, seasonal and year-to-year variations significantly affected the flavonoid content (Aryal *et al.*, 2019).

In TFC, the yeast fermented coffee extracts has significantly higher TFC than other. Yeast fermentation was effective in increasing the number of flavonoids in the coffee extracts. The increase in TFC might be due to the conversion of insoluble phenolic compounds into soluble flavonoids during fermentation. The ratio of TPC and TFC was lower in the yeast fermented coffee extracts. Fermentation was more effective in producing soluble phenolic compounds than flavonoids (Kwak *et al.*, 2018).

Coffee is high in antioxidants and flavonoids is also work as antioxidant in coffee beans (Jeszka-Skowron *et al.*, 2016).

According to the research of (Song.C., 2018) total flavonoid content of fermented and unfermented coffee beans are same. According to his research TFC of green coffee beans 7.8±0.5 mg QE/g of coffee beans.

2.21 Proximate component in green coffee beans

2.21.1 Moisture content

Green coffee beans are dried to moisture content of 9 to 12%. Drying is mainly concern for the degradation of the moisture content up to a certain limit12% (w.b). Drying diminishes the respiration rate of the product and increases the storage time with the minimum possible loss. If the beans are over dried it will be brittle in nature and if moisture content is more than safe storage moisture then there is a probability of mold growth in the beans for further processing (Venkatachalapathy, 2014).

2.21.2 Crude protein

Amino acids and proteins acts as precursors of many important aroma compounds found in roasted coffee. During the roasting process, pyrolitic reactions take place leading to the formation of particular volatile and semi-volatile aroma compounds responsible for the sensory qualities of roasted coffee. In this sense, the crude protein content (CPC) of the green coffee beans may constitute important information when deciding the best roasting conditions and blend composition (Rodrigues *et al.*, 2010). A wide range of protein contents (12.08–16.58 g per 100g) in green Arabica coffee beans have also been reported. The highest

protein values were found in green coffee beans from Brazil (14.34− 15.98 g per 100g), which were significantly higher than that of other countries. Then, Guatemalan and Chinese green coffee beans had second high protein content with values ranging from 14.33 to 14.75 g per 100 g and 13.95–15.75 g per 100 g , respectively, which were significantly higher than that of Indonesian, Kenyan, Ethiopian, Colombian, Honduran samples. On the contrary, the lowest protein contents were found in Kenyan green coffee beans (13.06− 13.67 g per 100g), which were significantly lower than that of other countries (Zhu *et al.*, 2021) also the content of fat and protein may contribute to volatile and non-volatile compound development during storage and roasting. Decomposition of nitrogen compounds in protein may contribute to pyridine, which gives a roasting aroma of carbohydrates, during roasting, polysaccharides will decompose and release di- and monosaccharides that may undergo caramelization and Maillard reactions (Kanitnuntakul *et al.*, 2015).

2.21.3 Crude fat

The lipid fraction of coffee is composed mainly of triacylglycerols, sterols and tocopherols, typical components found in all common edible vegetable oils. Additionally, the so-called coffee oil contains diterpenes of the kaurene family in proportions of up to 20 % of the total lipids. Diterpenes are of interest because of their analytical and physiological effects. The composition of the main lipid components of the two most important coffee species, Coffea Arabica and Coffea canephora var. Robusta is presented. In addition, the influences of typical processes like roasting and steaming on selected lipid components as well as the effects of the storage of green coffee beans under different conditions. Furthermore, new findings regarding the 5-hydroxytryptamides, the main parts of the coffee wax located on the outer layer of the bean and the recently identified components coffeadiol and arabiol. The two most important coffee species, Coffea Arabica and Coffea canephora var. Robusta, contain between 7 and 17 % fat. The lipid content of green Arabica coffee beans averages some 15 %, whilst Robusta coffees contain much less, namely around 10 %. Most of the lipids, the coffee oil, are located in the endosperm of green coffee beans only a small amount, the coffee wax, is located on the outer layer of the bean (Speer and Kölling-Speer, 2006).

The contents of crude fat and ash were lower in the Light-medium roasted coffee beans than in green coffee beans but increased as the roasting degree increased (Kim *et al.*, 2021).

2.21.4 Total Ash content

Ash is the inorganic residue after incineration of organic matter. The amount and composition depends on the methods used and the nature and type of samples. It represents all the minerals that do not volatilize. Ash and mineral contents in most food items are determined by first destroying the organic matter. The destruction can be carried out by two main methods, dry ashing, and wet ashing. The choice of the procedure depends on nature of the organic material, any inorganic constituent present, the metal to be determined and the sensitivity of the method used (KC and Rai, 2007). Normative Instruction No. 16 of the Ministry of Agriculture and Livestock (MAPA), which guarantees the quality of roasted ground coffee, establishes an acceptable level of up to 1% of impurities, sediments, and foreign matter in coffee. However, there are other compounds that, when present in the grains, could cause serious damage to the health of consumers, this is the case for metal contaminants.

Heavy metals are the most frequently evaluated elements in food, because of their ability to accumulate in the food chain. Thus, their maximum levels have become quality standards around the world. These elements are stable and, thus, persistent in the environment, accumulating in the soil as a result of factors associated with the weathering of rocks and soil formation, the soil soluble content, pH, plant species, environmental conditions, technological practices, and use of chemical products. Among the chemical products that contribute to increased heavy metal content in the soil are bio solids, which often contain high levels of cadmium, copper, chromium, lead, nickel, and zinc. Additional sources of contaminants include the use of phosphate fertilizers produced from sedimentary rocks and the application of micronutrients from industrial byproducts these materials are absorbed by plants and accumulate in food, thus becoming sources of contamination for humans (Pigozzi *et al.*, 2018).

According to study of (Kanitnuntakul *et al.*, 2015) Ash content of green coffee beans of different variety from different geographical location ranges from 3.98±0.07 to 4.43±0.13 gm. per 100gm. Coffee beans on dry matter basis.

2.21.5 Crude Fiber

"Crude fiber" is the term used to imply materials insoluble in dilute acid and alkali under specific conditions. The residue from crude fiber determination contains about 97% cellulose and lignin. Typically, it represents only about 50-80% of cellulose, 10-15% of lignin, and 20% of the hemicellulose of the original food. Crude fiber has been defined as the sum of all those organic components of the plant cell membrane and supporting structures which in chemical analysis of plant foodstuffs remain after removal of crude protein, crude fiber, and nitrogen-free extractives (KC and Rai, 2007). Coffea Arabica had 3.4% of crude fiber content. Intake of dietary fiber can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer (Sahrawat *et al.*, 2017).

Part III

Materials and methods

3.1 Collection of raw materials

The ripe coffee cherries (*Coffee Arabica L.*) of bourbon variety were brought from a single farm of Dadabazar-3 Dhankuta, Nepal (26.87°N 87.40°E).

3.2 Chemical required

All the used chemicals are enlisted in Appendices A.

3.3 Equipment required

All the used equipment are enlisted in Appendices A.

3.4 Methodology

Ripened coffee beans after transportation were sorted, floated beans were discarded (E.U, 2018) and coffee beans were then pulped by depulping machine.

The pulped coffee cherry were subjected to different fermentation process. 6.616 kg of beans with mucilage were subjected to 6 different types of fermentation methods as presented in Fig. 3.1.

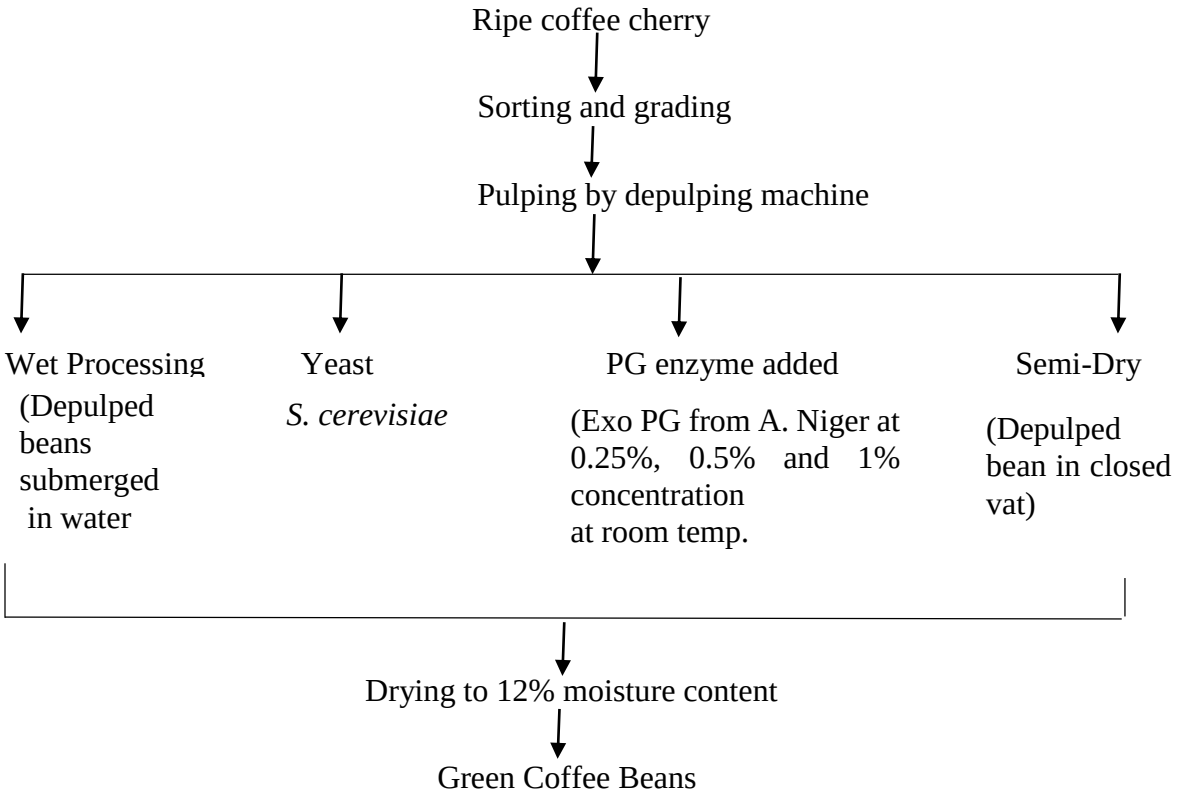


Fig. 3.1 Flow diagram for different processing methods of coffee used in study

3.4.1 Wet fermentation method

Wet processing gives pulped, peeled, and demucilated coffee beans. In this method, coffee pulp was removed by pulping and beans with mucilage were placed in vats containing water where processing takes place. Water was removed in fixed interval (Braham and Bressani, 1979). Coffee beans with mucilage were subjected to this type of fermentation, and time was noted.

3.4.2 Semi-Dry fermentation method

In this method, coffee beans with mucilage were added to fermentation without addition of water in closed vat and time for fermentation completion was also noted (Haile and Kang, 2019b).

3.4.3 Enzymes added fermentation

Different concentration (0.25%, 0.5% and 1%) of polygalacturonase enzyme was added for the fermentation (Haile and Kang, 2019a).

3.4.4 Yeast added fermentation

Yeast strain of *Saccharomyces cerevisiae* was added during fermentation process and fermentation time was noted (Haile and Kang, 2019a).

3.5 Analytical methods

3.5.1 Determination of physical properties of coffee beans

3.5.1.1 Length by breadth ratio

L/b ratio was used to determine the shape of the individual grain. The value of l/b ratio above 3 is generally considered as slender and below 3 is generally considered as bold and l/b ratio was measured by Vernier caliper (Rather *et al.*, 2016).

3.5.1.2 Bulk Density of coffee

The bulk density of green coffee bean was determined by measuring the weight of the sample in the fixed volume and unit was expressed as g of coffee sample per cm^3 (Nakilcioğlu-Taş and Ötleş, 2019).

3.5.1.3 Particle density

The particle density of green coffee bean was calculated by using the pycnometer method. 2.5 g of sample was placed in an empty pycnometer of 25 ml volume. It was filled with toluene and volume was measured. The particle density was determined as the total particle weight divided by its total volume (g/cm^3) (Krokida and Maroulis, 2001a).

3.5.1.4 Porosity

Bulk porosity of sample was determined by using value of bulk density and particle density.

$$\text{Porosity} = \frac{\text{particle density} - \text{bulk density}}{\text{particle density}} \times 100\%$$

Source: (Krokida and Maroulis, 2001b).

3.5.2 Determination of moisture content

Moisture content of bean after pulping was measured by hot air oven method (AOAC, 2005).

3.5.3 Determination of fermentation time

Fermentation completion time for different fermentation method was noted with the procedure given by National Tea and Coffee Development Board (NTCDB, 2020). Here, the processed beans were hand felt for demucilation by rubbing beans with each other and experiencing slimness.

3.5.4 Determination of yield after fermentation

For all methods, yield after fermentation was noted.

Yield %= (wt. of Sample after processing/ wt. of samples before processing)*100

3.5.5 Determination of total phenolic content of beans

TPC of the sample was determined by using spectrophotometric method with some modifications (Hussen and Al Ali, 2015). 0.5 ml of coffee extract, 2.5 ml of 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% of Na_2CO_3 was mixed and it was incubated at 45°C for 45 min. The absorbance at 765 nm on UV-Vis spectrophotometer for each samples was analyzed triplicate. The test results was correlated with standard gallic acid curve and TPC was expressed as mg Gallic acid equivalent (mg GAE/g) of dry matter in extract.

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= 0.9973) 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, 200 and 250 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 (Hussen and Al Ali, 2015).

Finally, total polyphenols were calculated using equation;

The Total Polyphenol Content (mg GAE/g) = C×V/m

Where; C= Concentration of polyphenols in mg/ml

V= Volume of sample taken for dilution in ml

m = mass of sample taken for analysis

3.5.6 Determination of tannin

The tannin was determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35 % Na2CO3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) was prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of (mg of GAE /g) of extract (Haile and Kang, 2019a).

3.5.7 Determination of caffeine

Caffeine content in the different coffee sample was determined by UV-Vis spectrophotometric method as per (Belay *et al.*, 2008) with slight modifications. Grinded coffee samples, 2.5 g each was poured to 200 ml of boiling water and stirred for 10 minute. After filtering through cotton wool, the extract was cooled at a room temperature and volume was made to 250 ml with distilled water. This solution was mixed with dichloromethane in ratio 1:1 (25:25 ml) for the extraction of caffeine from samples. It was stirred for 10 min and caffeine was extracted by dichloromethane from the solution with the help of separating funnel. Caffeine was extracted 4 times with 25 ml dichloromethane at each round and stored in volumetric flasks. The absorbance of the extracted solution was measured at 270 nm on UV/visible spectrophotometer. The test results were correlated with standard calibration curve of caffeine and it was expressed in percentage (%).

3.5.8 Preparation of caffeine standard

A 100 ppm stock standard of caffeine was prepared by dissolving 25 mg of caffeine in 250 ml purified dichloromethane 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 dichloromethane 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.

3.5.9 Determination of flavonoids (TFC)

TFC of the sample was determined by using a modified Aluminum chloride assay method (Barek *et al.*, 2015). 2 ml of the methanolic extract was mixed with 0.2 ml of $NaNO_3$ (5%, w/v) and after 5 min, 0.2 ml of $AlCl_3$ (2%, w/v) was added and allowed to stand for 6 min. This followed addition of 2 ml of 1N NaOH and finally volume was made up to 5 ml. After holding for 15 min at room temperature the absorbance was measured at 510 nm on UV-Vis spectrophotometer. The test result was correlated with standard Quercetin curve and TFC was expressed as mg quercetin equivalents (mg QE/g) of dry matter in extract.

3.5.10 Determination of DPPH radical scavenging activity

The DPPH radical scavenging activities (antioxidant activities) of the extract was determined as per the method described by (Vignoli *et al.*, 2011). 1 ml of the extract was mixed with 2 ml of DPPH (0.004% in methanol, corresponding to 100 μM) incubated at 37˚C in dark (wrapped with aluminum foil) for 20 min (for completion of reaction) before spectrophotometric analysis. The absorbance was measured at 517 nm on UV-Vis spectrophotometer after 30 min incubation in the dark.

$$\text{DPPH scavenging activity} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100\%$$

Where, Ac = Absorbance of control and As = Absorbance of test sample

3.5.11 Determination of crude protein

Protein in sample was determined by Kjeltron nitrogen estimation system (KC and Rai, 2007)

3.5.12 Determination of crude Fat

Crude Fat content was determined by Automatic Soxtron solvent extraction system(KC and Rai, 2007).

3.5.13 Determination of total ash content

The determination of total ash was done by incinerating all the organic matter of the coffee sample at 550°C (KC and Rai, 2007).

3.5.14 Statistical Analysis

All data obtained in this work was analyzed by statistical program known as Genstat and Rstudio (2008) and tested for 5% level of significance.

Part IV

Results and discussions

4.1 Physical Parameters of coffee cherries and green beans

4.1.1 Physical properties of coffee cherries

The physical property of coffee cherries was presented in Table 4.1

Table 4.1: Physical properties of coffee cherries

Parameters	Values
Length(mm)	11.09±0.54
Breadth(mm)	8.53±0.17
Thickness(mm)	4.96±0.43
L/B Ratio	1.298
Moisture content (%)	47±0.87

The length (11.09 mm) and width(8.53 mm) of coffee beans were similar to the result obtained by Sualeh and Dawid (2013) i.e. 15.5mm and 11.5mm respectively. L/B ratio of Arabica coffee bean was 1.298 which was comparable to the study of Severa *et al.* (2012a). L/B ratio is used to determine the shape of the individual grain. The value of l/b ratio above 3 is generally considered as slender and below 3 is generally considered as bold (Rather *et al.*, 2016).

Moisture content of coffee cherry after harvesting was 47% which was similar to the data obtained by Coradi *et al.* (2014) i.e. (48%). Moisture content at the stage of harvesting shows the maturity stage and also affect the quality of final product.

4.1.2 Physical properties of Green coffee beans

The physical property of green coffee beans is presented in Table 4.2

Table 4.2: Physical properties of green coffee beans

Parameters	Values
Bulk Density(g/cm^3)	0.60±0.01
Particle Density(g/cm^3)	0.97±0.05
Bulk Porosity	37.61±3.36
Moisture content (%)	11.76±0.26

4.1.2.1 Bulk density

Bulk density of Arabica coffee bean was found to be 0.60 g/cm^3 which was more than data obtained by Nakilcioğlu-Taş and Ötleş (2019).i.e. 0.43 g/cm^3. Particle density and bulk density is affected by pore space, texture and organic matter content so different coffee variety may have different physical properties. Bulk density is required for the design of storage, transport and separation systems. It has also been used to determine the dielectric properties (Ashwin Kumar *et al.*, 2017).

4.1.2.2 Particle density

The particle density or specific gravity of green coffee bean was found to be 0.97 g/cm^3 which was similar to the result obtained by Nakilcioğlu-Taş and Ötleş (2019). I.e. 0.92 g/cm^3. The loss of flavor compounds and the following change in flavor profile during storage are related to the pore structure of coffee due to which porosity in coffee is one of the major physical characteristics. Porosity of coffee is increased owing to both the degradation of the intercellular matrix and the destruction of the cells during roasting process. After roasting, the similar change in porosity of coffee samples has been also observed (Nakilcioğlu-Taş and Ötleş, 2019).

4.1.2.3 Bulk porosity

The bulk porosity of sample was found to be 37.61 g/cm^3 which was almost similar to the result obtained by Nakilcioğlu-Taş and Ötleş (2019). i.e. 37.78 g/cm^3.

4.1.1.4 Moisture content of green beans

Moisture content of all sample after fermentation and drying was noted and average moisture content of all sample was found to be 11.76% which lies within the range of 9 to 12% provided by Gautz *et al.* (2008). In range if 9 to 12 % moisture best quality of coffee beans is obtained above 12 percent mold growth and below 9 percent quality of beans is decreased due to brittleness (Afonso *et al.*, 2003).

4.2 Yield

Yield for different samples treated with different method was noted. Yield of coffee beans was found to be 84.15% after the removal of mucilage which was comparable to data obtained by Braham and Bressani (1979).

4.3 Fermentation completion time

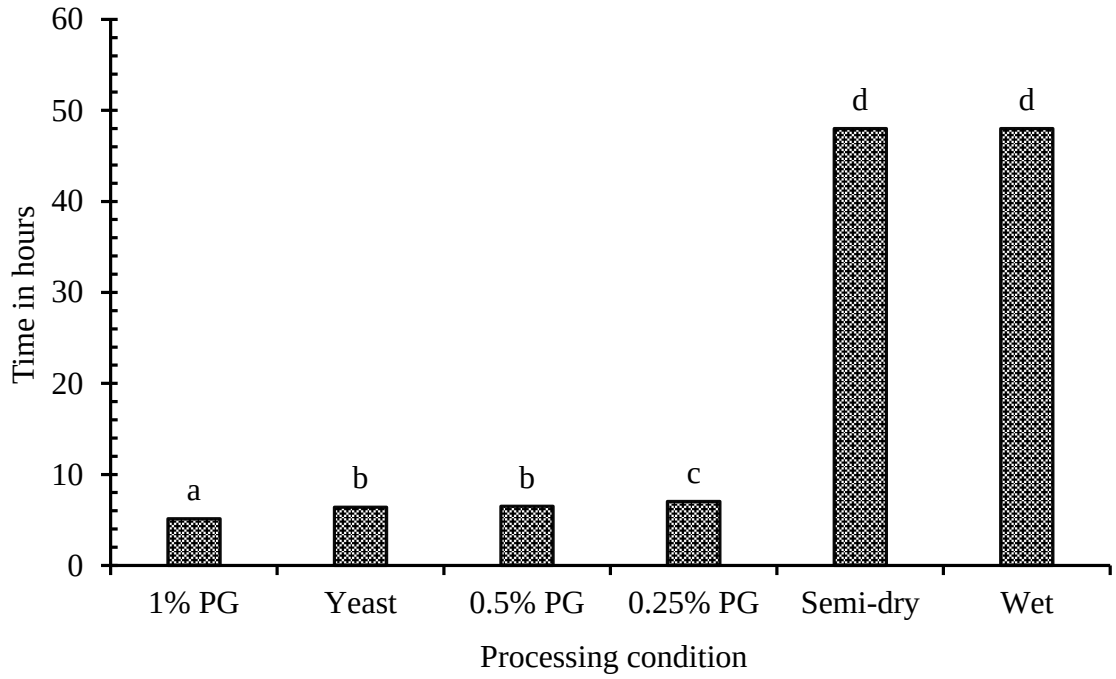


Fig. 4.1 Fermentation completion time of different samples

Processing time in (**Fig.4.1**) was highest for semi-dry process which is 48 h and lowest for1 % P.G treated sample which is 5.3 h for the completion of fermentation. According to study of Ruta and Farcasanu (2021) processing time for wet fermentation was reported to be 48-72 h and 7- 8 h for the sample with externally added yeast and p.g. enzyme. Short time for completion of fermentation is due to acceleration of breakage of mucilage which originally

present in coffee but by adding external agents time for breakage is reduces with reduced viscosity (Agate and Bhat, 1966).

4.4 Proximate composition of samples with different processing methods

Table 4.3: Proximate composition of coffee beans

Particulars	Wet	Semi-Dry	Yeast	0.25%PG	0.5%PG	1%PG
Moisture%	11.3 ±0.2[a]	11.5 ±0.13[a]	11.8 ±0.3[a]	12 ±0.2[a]	11.7 ±0.6[a]	11.4 ±0.5[a]
Crude protein%	12.28 ±0.1[a]	12.14 ±0.1[a]	12.54 ±0.2[a]	12.49 ±0.24[a]	12.5 ±0.2[a]	12.57 ±0.2[a]
Crude Fat%	6.82 ±0.05[a]	6.61 ±0.05[a]	6.71 ±0.06[a]	7 ±0.08[a]	6.7 ±0.05[a]	6.72 ±0.08[a]
Total Ash%	4.19 ±0.01[a]	4.25 ±0.01[a]	4.24 ±0.04[a]	4.27 ±0.03[a]	4.2 ±0.02[a]	4.24 ±0.03[a]
Crude Fiber%	3.23 ±0.04[a]	3.13 ±0.08[a]	3.23 ±0.04[a]	3.2 ±0.02[a]	3.2 ±0.02[a]	3.2 ±0.008[a]
Nitrogen free extract (%)	62.17	62.36	61.45	60.27	61.62	61.2

*Values are means of triplicate ± standard deviations.

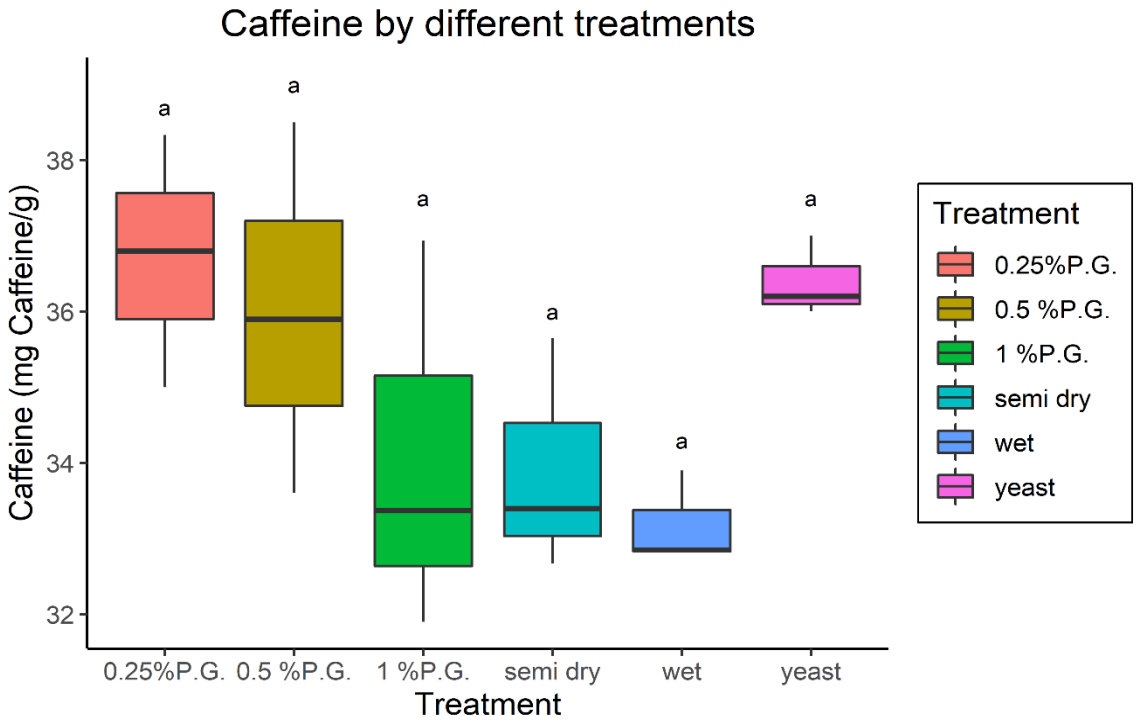
The moisture content of green coffee beans in all the samples was found to be average of 11.5% which was similar to the data obtained by Gautz *et al.* (2008). There was no significance difference for the crude protein content between the samples the data was comparable to the result obtained by Zhu *et al.* (2021) .i.e. 12.08–16.58 g per 100g of Arabica coffee beans the little variation may be due to the different geographical location and Difference in variety. Likewise, the data obtained for crude fat, total ash and crude fibre was

similar among the different sample which was around 6.7%, 4.2% and 3.2% respectively. The obtained data were comparable to the results reported by Speer and Kölling-Speer (2006), (Sahrawat *et al.*, 2017).

Amino acids and proteins acts as precursors of many important aroma compounds which leads to the formation of particular volatile and semi-volatile aroma compounds responsible for the sensory qualities of roasted coffee. In this sense, the crude protein content (CPC) of the green coffee beans may constitute important information when deciding the best roasting conditions and blend composition (Rodrigues et al., 2010). Crude fat in coffee beans is important because it has analytical and physiological effect on final quality of beans and also storage and some semi-volatile components, roasting and steaming is affected in beans due to fat content in beans (Speer and Kölling-Speer, 2006). The process like non-enzymatic browning reaction such as caramelization and Maillard in which the chemical reaction involved simple sugars, amino acids, and high temperature. Continuous heating would bring green coffee beans into the yellow stage and further into reddish-brown. Arabica coffee beans tend to show more yellow-reddish color than Robusta potentially due to more sugar content it has. Maillard would produce brownish pigments or intermediate products including melanoidins (Sunarharum et al., 2019).

4.5 Caffeine content

Caffeine content of different samples is shown in (**fig.4.2**)



[Boxplot with different alphabet at the top are significant different ($p<0.05$). The whisker shows the data distribution through their quartiles.]

There was no significant difference in caffeine content among different samples. However maximum value of caffeine content was seen in yeast treated sample and least value was found in wet processed sample. Similar data was reported by Jeszka-Skowron *et al.* (2016) i.e. 34.1–38.5 mg/g and (Rodriguez *et al.*, 2020) i.e. 35 mg/g in dry matter basis of caffeine content in coffee Arabica variety.

The addition of enzymes to ferment tea leaves helps in the synthesis of caffeine and caffeine content is increased slightly and when coffee beans are fermented with yeast that produces enzyme or externally added enzyme that will help to catalyze the biosynthesis of caffeine with help of component present in sample (Wang *et al.*, 2005b).

Slightly different level of caffeine content in different variety is may be due to difference in geographical location, different environmental factors such as height above sea levels and access to sunlight, method of analysis, coffee samples of different species , same species with different variety, grinding degree and extraction methods (Olechno *et al.*, 2021).

4.6 Total phenolic content

Total phenolic content of different samples is shown in (**fig.4.3**)

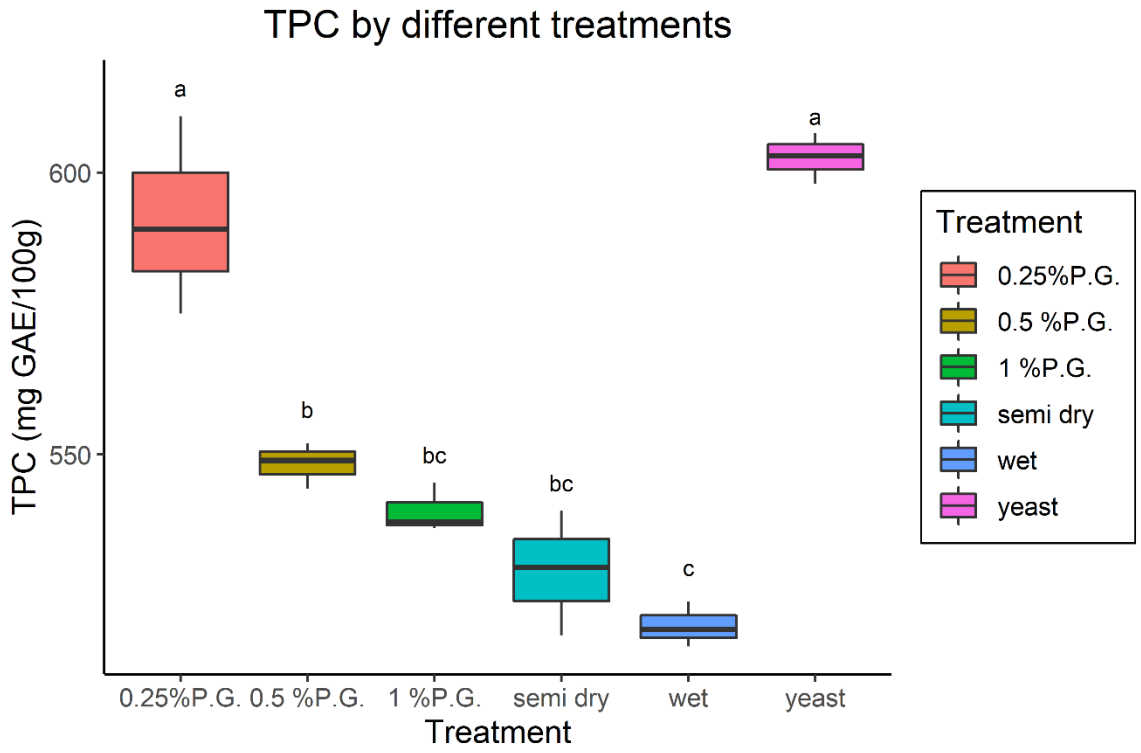


Fig.4.3 Total phenolic content (TPC) of different samples of methanolic extract. [Boxplot with different alphabet at the top are significant different ($p<0.05$). The whisker shows the data distribution through their quartiles.]

Total phenolic content was found higher on sample treated with yeast and least value on wet processed sample. The study of Asfaw and Tefera (2020),total phenolic content in green coffee varied from 580.9 mg GAE/100 g in 70% methanol extract to 640.3 mg GAE/100 g in 85% methanol extract and 640 mg GAE/g in 100% methanol. In our study higher value of TPC was found on samples treated with yeast and 0.25% PG i.e. 604 GAE/100g and 592 GAE/100g which was significantly different to other samples, higher value was may be due to fermentation of green coffee beans with yeasts and enzymes has positive impacts, increasing antioxidant activity and polyphenol and flavonoid contents (Haile and Kang, 2019a) and also according to study of Thakur and Gupta (2012) when samples is fermented with externally added pectinases increased level of polyphenols are found due to increase in level TF and TR.

Lower value of TPC was seen on processed by wet and semi-dry processing i.e. 548 GAE/100g, 529 GAE/100g respectively which was also significantly different to other processing methods and similar to the study of Asfaw and Tefera (2020) . Study of Kwak *et al.* (2018) shows that if coffee beans when processed for long than 6 h leaching of polyphenols in water occurs due to which slightly low level of polyphenol level was seen on sample processed with water for long time. Also Delgado *et al.* (2019) study shows TPC content was decreased when concentration of enzyme was increased which was may be due to TPC acts as substrate for enzyme. Also TPC varied with coffee species and variety, method of extraction, climate and soil (Olechno *et al.*, 2020).

4.7 Tannin

Total tannin content of different samples is shown in (**fig.4.4**)

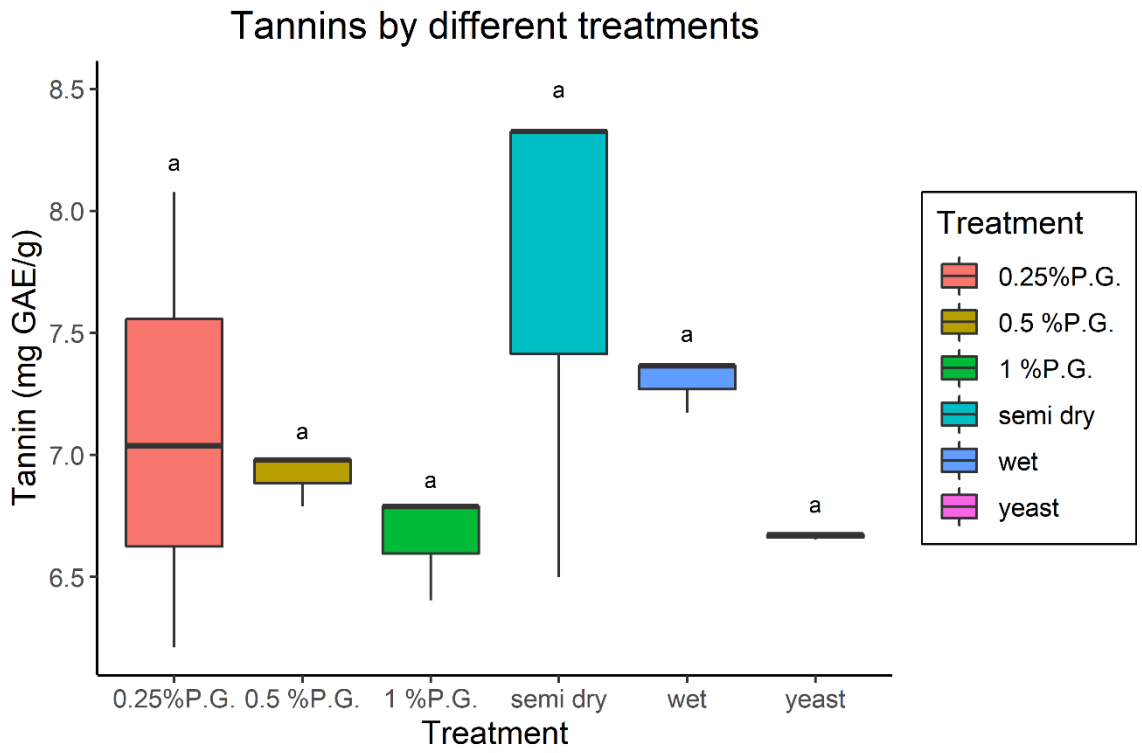


Fig.4.4 Tannin of different samples of methanolic extract.

[Boxplot with different alphabet at the top are significant different ($p<0.05$). The whisker shows the data distribution through their quartiles.]

There was no significant difference in tannin content between samples with different processing method however higher level of tannin was found on semi-dry process and least on 1% PG i.e. 7.71mgGAE/g ,6.60mgGAE/g on d.m basis. In the study of Savolainen (1992)

tannin content was found to be 6.8±0.6mgGAE/g which was similar to our study. According to research of Haile and Kang (2019a)tannin content was decreased while fermenting with externally added enzymes or microbes so low level of tannin content was may be due to treatment with externally added enzymes and yeast.

4.8 DPPH radicle scavenging activity

DPPH activity of different samples is shown in (**fig.4.5**)

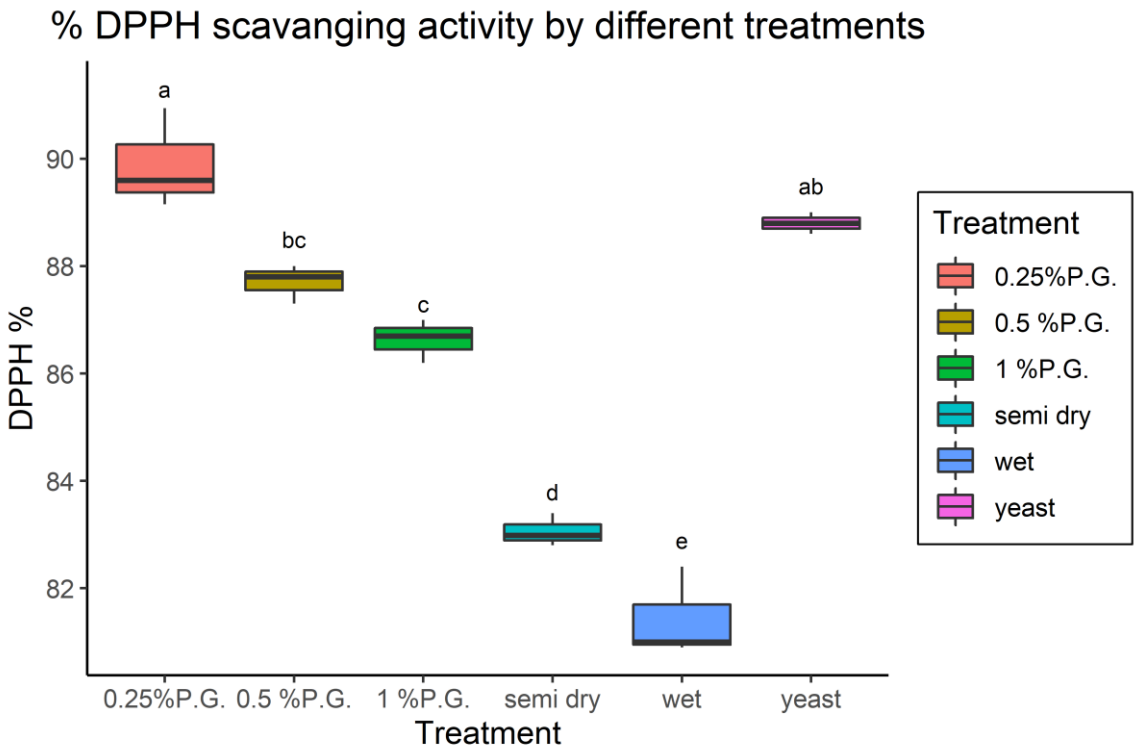


Fig.4.5 DPPH activity of different samples of methanolic extract.
[Boxplot with different alphabet at the top are significant different ($p<0.05$). The whisker shows the data distribution through their quartiles.]

DPPH activity was found highest on samples treated with PG and yeast and lowest antioxidant activity was found on sample processed from wet and semi-dry processing method i.e. 89.89±0.76% and 81.43±0.68%. According to the study of (Masek *et al.*, 2020) the total antioxidants content of green coffee beans of ethanolic extract was 78.5 ± 0.51% which was slightly lower than our study.

Significant difference between DPPH activities between samples with different processing method as shown **in (fig.4.5)** was may be due to difference in polyphenolic

content, melanoidins, chlorogenic acid, caffeine between different samples (Cammerer and Kroh, 2006) also difference in antioxidant activity was may be due to concentration difference of extract used in study and solvent used.

Liang and Kitts (2014) says that antioxidants property of coffee samples differ due to different maturity stage, same species with different variety, different species, soil condition, processing condition.

4.9 Total flavonoids content

Total flavonoids content of different samples is shown in (**fig.4.6**)

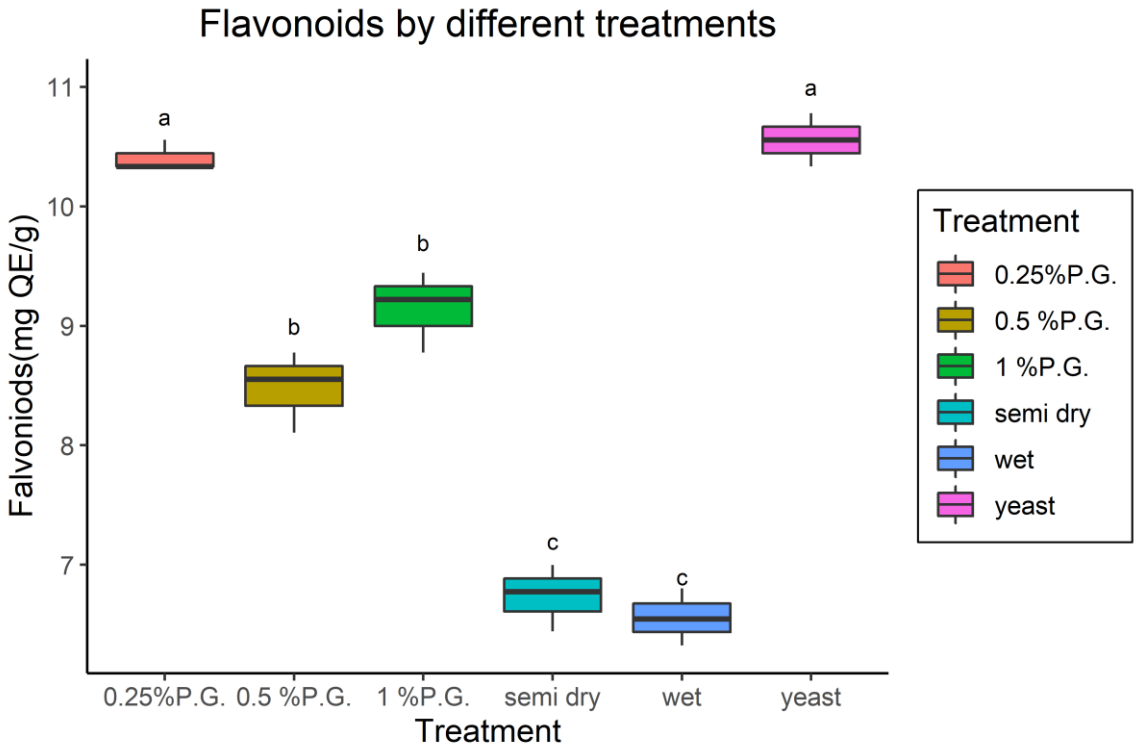


Fig.4.6 Total Flavonoids activity of different samples of methanolic extract. [Boxplot with different alphabet at the top are significant different ($p<0.05$). The whisker shows the data distribution through their quartiles.]

Total flavonoid content is higher on sample treated with 0.25% PG and yeast with the value of 10.55 mg QE/g, 10.4 mg QE/g on d.m. basis which is significantly different to samples processed with other methods. Haile and Kang (2019a) shows that total phenolic content and antioxidant activity of coffee samples with different enzyme treatment and microbes is high which may the reason for high value of TFC in 0.25% PG and yeast sample

also study of Kwak *et al.* (2018) shows that fermentation with externally added enzymes, insoluble phenolic component are changed to flavonoids which also may be the reason for high TFC content in samples treated with externally added agents.

TFC content in semi-dry and wet processed coffee was 6.73 QE/g and 6.55 QE/g on d.m. basis which was similar to result obtained by Song.C. (2018) i.e. 7.8±0.5 mg QE/g.

4.10 Principal component analysis (PCA)

PCA was done to select the best sample after processing with different fermentation methods from fig.4.6 the first principal component (Dim.1) was responsible for 71.3% variation with eigen value of 3.56.while second principal component was reported to 15.77% variation with eigen value of 0.78 and third component was responsible 9.13% for variation with eigen value of 0.45 percent and these three component together account for 96.15 percent variations.

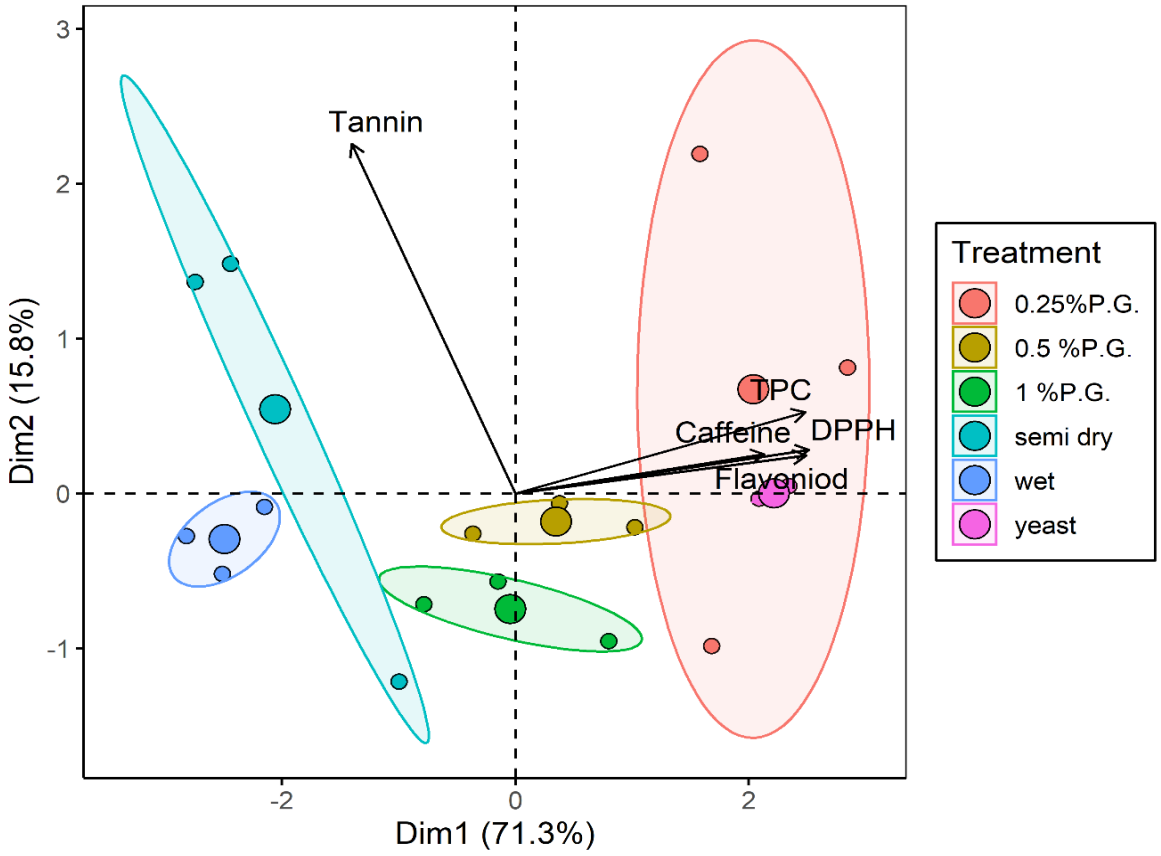


Fig.4.7 Biplot distributions of different processing samples with grouping caffeine, TPC, TFC, DPPH and tannin.

PCA shows the distribution of different coffee samples with different processing methods are influenced by caffeine, TPC, TFC, DPPH, Tannin. Semi-dry and wet processed coffee are on left side of(**Fig. 4.7)** while P.G and yeast treated samples are on right side of **(Fig. 4.7)** which shows that samples treated with yeast, 0.25% P.G., 0.5% P.G. are best than semi-dry and wet processed samples.

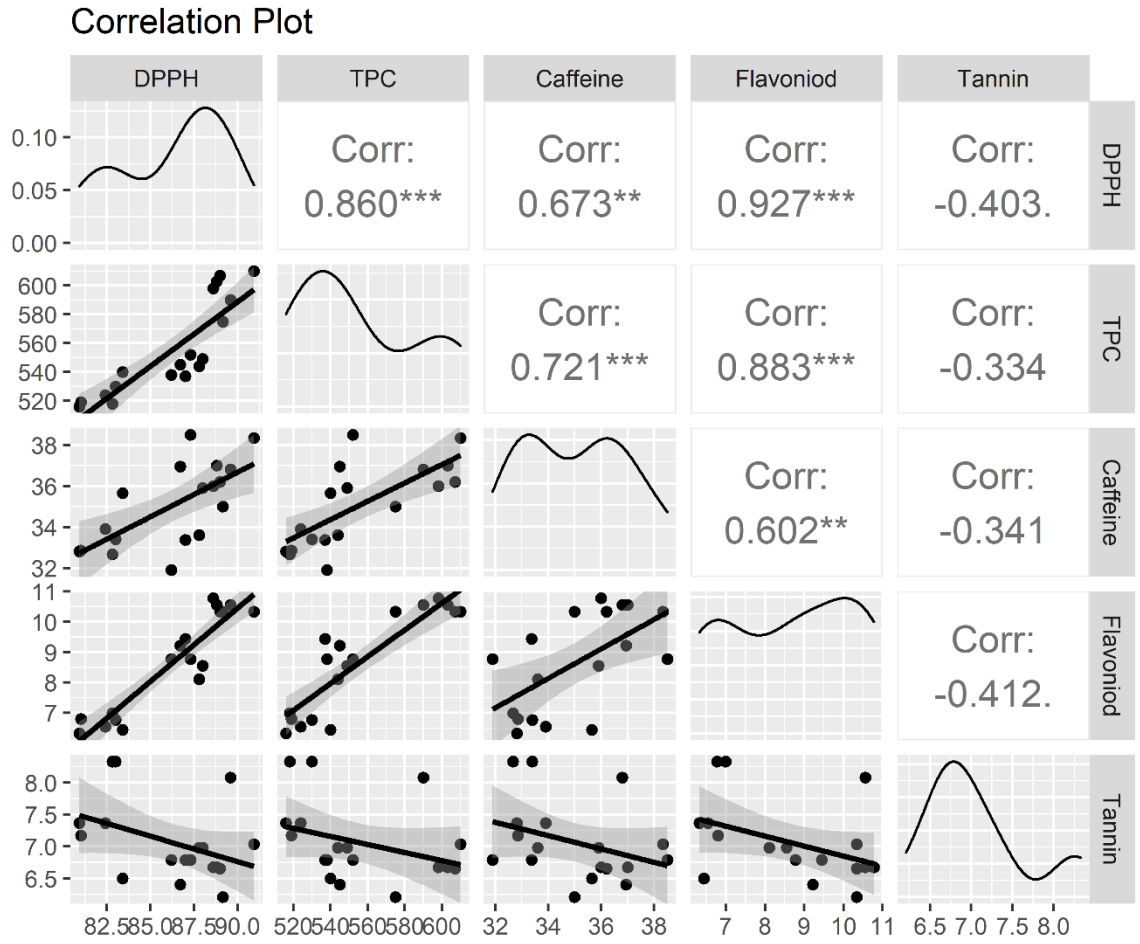


Fig.4.8 correlation plot between caffeine, TFC, TPC, DPPH, Tannin.

Correlations between different components was shown in(fig.4.8) this shows strong positive correlation between TPC and DPPH, Flavonoid and DPPH, Flavonoid and TPC, flavonoid and caffeine where negative correlation was seen between tannin and TPC, tannin and DPPH Positive correlation between DPPH and (TPC, Caffeine, and Flavonoids) may be due to their antioxidants activity (Kwak *et al.*, 2018).

Phenolic and flavonoid molecules are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to

free radicals. They also have ideal structural characteristics for free radical scavenging. Different literature reports indicate a linear correlation of total phenolic and flavonoid content with antioxidant capacity. The correlation of total phenolic and flavonoid content with antioxidant capacity is shown in Figure. High correlations between antioxidant capacity and total phenols (R^2=0.86) and total flavonoids (R^2=0.927) were observed at a 95% confidence level, By comparing the correlation coefficients (R-values), it is possible to suggest that phenolic and flavonoid groups are highly responsible for the antioxidant activity of the selected samples (Afonso *et al.*, 2003).

Part V

Conclusion and recommendation

5.1 Conclusion

1. Demucilation was fastest for sample treated with 1% polygalacturonase (PG) enzyme at room temperature (5 h) and slowest for sample treated using semi-dry method (48.5 h). Hence, adding polygalacturonase enzyme hasten the dumucilation process of depulped coffee beans.

2. PG treated beans especially at 0.25% had higher TPC, anti-oxidant activity and flavonoids than other processing methods i.e. wet, semi-dry and yeast. There was no significant difference in caffeine and tannin content using different processing method.

3. There was no significant difference in physical properties of samples processed with different processing methods.

5.2 Recommendation

1. Roasting of coffee beans processed with different methods and further analysis of physicochemical and organoleptic properties of brewed coffee can be assessed.

2. Fermentation kinetics of different processing methods can be carried out.

3. Variation in drying techniques and its effect on bioactive component can be done.

4. Using PG significantly decreases demucilation time.

Part VI

Summary

In this study, coffee cherry was taken from Dadabazar, Hileni, which is one of the place for commercial cultivation of coffee in Nepal. And other essential materials 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. Beans with mucilage were subjected to different processing methods and time for fermentation was noted and after completion of fermentation beans were washed with plenty of water, stinker beans was removed during washing and drying of coffee beans was done in mechanical drier until the moisture content of 12% was obtained. After drying coffee samples were dehulled by hand and are powdered to fine size in mixture and extraction of samples was done in Soxhlet apparatus by methanol for 24 hours and extracted sample was made of known concentration of 1mg/ml and for caffeine 2.5g sample in boil water was extracted with dichloromethane solution.

Analysis of physical and physiochemical property of samples was done and statistical analysis for normality, homogeneity and one way ANOVA, post-hocks test is done to compare means of all samples and significant difference between samples processed with different processing method was analyzed.

References

A. (2019). Horticultural Crop Species in Nepal: History, Investment, Achievement and Working Groups. Presented at Proceedings of a National Workshop on Working Crop Groups. Kathmandu. June, 2018.

Acharya, B. and Dhakal, S. C. (2014). Profitability and major problems of coffee production in palpa district, nepal *Int. J. Appl. Sci. Biotechnol.* **2** (4), 460-463.

Acharya, U. K. and Pun, U. (2016). Analysis of nepalese coffee industry: Production and postharvest issues *Nepalese Hort.* **11** (1).

Afonso, P. C., Correa, P. C., Pinto, F. A. C. and Sampaio. (2003). Shrinkage evaluation of five different varieties of coffee berries during the drying process. *BIOSYST ENG*. **86** (4), 481-485.

Agate, A. and Bhat, J. V. (1966). Role of pectinolytic yeasts in the degradation of mucilage layer of *Coffea robusta* cherries. *Appl. Microbiol.* **14** (2), 256-260.

Aldred, E. M., Buck, C. and Vall, K. (2009). Phenols. *In:* "Pharmacology". (E. M. Aldred, C. Buck and K. Vall, Eds.). pp. 149-166. Edinburgh. Churchill Livingstone. [(ISBN978-0-443-06898-0)].

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants (Basel)*. **8** (4), 96. doi:10.3390/plants8040096.

Asfaw, G. and Tefera, M. (2020). Total polyphenol content of green, roasted and cooked Harar and Yirgacheffee Coffee, Ethiopia. **24** (1), 187-192.

Asfew, Z. and Dekebo, A. (2019). Determination of caffeine content in Wollega Zones, Ethiopian coffee bean, pulp and leaves by high performance liquid chromatography. **3** (4), 261-274. [DOI:10.1002/9783527619627].

Ashwin Kumar, B., Rao, P. V. K. J. and Edukondalu, L. (2017). Acharya Physical properties of maize grains. *Int. J. Agric. Sci.* **9** (27), 4338-4341.

Author, S. and Vogt, M. (2019). "Variance in Approach Toward a ‘Sustainable’ Coffee Industry in Costa Rica." (1 ed.). Ubiquity Press Ltd. Costa Rica. [(ISBN9781911529767)].

Avallone, S., Guyot, B., Brillouet, J., Olguin, E. and Guiraud, J. P. (2001). Microbiological and biochemical study of coffee fermentation. *Current Microbiol.* **42** (4), 252-256.

Bade-Wegner, H., Bendig, I., Holscher, W. and Wollmann, R. (1996). Volatile compounds associated with the over-fermented flavour defect. *ASIC*. **17**, 176-182.

Barek, M. L., Hasmadi, M., Zaleha, A. and Fadzelly, A. M. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (Clinacanthus nutans Lind.) Leaves *Int. Food. Res. J.* **22** (2), 661.

Belay, A., Ture, K., Redi, M. and Asfaw, A. (2008). Measurement of caffeine in coffee beans with UV/vis spectrometer. *Food chem.* **108** (1), 310-315. [doi:10.1016/j.foodchem.2007.10.024].

Beniteza, V., Rebollo-Hernanza, M., Hernanza, S., Chantresa, S., Aguileraa, Y. and Martin-Cabrejasa, M. A. (2019). Coffee Parchment as a New Dietary Fiber Ingredient: Functional and Physiological Characterization *Food Res. Int.* **122**, 105-113.

Blinova, L., Bartosova, A. and Sirotiak, M. (2017). Biodiesel production from spent coffee grounds. *Mater. Sci. Technol.* **25** (40), 113. [doi:10.1515/rput-2017-0013].

Borem, F. M. (2008). Survey of existing risks to the safety and health of workers in post-harvest coffee (Coffea arabica) according to the requirements of regulatory standards. *Agric.sci*. [doi:10.1590/S1413-70542008000200018].

Braham, J. E. and Bressani , R. (1979). "Coffee Pulp: Composition, Technology, and Utilization". Vol. 95. Institute of Nutrition of Central America and Panama. Canada. [ISBN 978-0-889-36190-4].

Braham, J. E. and Bressani, R. (1979). "Coffee Pulp: Composition, Technology, and Utilization". IDRC, Ottawa, ON, CA. [ISBN 978-0-889-36190-4].

Bressani, A. P., Martinez, S. J., Sarmento, A. I., Borém, F. M. and Schwan, R. F. (2020). Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. *Food Res. Int.* **128**, 108773.

Burdan, F. (2015). Caffeine in coffee. *In:* "Coffee in health and disease prevention".). pp. 201-207. Elsevier. [[doi:10.1016/B978-0-12-409517-5.00022-X]].

Cammerer, B. and Kroh, L. (2006). Antioxidant activity of coffee brews. *Eur. Food Res. Techno.* **223** (4), 469-474.

Castro, A., Oda, F., Almeida-Cincotto, M., Davanco, M., Chiari-Andreo, B., Cicarelli, R., Peccinini, R., Zocolo, G., Ribeiro, P. and Correa, M. (2018). Green coffee seed residue: A sustainable source of antioxidant compounds. *J. Appl. Sci. Environ. Manage.* **246**, 48-57. [doi.org/10.1016/j.wasman.2019.04.030].

Chaudhary, J. N., Bhusal, Y. R., Adhikari, C. and Yadav, R. P. (2008). Performance evaluation of coffee genotypes in the western hills of Nepal. In: Proceedings of the fifth national seminar on horticulture.

Coradi, P. C., Borém, F. and Reinato, C. (2014). Coffee cherries drying process and the influence of environment relative humidity in the mathematical modeling, moisture content, and enthalpy of vaporization. *Energ. Agric., Botucatu.* **29**, 148. [doi:10.17224/EnergAgric.2014v29n2p148-157].

Coradi, P. C., Borém, F. M., Saath, R. and Marques, E. R. (2007). Effect of drying and storage conditions on the quality of natural and washed coffee. *coffee sci.* **2** (1), 38-47.

Corrêa, P. C., Resende, O. and Ribeiro. Drying characteristics and kinetics of coffee berry. **8** (1), 1-10.

Correa, P. C., Resende, O. and Ribeiro, D. M. (2006). Drying characteristics and kinetics of coffee berry. **8** (1), 1-10. [doi:10.15871/1517-8595%2Frbpa.v8n1p1-10].

Delgado, S. R., Arbelaez, A. F. A. and Rojano, B. (2019). Antioxidant capacity, bioactive compounds in coffee pulp and implementation in the production of infusions. *Acta Sci. Pol. Technol. Aliment.* **18** (3), 235-248. [doi:10.17306/j.Afs.0663].

E.U. (2018). Market System Analysis of Nepali Coffee [Report]. Transcend Vision Pvt. Ltd Sanepa, Lalitpur, Nepal.

Farah, A., Monteiro, A. C., Calado, V., Franca, A. S. and Trugo, L. C. (2006). Correlation between cup quality and chemical attributes of brazilian coffee. *food chem.* **98** (2), 373-380.

FareeyaKulapichitra, ChaleedaBorompichaichartkula, InthawootSuppavorasatita and KeithR.Cadwalladerb. (2019). Impactofdryingprocessonchemicalcompositionandkeyaroma componentsofarabicacoffee. **291**, 49-58.

Frank, H. A., Lum, N. A. and Cruz, A. S. D. (1965). Bacteria responsible for mucilage-layer decomposition in kona coffee cherries. *J. Appl. Microbiol*. **13** (2), 201-207.

Gautz, L. D., Smith, V. E. and Bittenbender, H. C. (2008). Measuring coffee bean moisture content.

Ghosh, P. and Venkatachalapathy, N. (2014). Processing and drying of coffee–a review. *J Int. J. Eng. Res. Techno* **3**(12), 784-794.

Gonzalez-Rios, O., Suarez-Quiroz, M. L. and Boulanger, R. (2007). Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. *J Food Compost Anal*. **20**, 289–296.

Gure, S., Mohammed, A. and Garedew, W. (2014). Effect of mucilage removal methods on the quality of different coffee

(coffea arabica l.) varieties in jimma, south western ethiopia. *World Appl. Sci. J.* **32** (9), 1899-1905. 10.5829/idosi.wasj.2014.32.09.457.

Haan, C. T., Barfield, B. J. and Hayes, J. C. (1994). "Design hydrology and sedimentology for small catchments". Elsevier. [0123123402].

Haile, M. and Kang, W. (2019a). Antioxidant activity, Total polyphenol, Flavonoid and Tannin Contents of Fermented Green Coffee Beans With Selected Yeasts. *Fermentation*. **5** (1), 29.

Haile, M. and Kang, W. (2019b). The Harvest and Post-Harvest Management Practices' Impact on Coffee Quality. *In:* "Coffee".). Kangwon National University. IntechOpen. [ISBN 978-1-83880-885-3].

Hicks, A. (2002). Post-harvest processing and quality assurance for speciality/organic coffee products. *AU J. Tech.* **5** (3).

Husniati, H. and Oktiani, D. (2019). Chlorogenic acid isolation from coffee as affected by the homogeneity of cherry maturity. *A Coffee Cocoa Res. J.* **35** (2), 119-124.

Hussen, F. and Al Ali, A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata decne*. *J. Mater. Environ. Sci.* . **6**, 1771-1778.

Ico. (2020). "Coffee development report 2020".

Jaiswal, R., Matei, M. F., Golon, A., Witt, M. and Kuhnert, N. (2012). Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. **3** (9), 976-984.

Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K. and De Peña, M. P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *Eur. Food Res. Technol.* **242** (8), 1403-1409. 10.1007/s00217-016-2643-y.

Kanitnuntakul, N., Meeasa, P. and Borompichaichartkul, C. (2015). Antioxidant properties and proximate analysis of green coffee beans from different plantations and processing methods in Thailand. *III Southeast Asia Symposium on Quality Management in Postharvest Systems*. 255-260.

Karki, Y. K. and Regmi, P. P. (2016). Consumer response and their level of satisfaction towards Nepalese coffee. *Int J Appl Sci Biotechnol*. **2** (4), 213-216. 10.3126/ijasbt.v4i2.14570.

KC, J. K. and Rai, B. K. (2007). Basic food analysis handbook,(1st edn), Prompt Printers Pvt.

Kim, I., Jung, S., Kim, E., Lee, J., Kim, C., Ha, J. and Jeong, Y. (2021). Physicochemical characteristics of Ethiopian Coffea arabica cv. Heirloom coffee extracts with various roasting conditions. *food sci. and biotechnol.* **30** (2), 235-244. [doi:10.1007/s10068-020-00865].

Krokida, M. K. and Maroulis, Z. (2001a). Structural properties of dehydrated products during rehydration. **36** (5), 529-538.

Krokida, M. K. and Maroulis, Z. B. (2001b). Structural properties of dehydrated products during rehydration. **36** (5), 529-538.

Kwak, H. S., Jeong, Y. and Kim, M. (2018). Effect of Yeast Fermentation of Green Coffee Beans on Antioxidant Activity and Consumer Acceptability. *J. Food Qual.* **2018**, 5967130. [doi:10.1155/2018/5967130].

Liang, N. and Kitts, D. (2014). Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action. *Molecules* **19**, 19180-19208. 10.3390/molecules191119180.

Masek, A., Latos-Brozio, M., Kałużna-Czaplińska, J., Rosiak, A. and Chrzescijanska, E. (2020). Antioxidant properties of green coffee extract. **11** (5), 557.

Murthy, P. S. and Naidu, M. M. (2011a). Improvement of robusta coffee fermentation with microbial enzymes. **3** (4), 130-139.

Murthy, P. S. and Naidu, M. M. (2011b). Improvement of robusta coffee fermentation with microbial enzymes. *EJAS*. **3** (4), 130-139.

Mutua, J. (2000). Postharvest handling and processing of green coffee in African countries. *IntechOpen*.

Myhrvold, N. Coffee. Encyclopædia Britannica, inc. Retrieved from https://www.britannica.com/topic/coffee.

Nakilcioğlu-Taş, E. and Ötleş, S. (2019). Physical characterization of Arabica ground coffee with different roasting degrees. **91**.

Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A., Feeley, M. and Contaminants. (2003). Effects of caffeine on human health. *J Food Additives*. **20** (1), 1-30.

Nepal, S. (2007). Nepal SIMI annual performance report [Report]. Nepal SIMI report.

NTCDB. (2020). "Coffee Trade Statistics in Nepal". Kathmandu. [Accessed 9/20/2021].

Olechno, E., Puścion-Jakubik, A., Markiewicz-Żukowska, R. and Socha, K. (2020). Impact of Brewing Methods on Total Phenolic Content (TPC) in Various Types of Coffee. *MDPI*. **25** (22), 5274. 10.3390/molecules25225274

Olechno, E., Puścion-Jakubik, A., Zujko, M. E. and Socha, K. (2021). Influence of Various Factors on Caffeine Content in Coffee Brews. **10** (6), 1208. 10.3390/foods10061208.

Oliveira, D., de Oliveira Neto, J., Nascimento, N., Gallo, S., Pessoa, V. and Terra, L. (2011). Administração estratégica. *São Paulo, Atlas*.

Ortega-Rivas, E. (2009). Bulk properties of food particulate materials: an appraisal of their characterisation and relevance in processing. *bioprof. technol*. **2** (1), 28-44.

Pigozzi, M. T., Passos, F. R. and Mendes, F. (2018). Quality of Commercial Coffees: Heavy Metal and Ash Contents. *J. Food Qual.* **2018**. [doi:10.1155/2018/5908463].

Poudel, K., Nepal, A., Dhungana, B., Sugimoto, Y., Yamamoto, N. and Nishiwaki, A. (2009). Capital Budgeting Analysis of Organic Coffee Production in Gulmi District of Nepal [Report].

Pradeep, S., Rameshaiah, G. and Ashoka, H. (2015). Caffeine extraction and characterization. *Int. J. Curr. Res.* **7** (9), 16.

Rather, T. A., Malik, M. A. and Dar, A. H. (2016). Physical, milling, cooking, and pasting characteristics of different rice varieties grown in the valley of Kashmir India. *Cogent Food & Agriculture*. **2** (1). doi:10.1080/23311932.2016.1178694.

Rodrigues, C., Maia, R. and Máguas, C. (2010). Comparing total nitrogen and crude protein content of green coffee beans (coffea spp.) from different geographical origins. *coffee sci.* **5**, 197-205.

Rodriguez, Y., Guzman, G. and Hernandez, J. (2020). Effect of the postharvest processing method on the biochemical composition and sensory analysis of arabica coffee. **40**, 177-183.

Ruta, L. L. and Farcasanu, I. (2021). Coffee and Yeasts: From Flavor to Biotechnology. *MDPI*. **7** (1), 9. [doi:10.3390/fermentation7010009].

Sahrawat, N., Gupta, D. and Chaturvedi, N. (2017). Nutritional composition and sensory attributes of functional aqualetes fortified with Cymbopogon citratus and Coffea arábica. *Asian J. Biol. Life Sci.* **6** (2).

Savolainen, H. (1992). Tannin content of tea and coffee. **12** (3), 191-192.

Schwan, R., Silva, C. F. and Batista, L. R. (2012a). Coffee fermentation. 677-690.

Schwan, R. F., Silva, C. F. and Batista, L. (2012b). Coffee fermentation. *Handbook of plant-based fermented food and beverage technology*. 677-690.

Severa, L., Buchar, J. and Nedomova, S. (2012a). Shape and Size Variability of Roasted Arabica Coffee Beans. *Int. J. Food Prop.* **15** (2), 426-437.

Severa, L., Buchar, J. and Nedomová, Š. (2012b). Shape and size variability of roasted Arabica coffee beans. **15** (2), 426-437.

Shrestha, P. M., Sharma, P. and Mishra, R. (2008). Production, Processing and Marketing of Coffee in Nepal. *Proceedings of the Fifth National Seminar on Horticulture, June*. 9-10.

Silva, C. F. (2014). Microbial activity during coffee fermentation. *Cocoa and Coffee Fermentations*. 368-423.

Silva, C. F., Abreu, L. R. B. M., Dias, E. S. and Schwan, R. F. (2008). "Succession of bacterial and fungal communities during natural coffee (coffea arabica) fermentation. *Food Microbiol.* **25** (8), 951-957.

Silva, C. F., V., D. M., de Souza Cordeiro, C., Duarte, W. F., Dias, D. R. and Schwan, R. F. (2013). Evaluation of a potential starter culture for enhance quality of coffee fermentation. *World J. Microbiol. Biotechnol.* **29** (2), 235-247.

Singh, P. and Kumar, S. (2019). Microbial enzyme in food biotechnology. *In:* "Enzymes in food biotechnology".). pp. 19-28. Elsevier.

Song.C. (2018). Fermentation of Green Coffee Bean with Rhizopus oryzae and Its Biochemical Properties. Master in agriculture science dissertaion. 서울대학교 대학원,

Speer, K. and Kölling-Speer, I. (2006). The lipid fraction of coffee bean. *Braz. J. Plant Physiol*. **18**. [doi:10.1590/S1677-04202006000100014].

Sualeh, A. and Dawid, J. (2013). Relationship of fruit and bean sizes and processing methods on the conversion ratios of arabica coffee (*Coffea arabica*) cultivars. *Time J. Agr. Vet. Sci.* **2**, 70-74. ISSN:2360-736X.

Subba, R. (2021). Byproduct utilization of coffee cherry as an alcoholic beverage. Department of Food Technology Central Campus of Technology Institute of …,

Subedi, R. N. (2010). Comparative analysis of Dry and Wet Processing of Coffee With Respect to Quality in Kavre District, Nepal. M. Tech Wageningen Univ., The Netherlands.

Sunarharum, W., Yuwono, S. and Aziza, O. (2019). Study on the effect of roasting temperature on antioxidant activity of early-roasted Java coffee powder (Arabica and Robusta). *Environ. Earth Sci.* **230**. [doi:10.1088/1755-1315/230/1/012045].

Tai, E., Hsieh, P. and Sheu, S. (2014). Effect of polygalacturonase and feruloyl esterase from *Aspergillus tubingensis* on demucilage and quality of coffee beans. *pro. Biochem.* **49**. 10.1016/j.procbio.2014.05.001.

Teketay, D. (1999). History, botany and ecological requirements of coffee. *Walia*. **20**, 28-50.

Tesfa, M. (2019). Review on post-harvest processing operations affecting coffee (*Coffea arabica l.*) quality in Ethiopia. *J. Environ. Earth Sci.* **6**, 25-35.

Thakur, J. and Gupta, R. (2012). Improvement of tea leaves fermentation through pectinases. **59** (3), 321-334. DOI: 10.1556/AMicr.59.2012.3.3.

Tiwari, K. P. (2010). Agricultural policy review for coffee promotion in nepal. *J. Agric. Environ*. **11**.

Tuli, H. S. (2019). "Current Aspects of Flavonoids: Their Role in Cancer Treatment". Springer. [9811358745].

Ulloa Rojas, J., Verreth, J., Weerd, J. H. and Huisman, E. A. (2002). Effect of Different Chemical Treatments on Nutritional and Antinutritional Properties of Coffee Pulp. *Anim. Feed Sci. Technol.* **99**, 195-204.

USDA. "Tannins". Washington D.C.

Vaast, P., Bertrand, B., Perriot, J. J., Guyot, B. and Genard, M. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (coffea arabica l.) under optimal conditions. *J. Sci.* **86** (2), 197-204.

Valentin, J. L. and Watling, R. J. (2013). Provenance establishment of coffee using solution ICP-MS and ICP-AES. *J. Appl. Sci. Environ. Manage.* . **141** (1), 98-104.

Venkatachalapathy, D. N. (2014). Processing and drying of coffee – a review. *IJERT*. **3** (12). 2278-0181.

Vignoli, J., Bassoli, D. and Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chem.* **124** (3), 863-868.

Wang, X., Hu, S., Wan, X. and Pan, C. (2005a). Effect of microbial fermentation on caffeine content of tea leaves *J. Agric. Food Chem.* **53** (18), 7238-7242.

Wang, X., Hu, S., Wan, X., Pan, C. J. J. o. a. and chemistry, f. (2005b). Effect of microbial fermentation on caffeine content of tea leaves. **53** (18), 7238-7242.

Wang, X. and Lim, L. T. (2015). Physicochemical characteristics of roasted coffee. *In:* "Coffee in health and disease prevention".). pp. 247-254. Elsevier.

Willson, K. C. (1999). "Coffee, Cocoa and Tea". CABI Pub. New York. [0851989195 9780851989198].

Wintgens, J. N. (2009). The coffee plant. 3-24. DOI:10.1002/9783527619627.

Yahia, E. M. and Carrillo-Lopez, A. (2018). "Postharvest physiology and biochemistry of fruits and vegetables". Woodhead publishing. [0128132795].

Zhu, M., Long, Y., Chen, Y., Huang, Y., Tang, L., Gan, B., Yu, Q. and Xie, J. (2021). Fast determination of lipid and protein content in green coffee beans from different origins using NIR spectroscopy and chemometrics. *J Food Compost. Anal.* **102**, 104055. [doi10.1016/j.jfca.2021.104055].

Appendices

Appendix A

Chemical required

- Yeast food (malthouse)
- Yeast strain (*Saccharomyces cerevisiae*)
- Ploygalacturonase Enzyme (from Aspergillus Niger)
- Gallic acid Standard (99% Assay)
- Tannic acid Standard(≥ 99.9 %,merck)
- Methanol (assay:>99%, Emplura®)
- Aluminum chloride (assay: 98.5%, Qualigens, India)
- Folin-ciocalteau's reagent (A.R. grade, Fisher Scientific, India)
- Sodium hydroxide (purity 97%,Alfa chemicals)
- Quercitin (assay: 98%, Himedia, India)
- Dichloromethane (assay: >99.5%, Himedia, India)
- DPPH assay: (95% Sisco Research Laboratories. Pvt. Ltd., India)
- Standard Caffeine (M.W. 194.19 g/mol, Sisco Research Laboratories. Pvt. Ltd, India)
- Sodium Carbonate (≥ 99.9 %,merck)

Equipment required

- Hot air oven
- Water bath (serological)
- Weighing balance (AMPUT Electronic Balance Model No-457, Sensitivity±0.01 g)
- Fermentation jars
- Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO- 291)
- Soxhlet apparatus (Tulin SoX-2)
- Heating arrangement
- Glassware and utensils required
- Beaker, Conical Flask, Volumetric flask, Separating Funnel, Burette, Pipette, Test tube, Measuring Cylinder, Stand, Petri plate.

Analysis of variance of different samples

1. DPPH

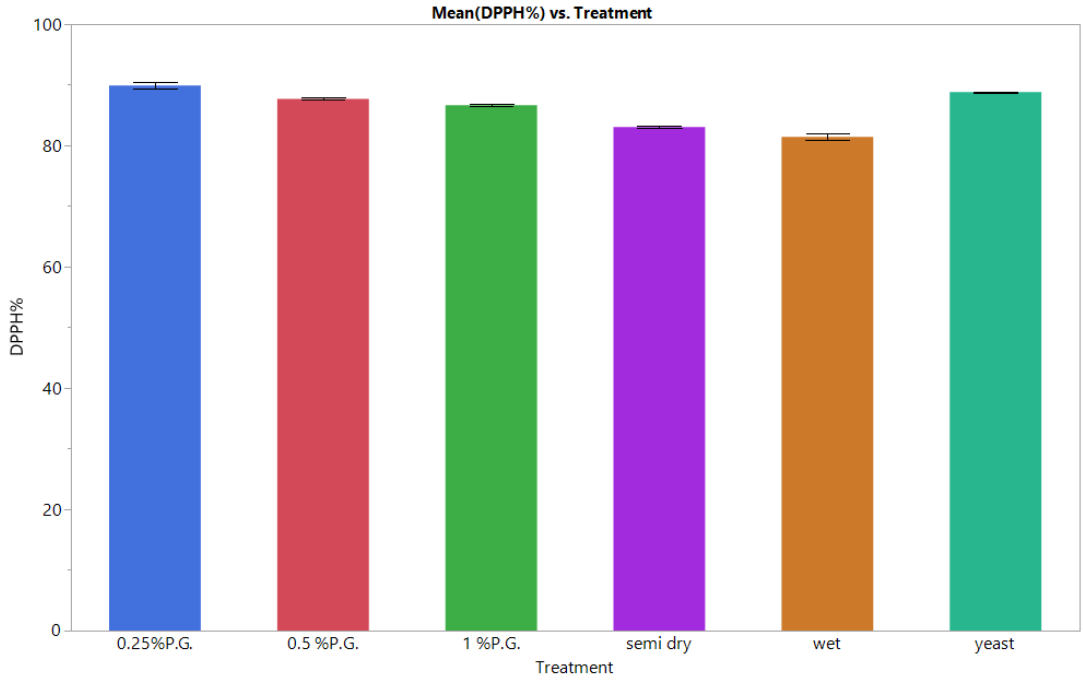
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	166.22821	33.2456	99.8057	<.0001*
Error	12	3.99724	0.3331		
C. Total	17	170.22545			

Connecting Letters Report

Level						Mean
0.25%P.G.	A					89.895600
yeast	A	B				88.800000
0.5 %P.G.		B	C			87.700000
1 %P.G.			C			86.633333
semi dry				D		83.061447
wet					E	81.433333

Levels not connected by same letter are significantly different.



2. TPC.

Analysis of Variance

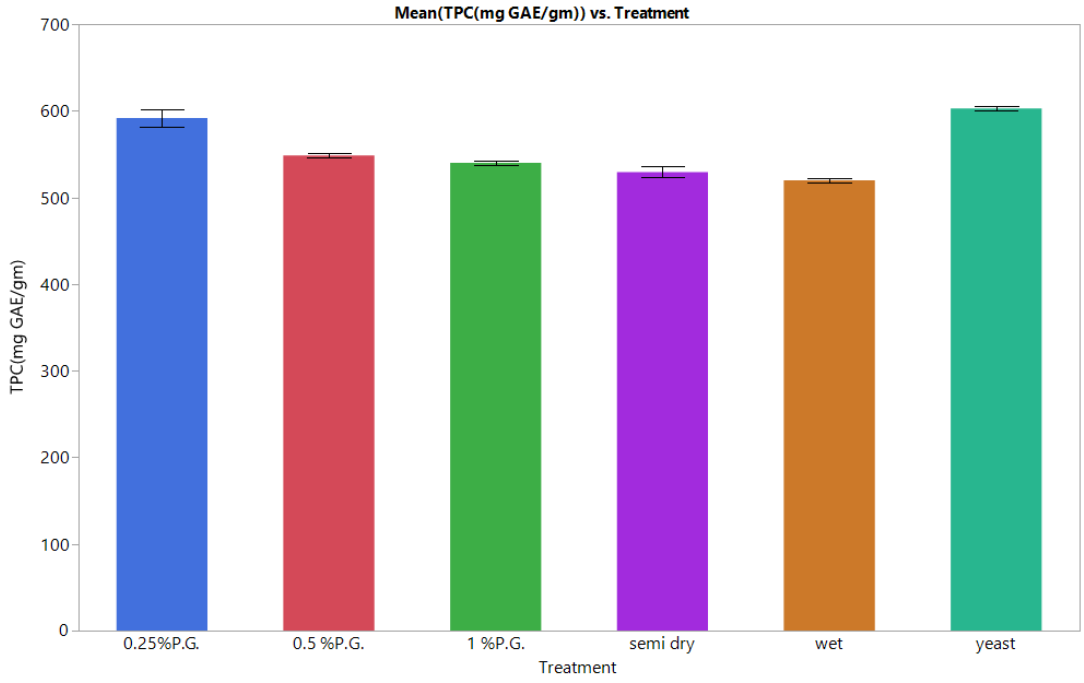
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	17378.278	3475.66	41.5693	<.0001*
Error	12	1003.333	83.61		
C. Total	17	18381.611			

Connecting Letters Report

Level		Mean
yeast	A	602.66667

Level			Mean
0.25%P.G.	A		591.66667
0.5 %P.G.		B	548.33333
1 %P.G.		B	C	540.00000
semi dry		B	C	529.33333
wet			C	519.66667

Levels not connected by same letter are significantly different.



3.Tannin:

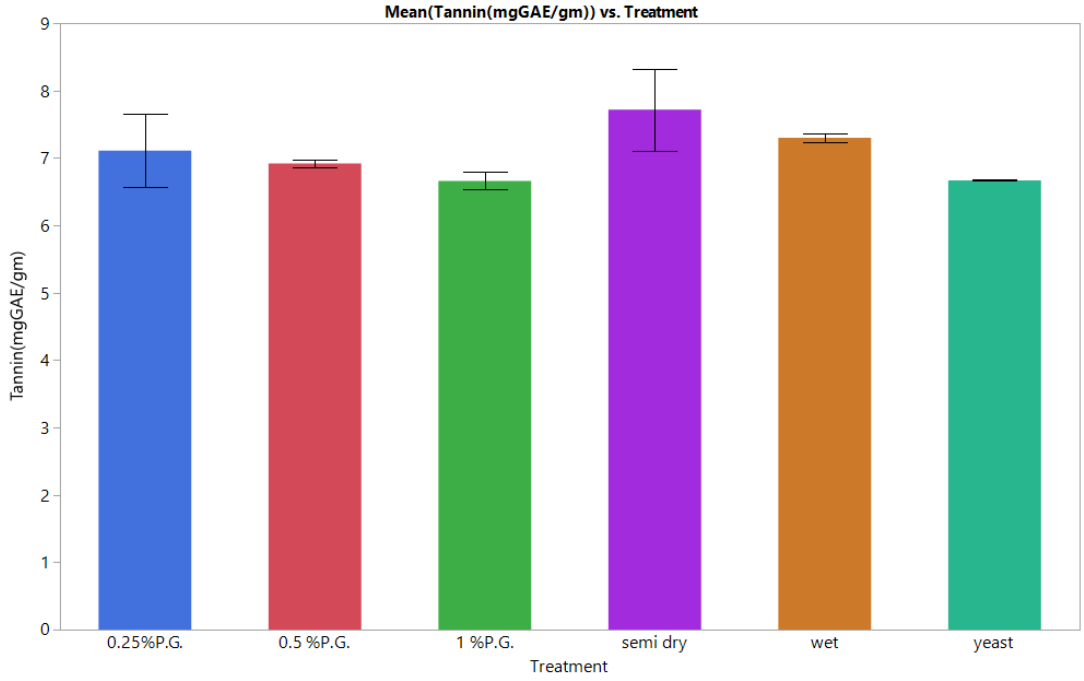
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	2.4856180	0.497124	1.4477	0.2769
Error	12	4.1205622	0.343380		
C. Total	17	6.6061802			

Connecting Letters Report

Level		Mean
semi dry	A	7.7179487
wet	A	7.3012821
0.25%P.G.	A	7.1089744
0.5 %P.G.	A	6.9166667
yeast	A	6.6666667
1 %P.G.	A	6.6602564

Levels not connected by same letter are significantly different.



5. **Caffeine**:

Analysis of Variance

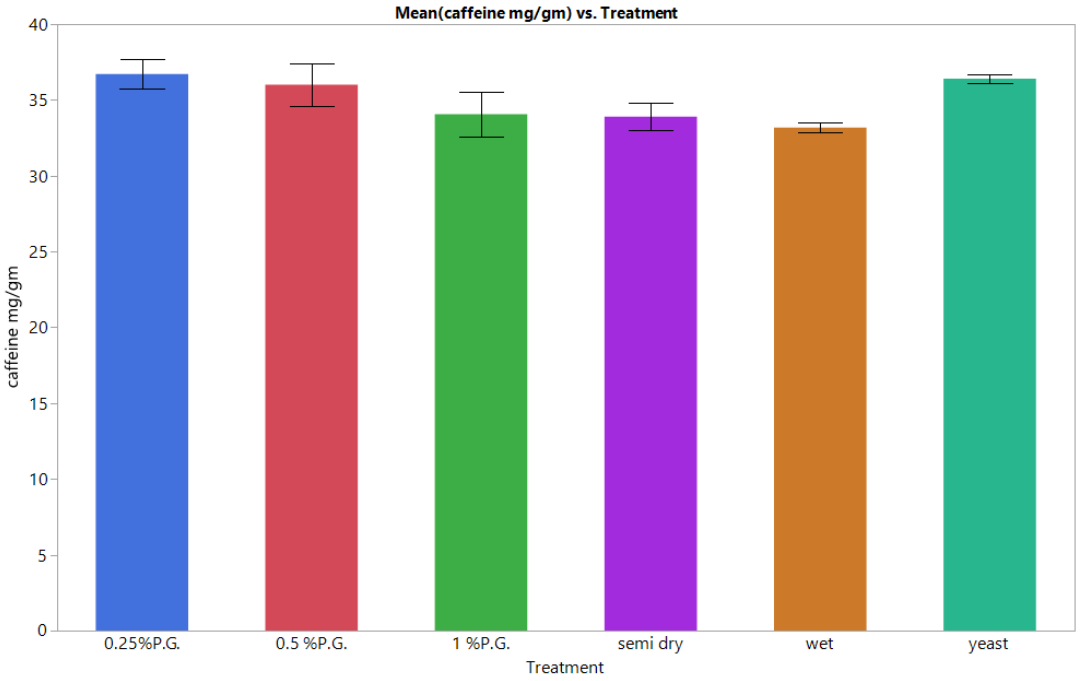
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	33.645444	6.72909	2.1731	0.1257
Error	12	37.159051	3.09659		
C. Total	17	70.804495			

Connecting Letters Report

Level		Mean
0.25%P.G.	A	36.710000
yeast	A	36.400000

Level		Mean
0.5 %P.G.	A	36.000000
1 %P.G.	A	34.070000
semi dry	A	33.906667
wet	A	33.188000

Levels not connected by same letter are significantly different.



5. Flavonoids

Analysis of Variance

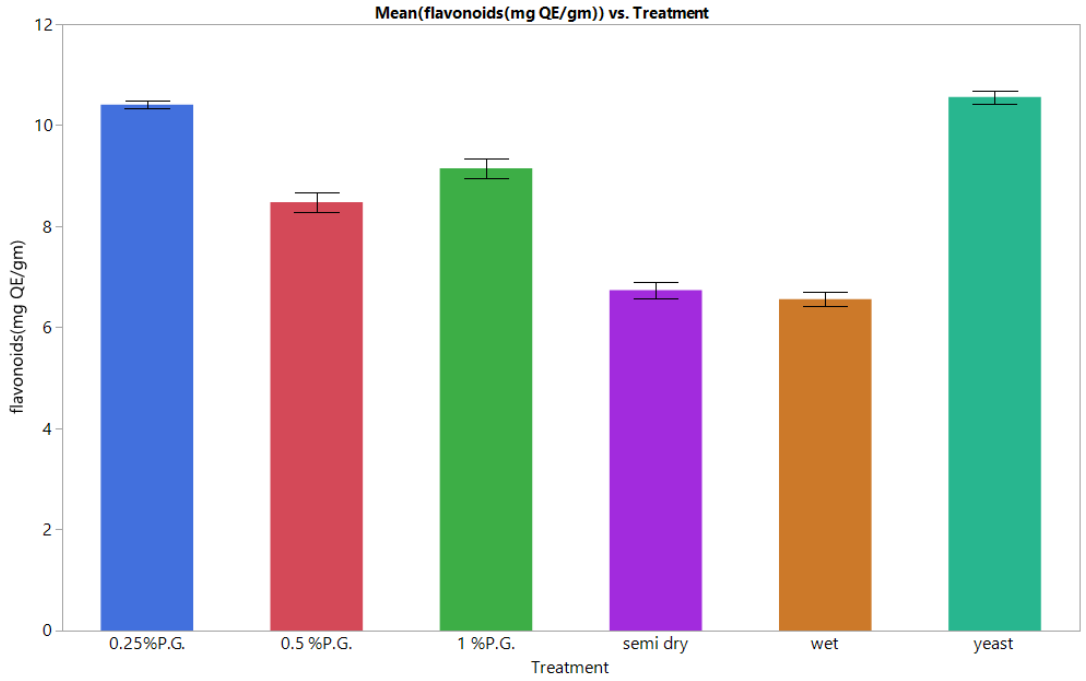
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	45.092506	9.01850	125.1035	<.0001*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Error	12	0.865060	0.07209		
C. Total	17	45.957566			

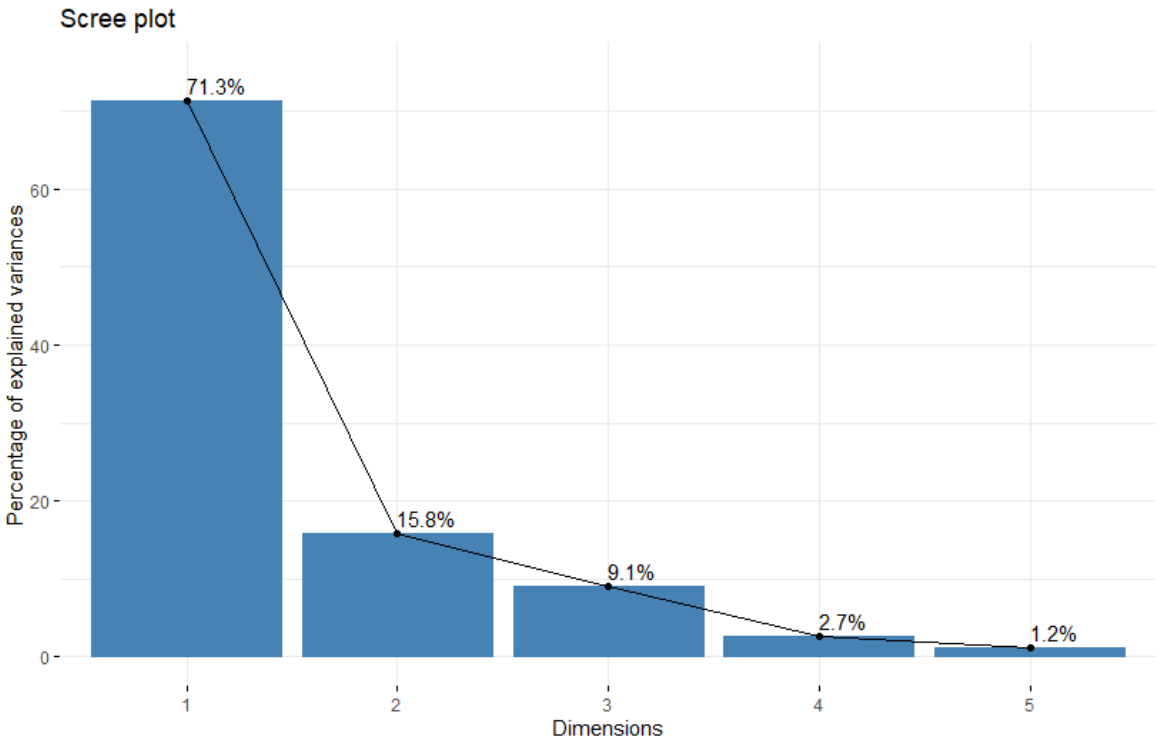
Connecting Letters Report

Level			Mean
yeast	A		10.556793
0.25%P.G.	A		10.408315
1 %P.G.		B	9.146251
0.5 %P.G.		B	8.478099
semi dry		C	6.738679
wet		C	6.558500

Levels not connected by same letter are significantly different.


Mean(flavonoids(mg QE/gm)) vs. Treatment
flavonoids(mg QE/gm)
12
10
8
6
4
2
0
0.25%P.G.
0.5 %P.G.
1 %P.G.
semi dry
wet
yeast
Treatment

scree plot


Scree plot
71.3%
15.8%
9.1%
2.7%
1.2%
Percentage of explained variances
60
40
20
0
1
2
3
4
5
Dimensions

PCA:

1. **Eigen value**

	eigenvalue	variance.percent	cumulative.variance.percent
Dim.1	3.56257849	71.251570	71.25157
Dim.2	0.78882249	15.776450	87.02802
Dim.3	0.45657661	9.131532	96.15955
Dim.4	0.13364815	2.672963	98.83251
Dim.5	0.05837426	1.167485	100.00000

Appendices B

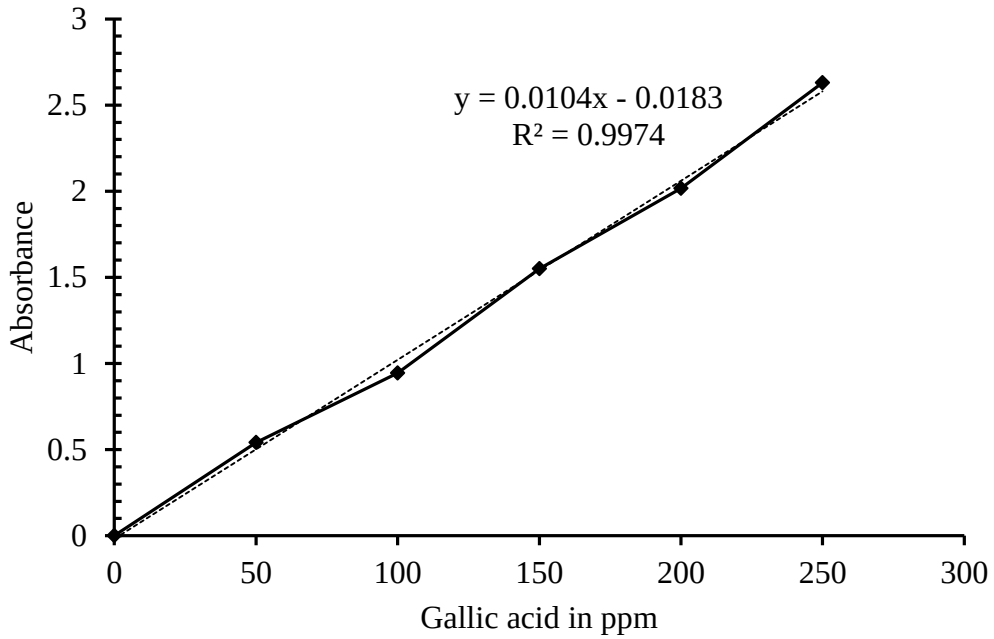


Fig.B1 calibration curve for total phenolic content

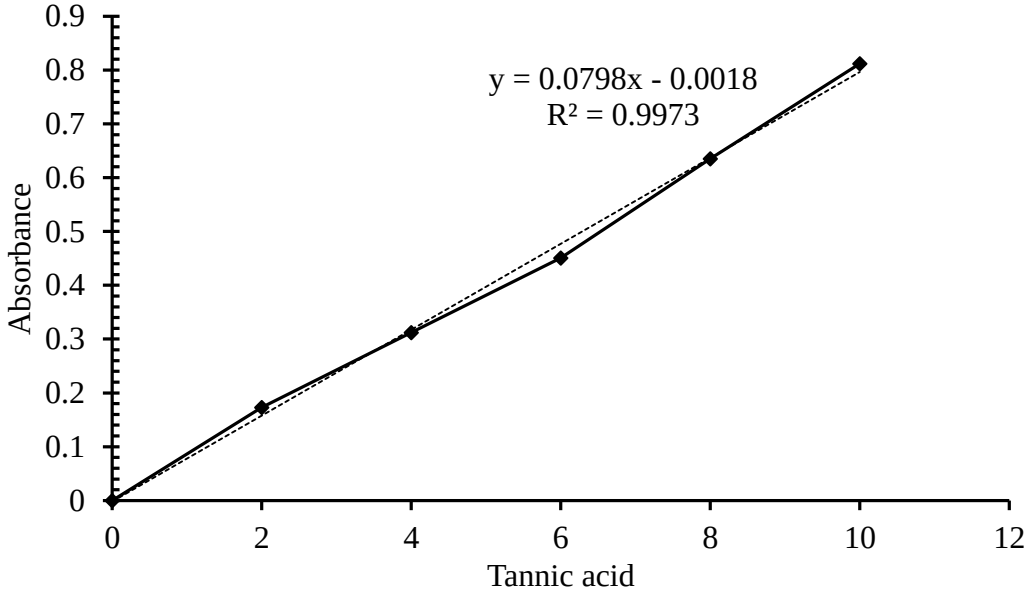


Fig.B2 Calibration curve for tannin

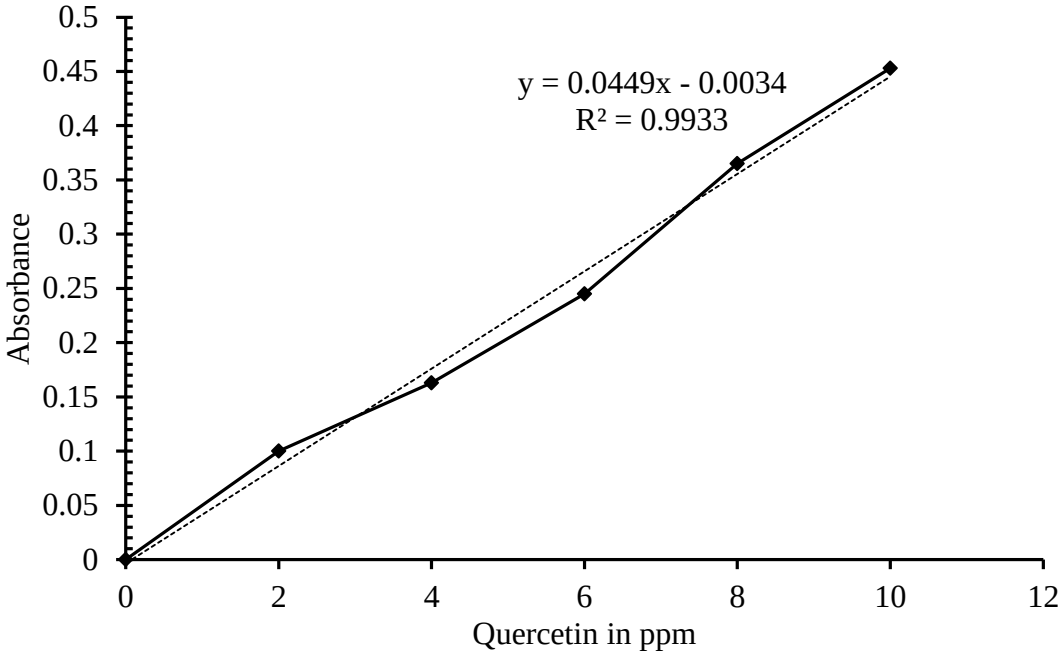


Fig.B3 calibration curve for flavonoids

Color plates

Pic.1 Fermentation by different methods

Pic.**2**drying of beans

Pic.3 extraction of phytochemicals

pic.4 caffeine Extraction

Pic.5 Analysis of protein and fat

pic.6 Crude fibre analysis