EFFECT OF DRYING TEMPERATURE ON PHYSICOCHEMICAL PROPERTIES AND BIOACTIVE COMPONENTS OF BEETROOT (*Beta vulgaris*)

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Approval Letter

This *dissertation* entitled *Effect of Drying Temperature on Physicochemical Properties and Bioactive Components of Beetroot (Beta vulgaris)* presented by Prakash Sapkota has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

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(Prakash Sapkota)

Abstract

The aim of the study was to determine the effect of drying temperature on the bioactive components and physicochemical characteristics of beetroot dried at various temperatures. Beetroot was purchased and pretreated with sorting, cleaning, and cutting procedures. It was then dried in a cabinet dryer at 50°C, 55°C, 60°C, 65°C and 70°C until the weight of the beetroot was constant. The dried beetroot was then powdered and then sieved through 40 mesh size to a fine consistency.

Both fresh and dried samples were examined for the bioactive components, such as antioxidant activity, total phenolic content, total flavonoid content, betalain content and total tannin content, and physicochemical properties such as bulk density, solubility, oil absorption capability, and swelling capacity, were also evaluated. The results demonstrated a significant decline in bioactive components as beetroot underwent drying at progressively increasing temperatures. However, drying at 50°C revealed a smaller loss of bioactive components as compared to other temperatures. Reduction in the concentration of bioactive components was noted, corresponding with the increasing temperature. In comparison between fresh sample to 70°C sample, the TPC, TFC, AA, TTC, and betalain content all dropped by 63%, 44%, 16%, 74% and 54%, respectively. Acording to analyses of this study for physicochemical attributes, higher temperatures improved the bulk density, oil absorption capacity, swelling capacity, and solubility index of beetroot powder. All of the study's sample analyses revealed that the lower temperature drying i.e at 50°C, retained more bioactive components.

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List of Abbreviations

Abbreviations	Fullform
AA	Antioxidant activity
AOAC	Association of Analytical Communities
ANOVA	Analysis of variance
BLC	Betalain content
CA	Controlled atmosphere
DOPA	Dihydroxyphenylalanine
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DE	Dry extract
FAO	Food and Agriculture Organization
GAE	Gallic acid equivallent
IBM	International Business Machine
LDL	Low density lipoprotein
LSD	Least significance difference
OAC	Oil absorption capacity
QE	Quercetin equivalent
SPSS	Statistical Package for Social Sciences
TAE	Tannic acid equivallent
TSS	Total soluble solid
TPC	Total phenolic content
TTC	Total tannin content
TFC	Total flavonoid content

Part I

Introduction

1.1 General introduction

Beetroots have spread from North Africa to the seashores of Asia and Europe via the Mediterranean Sea route. It is grown in temperate regions all over the world, with the majority of output concentrated in North America, Europe, Asia, and North Africa. Around the world, the red beetroot (*Beta vulgaris L. var. esculenta L. Cylindra*) is a well-known and traditional vegetable. It can be used in many different ways as red food colorings, such as dry forms like chips, tea, powder in bakeries, food supplements, and more. Examples of foods where it is utilized include tomato paste, sauces, desserts, jams and jellies, ice cream, sweets, and cereals (Nistor *et al.*, 2017).

Red beetroot (*Beta vulgaris L.*) has higher levels of bioactive substances such betacyanin, betaxanthin, betalains, polyphenols, flavonoids, ascorbic acid, and carotenoids, among others which makes it a popular root vegetable with various health benefits. Various colors and bioactive components make it suitable for use in a range of therapeutic applications. It aids in the industry's creation of functional food products (Malakar *et al.*, 2022). Beetroots contain reddish-purple betacyanins (like betanin and isobetanin) and yellow-orange betaxanthins (like vulgaxanthin and miraxanthin), which have advantageous effects on human health. These include boosting the immune system and hematopoietic system, as well as having antitumor, anti-inflammatory, and hepatoprotective properties (Liu *et al.*, 2020).

Beetroot (*Beta vulgaris*), a natural alternative to manufactured colors, is the main source of the betalains pigment. As the 10^{th} most potent vegetable with antioxidant qualities, beetroot contains phenolic compound, carotenoids, betalains, vitamins, and minerals, all of which are significant biocompounds and micronutrients (Kushwaha *et al.*, 2018). There are three key factors that make sugarbeet essential to farmers. First off, it is a solid income crop; second, it improves soils afflicted by salt by boosting soil fertility via good agricultural techniques; and third, the by-products supply nourishing cattle feed during the warmer months of the year when green fodder is not easily accessible (Pathak *et al.*, 2014). One of the most popular drying techniques is hot air drying. High drying efficiency, ease of use, affordability, and minimal environmental impact are just a few of its numerous benefits. Few studies have been conducted to examine how the physicochemical qualities and drying

characteristics of beetroot slices are affected by varying hot air drying temperatures (Liu *et al.*, 2020).

Drying enables the use of fresh fruits and vegetables throughout the off-season as an alternative to eating them fresh. The dried beetroot powder functions as a brightly colored pigment, has a longer shelf life, a lower risk of microbiological risks, and can be utilized as a value-added component in a variety of culinary products. The two qualities that have the greatest impact on how much a consumer would accept a product are its color and flavor in the case of dried red beetroot (Bunkar *et al.*, 2020).

1.2 Statement of the problem

With beetroot's special blend of vitamins, minerals, and antioxidants, beetroot is an underappreciated crop that is rich in nutrients. Although beets are grown economically in many places of Nepal, they aren't being used to their full potential in the market (Paudel, 2022).

Vegetables are sensitive to a variety of spoiling organisms, such as numerous kinds of bacteria and fungi, due to certain characteristics like high water activity (Kale *et al.*, 2018) and near neutral pH (Tournas, 2005). Additionally, the chemical and mineral composition of the beetroot was reported, with results revealing that its betalain concentration was 14.20 mg/100g, moisture content of 87.4%, carbohydrate content of 7.59%, protein content of 1.35%, and fat content of 0.3% in weight basis (Kale *et al.*, 2018).

Because of its disagreeable flavor and aroma, beetroot has not been in particularly high demand as a functional food ingredient. Beet tubers are typically ingested in the form of juice or fruit juice (Aznury *et al.*, 2020). Nitrates are prevalent in beetroots, however. The early deterioration of fresh, raw beetroot is caused by microbial attacks because of the high concentration of nitrates (Nistor *et al.*, 2017). This study particularly identifies the bioactive components of raw beetroot and the impact of different controlled drying temperatures on them which is important for consistent product quality and is currently absent. The drying temperature-related variations in bioactive components have not been researched as much as other topics.

1.3 Objectives

1.3.1 General objectives

The general objective was to study effect of drying temperature on physicochemical properties and bioactive components of beetroot (*Beta vulgaris*).

1.3.2 Specific objectives

The specific objectives of the study were as follow:

1. To evaluate the effect of various drying temperatures on bioactive components of beetroot.
2. To evaluate the physicochemical properties of dried beetroot powder.
3. To find the optimum drying temperature for beetroot on the basis of effect of temperature on its physicochemical properties and bioactive components.

1.4 Significance of the study

The study can aid in determining the optimum drying temperature for beetroot, which ensures efficient water removal while minimizing the degradation of bioactive components. By identifying the temperature range that maintains the desired physical properties and bioactive content, researchers can help optimize the drying processes for beetroot, leading to improved product quality and energy efficiency. After the completion of this work, by understanding the effect of drying temperatures, companies can develop innovative processing techniques, modify existing drying protocols, and formulate new products that cater to consumer preferences while maintaining nutritional quality.

1.5 Limitations of the study

1. Only the change in total phenolic content, total flavonoid content, tannin content, betalain content and anti-oxidant activity were studied.
2. Only one variety of the beetroot was used for the study.

Part II

Literature review

2.1 Historical background

The history of beetroot dates back to 8500 B.C. on the European coasts. The first part of a plant that prehistoric men used as a culinary commodity was its leaves. Its roots can produce sugar, which was found and discovered in 1705, but it was not heavily used. Later, Andreas Marggraf made the first scientific discovery that the crystals found in sugarcane stalks and powdered sugar beet root are the same. When the British blockade cut off the French Empire's supply of raw cane sugar from the West Indies in 1811, the significance of sugar beet for sugar became apparent, which sparked Napoleon's interest in it. This crop was subsequently brought to North America around 1830. The United States' first prosperous sugar beet factory was constructed in 1840 after the factories were resurrected. While sugar beet production first reached Russia and Ukraine in 1850, it was brought to India as a new commercial crop in the 1950s (Pathak *et al.*, 2022).

Beta vulgaris was valuable for its leaves and the meaty, lengthy leaf midribs that make chard during its progress on cultivation in the past. Since the beginning of written history, leaf beets, including chard, have been a common food crop throughout Europe, North Africa, and the Middle East. Around 800 BC, beet (silga), one of the wonders of the ancient world, was mentioned in an Assyrian document as growing in the Hanging Gardens of Babylon. In Middle Eastern regions, it was also known by other names such as selg, silq, silig, seig, or salk. Leaf beets were grown by the ancient Greeks and Romans as a pot herb (Nottingham).

2.2 Beetroot

The beetroot (*Beta vulgaris*), an alkaline meal with a pH range of 7.5 to 8.0, has received praise for its health advantages, particularly for its capacity to fight disease-causing free radicals, considerable amounts of vitamins C and B_1, B_2, niacin, B_6, and B_{12}, and great source of vitamin A (Kharode *et al.*, 2019). Kidney stone could be treated by drinking two glasses of beetroot rhizome juice per day for seven days (Sharma *et al.*, 2011).

2.3 Taxonomy

Beta vulgaris is a combination of domesticated and untamed plants that Linnaeus included in the Species plantarum protologue (1753). Starting with var. perennis, the species is treated. Initially, according to Linnaeus, domesticated beets were created from wild plants; the primary source for this theory is the var. perennis, or "Habitat in Angliae & Belgii litoribus maris." According to pre-Linnaean botanist Caspar Bauhin, the variety perennis is equivalent to Beta sylvestris maritima.

On the basis of a lack of understanding of the continuous variation pattern over a wide distribution range, small variants of wild taxa were classified in early taxonomic treatments. Most items are classified in recent taxonomies treatments according to subspecies or varieties in an effort to recognize them. They view *Beta vulgaris* L. in its natural state as a highly variable taxon where 'clinal variation' and the development of 'biotypes' are common in some regions of the distribution range. in order to have a better understanding of the genetic diversity structure in the widespread *B. vulgaris* species as well as the taxonomic organization of this complicated group (Letschert, 1993).

Table 2.3 The taxonomic position of beetroot

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cryophyllales
Family	Amaranthaceae
Genus	*Beta*
Species	*vulgaris*

Source: Ceclu and Nistor (2020)

2.4 Varieties of beetroot

Beetroot is a root vegetable rich in carotenoids, nitrates, flavonoids, vitamins, minerals like potassium, sodium, phosphorous, calcium, magnesium, copper, iron, zinc, and manganese, as well as water-soluble betalains like betacyanins (reddish-violet color) and betaxanthins (yellowish-orange color), which have a variety of nutritional and health benefits.

Beetroot comes in four different primary varieties: Detroit Dark Red, Crimson Globe, Crosby Egyptian, and Early Wonder. Detroit Smooth, homogeneous roots with dark red flesh make up the dark red beet. The flesh of Crimson Globe beetroots is a medium-dark crimson with a variety of zones, and they have small shoulders. The Crosby Egyptian beetroot is a flat globe with a dark purple red interior and hazy zones. After seeding, they attain maturity 55–60 days later. The inside flesh of Early Wonder roots is dark red with occasional lighter red zones. The root is flattened. The highest is a smooth-textured, heavy-leafed globe with green leaves and crimson veins (Chhikara *et al.*, 2019).

2.5 Morphology of beetroot

The beetroot is a true biennial, producing thicker roots and a rosette of leaves the first year, followed by flowers and seeds the following year. The enlarged root of beets is the primary reason they are grown. Simple leaves that are placed in a closed spiral are produced by the short, plate-shaped stalk. Heart-shaped leaves are present. The leaves can also be consumed like spinach. Flowers are produced in dense spikes and have a 3 to 5 mm diameter. They have five petals and are green or have reddish undertones. Fruit is a dark-colored cluster of tough nuts (Neha *et al.*, 2018).

2.6 Climates and soil

It requires soil temperatures between 4.5 to 30°C for the germination of red beetroot seeds, making it a cool-weather crop. With a pH range of 5.8 to 7.0 and a tolerance of up to pH 7.6, deep, well-drained, loose, loamy, sandy soils are ideal for growing red beetroot. Red beets are multiplied from seeds, and the timing of the sowing process varies depending on the production area (Akan *et al.*, 2021).

2.7 Cultivation

Even though they are typically produced as annuals, cultivated beets (*Beta vulgaris*) are biennials. During its first growth season, beetroot generates green tips and a swelling taproot. In the second season, the taproot's nutrients are used to generate blooms and seeds. *Beta vulgaris* cultivars must be grown from seeds. The seed can be found as a seed cluster, glomerular, or seed ball. Depending on the temperature and other factors, germination typically takes 10 to 24 days, though it can happen sooner in the right circumstances. When temperatures are high, beetroot germinates rather well; but, when temperatures are low, germination is inconsistent and slow. Compared to other crop seeds, beetroot seed has a comparatively low rate of germination (Nottingham, 2004).

2.8 Production in the world

In 2017, 301 million tons of beet roots were produced worldwide on 4.89 million hectares of harvested land. In terms of beet root production, Europe leads the world with 207.9 tons, or 69% of the total amount produced globally. Asia is second with 42.7 tons, America is third with 34.3 tons, and Africa is in last place with 15.9 tons (de Oliveira *et al.*, 2021). The global production of beetroot was roughly 274 million tonnes in 2018, according to FAO statistics (El-Beltagi *et al.*, 2022). In 2020, there were about 252,968,843 tons of beetroot produced overall. Additionally, the US (approximately 30,497,740 tons) and Russian Federation (about 33,915,086 tons) were the two countries with the highest beetroot production in 2020 (Punia Bangar *et al.*, 2022).

2.9 Storage

At 1-2°C and 98%-100% RH, topped beetroots can be kept for 8 to 10 months. Black spots brought on by Phoma betae Frank grow more quickly when the temperature is lower. Because the benefits of CA are minimal, it is not advised for long-term beetroot storage. But for 4.5 months at 1°C, beetroots can withstand 3%–5% CO_2. As it encourages fungal development and gives geosmin a fusty, earthy odor and flavor, storage atmospheres with more than 10% CO_2 are not advised. To keep root texture and turgidity in tact, packaging should be done in films with low water vapor transfer rates, like polyethylene films.

Optimal storage circumstances

The ideal conditions are between 1-2°C and 98-100% RH. considerations:

- Benefits: >17% O_2 and 4% CO_2

- Tolerances: >10% O_2 and 10% CO_2 for 1 month

- Injuries: 10% O_2 and >10% CO_2

Beetroot quality is negatively impacted by low RH (95%) for an extended period of time because the cell turgor drops, the tissue loses water, and the roots get dehydrated and lose weight. Beetroot may sustain weight loss of up to 7% and still be marketable (Edelenbos *et al.*, 2020).

2.10 Nutritional composition

Beetroot (*Beta vulgaris*) is a significant plant-based basic material that has been shown to have health benefits for people. They can be boiled, steamed, roasted, or eaten raw. Minerals like manganese, iron, sodium, potassium, magnesium, and copper are abundant in red beetroot. Beetroot is rich in antioxidants, vitamins (A, B, and C), fiber, and natural colors. Additionally abundant in phenol compounds with antioxidant capabilities is red beetroot. These vibrant root vegetables provide defense against heart disease and some malignancies, including colon cancer (Mudgal, 2022).

Raw beetroot was found to have the following nutritional values: moisture (85.56%), protein (1.41%), fat (0.21%), carbohydrate (9.05%), ash (1.18%), fiber (2.2%), and betalain content (291 mg/100g), all in that order (Sakhare *et al.*, 2019). Another study conducted by Niroula *et al.* (2022) found the proximate composition of fresh beetroot to be moisture content 89.49%, protein 10.99% (db), crude fat 1.32% (db), total ash 11.08% (db), crude fiber 9.8% (db), and carbohydrate 66.8% (db).

2.11 Bioactive components

Bioactive components are those found in meals or dietary supplements that, in addition to meeting basic nutritional requirements, cause positive changes in health status (Guaadaoui *et al.*, 2014).

In various regions of the world, red beetroot is extremely well-liked and frequently utilized for cancer patients. Despite the lack of evidence supporting its direct role in cancer treatment and chemoprevention, patients with cancer may benefit from its antioxidant, anti-inflammatory, and other supportive effects at specific stages of the disease, such as during

chemotherapy and after chemotherapy. This narrative review intends to highlight the important phytochemical components found in red beetroot, the state of the science regarding its potential health advantages as a chemopreventive functional food, and to pinpoint any remaining research gaps in this field (Tan and Hamid, 2021).

The highest concentrations of total phenol were found in raw beets and beetroot juice (98.08 ± 8.16 mg/g and 98.08 ± 5.77 mg GAE/g), respectively. The least amount of total phenol was found in oven-dried beetroot (94.23 ± 2.72 mg GAE/g). The maximum concentration of flavonoids, 96.67 ± 10.10 mg GAE/g, was found in the heat-treated beetroot. Raw beetroot had the least flavonoids (63.34 ± 4.72 mg QE/g. Beetroot juice and oven-dried produce both had a total flavonoid value of 83.34 ± 3.34 mg GAE/g (Olumese *et al.*, 2016). A 100 g serving of beetroot contains 43 Kcal of calories, 9.56 g of carbs, 1.61 g of protein, 0.17 g of total fats, 6.76 g of total sugars, alkaloids (127.8 mg), steroids (16.4 mg), flavonoids (6.15 mg), terpenoids (115.5 mg), and saponins (3.789 mg). Additional phenolic acid compounds comprise chlorogenic acid, epicatechin, and 4-hydroxybenzoic acids. Further examples of flavonoids encompass betagarin, betavulgarin, cochliophilin A, and dihydroisorhamnetin (Thiruvengadam *et al.*, 2022).

2.11.1 Phenolic compounds

Phenolic chemicals are a broad category of secondary plant metabolites that are important for the nutritional value of plant-based diets. High concentrations of flavonoids and phenolic chemicals can be found in beetroot. According to reports of Kathiravan *et al.* (2014), beetroot has 50–60 mol/g dry weight of phenolic acids in its whole. Additionally, the second-highest dry weight concentration of total phenols is found in beetroot peel. The very unstable phenolic compounds isolated from the peel of the red beetroot were 5,50,6,60-tetrahydroxy-3,30-biindolyl; a dimer of 5,6-dihydroxyindolecarboxylic acid and betalains composed of vulgaxanthin I, vulgaxanthin II, indicaxanthin, prebetanin, isobetanin, betanin and neobetanin. Additionally, the seed wall of beetroot was used to isolate the phenolic amides N-trans-feruloyltyramine and N-trans-feruloylhomovanillylamine (Nemzer *et al.*, 2011). The two main groups of phenolic acids, hydroxybenzoic and hydroxycinnamic acid derivatives, were found to be significantly present in *Beta vulgaris* var. cicla. These phenolic acids include proline, monoterpenedehydrovomifoliol, vanillic, p-coumaric, protocatechuic, caffeic acid, catechin hydrate, and epicatechin (Maraie *et al.*, 2014). Betalain extracts from intact *B. vulgaris* cv. Detroit Dark Red plants contain 4-hydroxybenzoic acid (0.012 mg/g),

chlorogenic acid (0.018 mg/g), caffeic acid (0.037 mg/g), catechin hydrate (0.047 mg/g), epicatechin (0.032 mg/g), and rutin (0.0 mg/g), whereas extracts from hairy root cultures contain 4-hydroxybenzoic acid (0.396 mg/g), and chlorogenic acid (0.0 mg/g) (Georgiev *et al.*, 2010). In general, phenolic chemicals are least abundant in root portions. Due to the loss of compounds during the drying process, beet juice (3.67 GAE mg/g) and cooked beet (2.79 GAE mg/g) were found to have greater total phenolic content values than beet chips (0.75 GAE mg/g) and powder (0.51 GAE mg/g) (Vasconcellos *et al.*, 2016).

There are two reasons for the reduction in phenolic compounds after drying. First of all, using various drying temperatures may cause some phenolic chemicals to degrade. Second, as the material dries, the parts of the cells tend to stick together in the absence of water, making solvent extraction more difficult and lowering the overall recovery of phenolic chemicals (Ghanem *et al.*, 2012). A study conducted by Niroula *et al.* (2022) reported the total phenolic content of beetroot as 1966.46 ± 30.92 mg GAE/100g db.

2.11.2 Flavonoids

Flavonoids are physiologically active substances with strong antioxidant potential and a wide range of health advantages (Chhikara *et al.*, 2018). The primary flavonoid classes found in beetroot were dihydroisorhamnetinas, betagarin, betavulgarin, and cochliophilin A The betagarin (5,2-dimethoxy-6,7-methylenedioxyflavanone) and betavulgarin (2′-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone), flavanones were extracted from beetroot leaves. 3,5-dihydroxy-6,7-methylenedioxyflavanone, 5-hydroxy-6,7-methylenedioxyflavone, 2,5-dihydroxy-6, and 7-methylenedioxyisoflavone were other flavonoid compounds extracted from beetroots (Lim, 2016). Quercetin, rutin, and 4-hydroxy-5-methoxy-6,7-methylenedioxy flavanone arefound in the *B. vulgaris* ssp. perennis ethyl acetate fraction (Maraie *et al.*, 2014). The study conducted by Lin and Tang (2007) reported total flavonoid content of fresh beetroot as 392.5 mg QE/100g db.

2.11.3 Tannins

Tannins are a group of intricate polyphenolic macromolecules produced by a wide range of plants, and they are employed as pesticides and antipredators (Mathias *et al.*, 2016). Tannins are thought to have physiological effects on the body, including the ability to speed up blood clotting, lower blood pressure, lower serum cholesterol levels, cause liver necrosis, and modify immunological responses. The antioxidative characteristic of tannins, which is

crucial in preventing cellular damage including lipid peroxidation, may be related to their anticarcinogenic and antimutagenic potential. Tannins have physiological effects on blood coagulation, blood pressure, serum lipid levels, liver necrosis, blood pressure reduction, and immune response modulation as reported by Chung *et al.* (1998). Hydrolysable tannins and condensed tannins are the two categories of tannins. When hydrolyzed, hydrolysable tannins yield ellagic and gallic acids; depending on the acid produced, the hydrolysable tannins are referred to as egallitannins or gallo tannins. They become pyrogallic acid upon heating. The phenolic group in tannins is what gives them their antibacterial properties. Typical examples of hydrolysable tannins include theaflavins (found in tea), daidezein, genistein, and glycitein (Doughari, 2012).

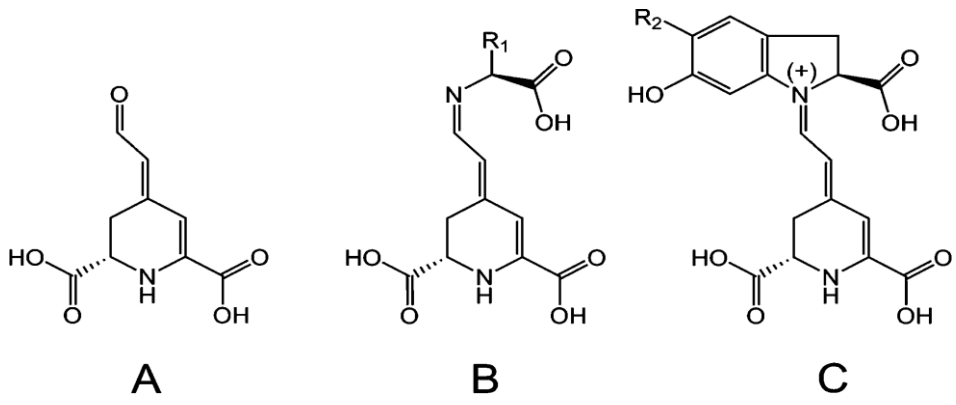
Condensed and hydrolyzable tannins have been reported to have comparable antibacterial and antifungal effects, though the hydrolyzable tannins appeared to be more effective against yeasts. The ellagitannins corilagin, phyllanthusiin, and pelargoniin B's anticryptococcal activity depended critically on the presence of a hexahydroxydiphenoyl group or its oxidatively changed forms. As a larger molecule would more effectively bind to proteins, it has also been suggested that tannin lethality might be correlated with molecule size (Gupta and Pandey, 2020). A study conducted by El-Beltagi *et al.* (2018) reported total tannin content of fresh beetroot as 513 mg TAE/100g.

2.11.4 Betalains

Betalains are nitrogenous plant pigments that are water soluble. Based on their chemical compositions and structures, two betalains—betacyanin (a red pigment) and betaxanthin (a yellow pigment)—have been identified. One of the most abundant sources of the coveted red or yellow betanin pigment is beetroot. The proportion of betacyanin and betaxanthin determines the types and level of redness of beetroot (Szopińska and Gawęda, 2013). The two kinds of betaxanthin, vulgaxanthin-I and vulgaxanthin-II, are further divided into (Ravichandran *et al.*, 2013). The peel of beets included a number of betacyanins, including betanin, prebetanin, isobetanin, and neobetanin (Nemzer *et al.*, 2011). The active components of beetroot are thought to make up between 75-95 % of the total betacyanin. Tyrosine is the starting point for the synthesis of betalain. Tyrosinase catalyzes the diphenol/DOPA oxidase activity that transforms the dihydroxyphenylalanine (DOPA) produced by the enormous accumulation of tyrosine (hydroxylated by tyrosine hydroxylase)

into cyclo-DOPA. The aromatic ring of DOPA is broken down to create betalaimic acid (Hatlestad *et al.*, 2012).

Both betaxanthin and betacyanin are produced through the condensation of betalamic acid and cyclo-DOPA, respectively. Non-glycosylated betanidin or isobetanidine chromophores are betacyanins. Tyrosinase and DOPA are two essential enzymes that are involved in the entire biosynthesis process. An early study examined the amount of betalain present in betalain extracts from whole *B. vulgaris* cv. Detroit Dark Red plants and hairy root cultures. The whole beetroot plant extracts yielded 39.760.98 mg/g of dry extract (DE) of betalains (20.75 mg/g of DE betacyanins and 19.01 mg/g of DE betaxanthins), whereas hairy root extract has 47.11 mg of betalains/g DE (16.33 mg/g DE betacyanins and 30.78 mg/g DE betaxanthin). In comparison to whole *B. vulgaris* cv. Detroit Dark Red plants, hair root extracts has more betalain as reported by Georgiev *et al.* (2010b). Betanin (312.5 mg/100 g), isobetanin (71.3 mg/100 g), vulgaxanthin-I (104.1 mg/100 g), vulgaxanthin-II (57.4 mg/100 g), betanidin (18.2 mg/100 g), and isobetanidin (4.6 mg/100 g) are the individual betalain contents that were discovered in the beetroot juice that was extracted. The amount of betalain in total is determined to be 606.34 mg/100 g of dry matter (Slavov *et al.*, 2013).



Source: Gandía-Herrero *et al.* (2012)

Fig. 2.1 General structures of betalmic acid (A), betaxanthins (B), and betacyanins (C).

According to K. Herbach *et al.* (2004), betalain in beetroots can experience a variety of degradation processes during heat treatment, including isomerization, decarboxylation, and cleavage by heat and acids. It is possible for betanin to degrade in a number of ways after thermal treatment.A study conducted by Liu *et al.* (2020) reported betalain content at 50°C as 441.51 mg/100g.

2.11.5 Antioxidant activity

Like many other colorful vegetables, beetroot is a veritable gold mine of antioxidants (Chhikara *et al.,* 2019). In comparison to citrus fruits, yellow passion fruit, apple, and cranberry, beetroot juice (5.45 pH, 9 °Brix) has a better antioxidant profile in terms of DPPH (325 mg ascorbic acid equivalent/L) due to its higher amounts of total phenolics (1169 mg GAE/L), flavonoids (925 mg catechin equivalent/L), and pigments (854 mg/L) (Fidelis *et al.,* 2017). It has been discovered that betanin and its aglycone betanidine have significant antioxidant action (Wootton-Beard and Ryan, 2011), and lipid peroxidation has been successfully avoided by this method (Kathiravan *et al.,* 2014). Beetroot juice (80.48%), beetroot chips (95.70%), beetroot powder (95.31%), and cooked beetroot (85.79%) all have a combined antioxidant activity. Beetroot powder and chips had similar values to cooked beets and beet juice, and there was no discernible difference between them (Vasconcellos *et al.,* 2016).Similarly a study was conducted by Georgiev *et al.* (2010) in which AA was reported as 90.7% inhibition.

Antioxidants work by scavenging "free-oxygen radicals," which creates a relatively "stable radical." The body's own natural antioxidant defenses, such as glutathione or catalases, can eliminate free radicals produced by the body. Therefore, this shortfall needs to be made up for by using exogenous natural antioxidants including vitamin C, flavones, beta-carotene, and natural plant products. Many different compounds that can scavenge free radicals are found in plants, including phenols, flavonoids, vitamins, and terpenoids, which have high antioxidant activity. Ascorbic acid, vitamin E, carotenoids, flavanols, and phenolics, which are abundant in plants, citrus fruits, and green vegetables, can scavenge free radicals in the human body. Phytochemicals have been found to have important antioxidant capabilities that are essential for lowering the incidence of various diseases (Omojate Godstime *et al.*, 2014).

The free radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), which is frequently employed to test a compound's capacity to act as a free radical scavenger or hydrogen donor and to evaluate antioxidant activity, can be used as a quick, easy, and affordable way to measure the antioxidant capacity of food. A stable free radical called DPPH is reduced as part of the DPPH test procedure. At 517 nm, the free DPPH radical with an odd electron produces absorption (purple color). DPPH is reduced to DPPH-H and, depending on how many electrons are caught, decolorizes to a yellow color when the antioxidants in plant extract

react with it. The relationship between the color absorbance and the sample extract's capacity to scavenge free radicals is inverse.

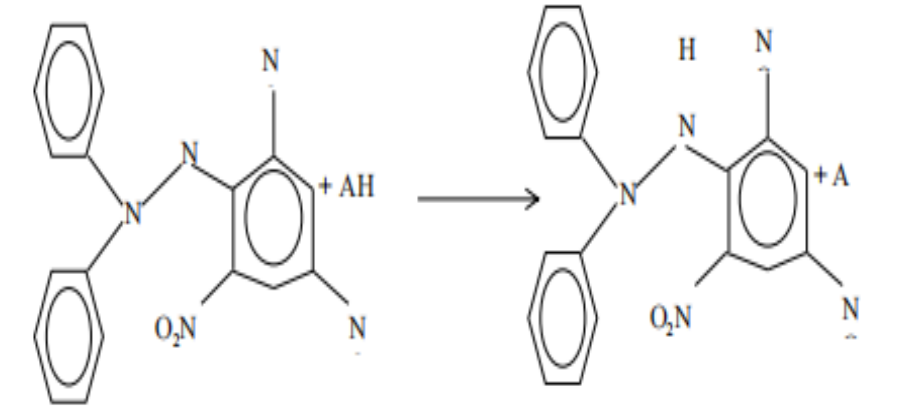
The radical scavengers' DPPH scavenging is best described as:

$$DPPH^{\bullet} + FE \longrightarrow DPPH - H + A^{\bullet}$$

$$DPPH + A^{\bullet} \longrightarrow DPPH - A$$

$$A^{\bullet} + A^{\bullet} \longrightarrow A - A$$

Where FE is an extract scavenger and A• is a radical. The freshly created radical (A•) can primarily be produced through radical-radical contact, collision of radicals, and the abstraction of an atom by one radical from another equations (Tailor and Goyal, 2014).



2, 2-diphenyl-1-picrylhydrazyl 2, 2-diphenyl-1-picrylhydrazine

Fig. 2.2 Reaction of antioxidant radical activity

In most cases, changes in TPC come after changes in the antioxidant capacities of the samples. An increase in drying temperature may affect the molecular makeup of phenols, which could lead to a decrease in TPC (Mella *et al.*, 2022).

2.12 Physicochemical properties

2.12.1 Bulk density

Bulk density is a way to describe how bulky a flour is, and it's a crucial factor in determining whether a flour is suitable for making baby formula or for foods that need to be packaged and transported easily. Low bulk density flours are suitable for these purposes (Nelson-Quartey *et al.*, 2007). Particle size influences bulk density, and the relationship between the two is inverse (Omimawo and Akubor, 2012). Different bulk densities of flours may result

from variations in particle size. Powders' bulk densities are dictated by their particle densities, which are in turn determined by the solid density and internal porosity of the particles in the container. Powders can be measured for their "loose bulk density" after being freely poured into a container, and they can also be measured for their "compact density" following mechanical pressure, vibration, and/or impact (Sapkota and Sherpa, 2018).A study carried out by Kerr and Varner (2020) discovered the bulk density of beetroot powder as 0.548 ± 0.0041 g/ml at temperature 53℃.

2.12.2 Solubility

One of the most significant physiochemical and functional characteristics of protein concentrates is solubility. Due to its high solubility, powder has the potential to be used in food systems that have been specially designed so that the product has a pleasing appearance and texture (Kanpairo *et al.*, 2012). A study conducted by Kaur and Singh (2014) reported solubility as 24% at 55℃.

2.12.3 Swelling capacity

The term "swelling capacity" refers to a molecule's ability to expand in response to water uptake up until a colloidal suspension is obtained or up until intermolecular interactions in the swollen particle preclude further expansion and uptake (Houssou *et al.*, 2002). The ability of flour granules to swell is a sign of how strong the associative forces are inside the granule (Moorthy and Ramanujam, 1986). The difference in swelling capacity reveals the degree of exposure of the internal structure of the starch found in flour to the action of water (Ruales *et al.*, 1993). Hydrogen bonds between water molecules and the exposed hydroxyl groups of amylose and amylopectin are broken when starch is cooked in an environment with too much water, disrupting the crystal structure. Granule swelling and solubility are increased as a result of this (Garau *et al.*, 2007).

The degree of contact between starch chains in the amorphous and crystalline domains, as well as association bonding inside the granules of sorghum starches, are demonstrated by swelling and solubility. The associative forces are smaller the bigger the swelling capacity (Jimoh *et al.*, 2009). The ratio of amylose to amylopectin as well as the properties of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation all have an impact on the intensity of this interaction (Hoover, 2001). Amylose-lipid complex formation can limit edema and solubilization (Swinkels, 1985). The

maximal increase in volume and weight that the starch experiences when allowed to expand freely in water is referred to as the swelling power (Moorthy, 2018). A study conducted by Sahni *et al.* (2017) reported swelling capacity of beetroot as 7.8 g/g at 65°C.

2.12.4 Oil absorption capacity

Oil absorption capacity is attributed mainly to the physical entrapment of oils. It is an indication of the rate at which protein binds to fat in food formulations (Omimawo and Akubor, 2012). Different flours have different bulk densities, which may be due to variations in particle size. Powders' particle densities, which are influenced by the solid density and internal porosity of the individual particles as well as the container's solid content, dictate the bulk densities of the materials. Powders have two different densities: "loose bulk density," or the density after a powder has been freely poured into a container, and "compact density," which is the density after a powder has been allowed to compress due to mechanical pressure, vibration, and/or impact (Micha, 1983). The mouth feel of food is improved by fat, which also functions as a flavor retention. The texture of the baked good is enhanced by fat, which boosts the baking powder's ability to leaven (Isah *et al.*, 2013). It is particularly desired for flour products to have low fat absorption. The quantity of flour needed to generate decent dough is determined by this functional feature (Abou-Arab *et al.*, 2017). Oil absorption capacity is influenced by the polysaccharide composition of the plant, its surface characteristics, overall charge density, thickness, hydrophobicity of the fiber particle, particle size, and drying (Carme *et al.*, 2007).

2.13 Health benefits of beetroot

Beetroot has a wide range of bioactive substances that naturally have antianemic, anti-inflammatory, anti-hypertensive, antioxidant, anti-carcinogenic, antipyretic, antibacterial, detoxicant, and diuretic activities (Hobbs *et al.*, 2013). Betalains have a wide range of medicinal, anticarcinogenic, hepatoprotective, and anticancer effects (Wootton-Beard *et al.*, 2011) by insulating the damaged tissue exhibited no apparent effects on normal cell lines (Nowacki *et al.*, 2015). It has been shown and reported that the ability of betalains pigments to inhibit the cell proliferation of different human tumor cells can be used in the chemotherapy prevention of lung and skin cancers. A number of beetroot flavonoids, including vitexin, vitexin-2-O-rhamnoside, and vitexin-2-O xyloside, have been shown to have excellent antiproliferative properties in cancer cell lines (Slavov *et al.*, 2013). They

have anticancer effects, modestly lower inflammatory responses, and alter immunological responses (Iglesias *et al.*, 2015).

The nitrates in beetroot can reduce blood pressure, prevent ischemia-reperfusion injury, and modulate mitochondrial activity (Satyanand *et al.*, 2014). It normalizes blood pressure, lowers oxidized LDL cholesterol and bad cholesterol (Guldiken *et al.*, 2016). Beetroot extracts have hypoglycemic and antihypertensive properties (Ninfali and Angelino, 2013). Betalains have the potential to lower homocysteine levels, which regulates vascular homeostasis and preserves platelet function, thrombotic activity, vascular tone, and delicate stability between the release of vasodilating and vasoconstricting agents (Machha and Schechter, 2011). Heart problems, including hypertension and atherosclerosis, are a risk factor for endothelial dysfunction (Krajka-Kuźniak *et al.*, 2012). Beetroot consumption lowers the likelihood of inflammation (an innate response characterized by infection, erythema, edema, trauma, fever, and pain that results from cell damage by antigens) in the body (Monteiro and Azevedo, 2010).

While current pharmaceutical treatments are linked to negative side effects, betalains extracts protect the thin lining of one's blood vessels and reduce inflammation (Miraj, 2016). Acording to El Gamal *et al.* (2014), the anti-inflammatory action of beetroot ethanolic extract on gentamicin-induced nephrotoxicity was clarified. When beetroot is boiled, the water is a great treatment for skin infections, pimples, and pustules. The entire digestive system benefits from beetroot's beneficial effects. The possible mechanism is supported by a considerable decrease in cleaved caspase 3, Bax, and enhanced Bcl-2 protein expression (El Gamal *et al.*, 2014). Red beet contains phytochemicals that support the immune system, kidney and liver health, as well as hematopoietic development (Miraj, 2016). The phytochemicals in beetroot help to sustain cognitive abilities like perception, learning, communication, and decision-making while also lowering age-related oxidative stress. Nitric oxide (NO) is produced by beetroot, which has the ability to increase cerebral blood flow (Presley *et al.*, 2011). The effects of dietary nitrate (NO_3^-) supplementation on cerebral hemodynamics have been documented (Haskell *et al.*, 2011), boost neurovascular coupling in response to visual stimuli and increase blood flow to executive function-related brain regions (Aamand *et al.*, 2013). Acording to Satyanand *et al.* (2014), beetroot ingestion increased plasma nitrate level by 96%. When ingested into the stomach's acidic environment,

some nitrite is transformed into nitric oxide, while the remainder is absorbed to increase the amount of circulating plasma nitrite (Wylie *et al.*, 2013).

Beetroot juice is good for the skin, and a mixture of a little vinegar and beet juice gets rid of dandruff and heals ulcers and running sores. Additionally, it contains significant amounts of boron, which is directly associated to the synthesis of human sex hormones. Beetroot juice consumption has a good effect on the body's biochemical responses to exercise and enhances cardiovascular health (Wylie *et al.*, 2013). Nitric oxide is quickly produced by the body, which gives these qualities (Gilchrist *et al.*, 2013). Due to its high iron content, red blood cell regeneration, and reactivation, beetroot juice is said to aid in blood purification and develop healthy blood. It also provides the body with new oxygen (Coles and Clifton, 2012). The remarkable physiological qualities of beetroot are due to the macronutrients and micronutrients that it contains. In conjunction with vitamin B, the folic acid in beets helps to prevent cancer and supports the neurological system's healthy operation (Székely *et al.*, 2016). Regular consumption of foods containing beetroot products helps maintain healthy digestion and offers protection from diseases linked to oxidative stress (Chandran *et al.*, 2014). Beetroot's copper concentration contributes to the body's increased ability to absorb iron. Constipation and fevers are treated with beetroot (Chhikara *et al.*, 2019).

Beetroot is favorable and useful in the treatment of a variety of illnesses, according to pharmacological study by numerous researchers. Additionally, beetroot extract from the leaves was tested in rats for its potential to reduce stress, anxiety, and depression. Along with its beneficial antioxidant properties, it has anxiolytic and antidepressant action in stressed rats. By modifying mood and relieving physical tension, uridine, a sugar beetroot extract, can be taken with omega-3 to relieve anxiety or prevent depression (Miraj, 2016; Sulakhiya *et al.*, 2016). The antiviral, antimicrobial effects (Slavov *et al.*, 2013) and antiradical activities (Slavov *et al.*, 2013) of betalain pigments have been reported. Beetroot saponins have a significant impact on a variety of human malignancies, including prostate, kidney, breast, colon, lung, leukemia, and melanoma (Podolak *et al.*, 2010).

2.14 Uses of beetroot

Beeteoots with a deep red hue are consumed by humans as a food source, both raw in salads and cooked in stews. Beetroot is eaten all around the world. Beetroot soup is a common dish in Eastern Europe, while pickled beets are a typical South American dish. Today, a

significant amount of beetroot is used commercially in the making of pickles. Beetroot is used in small amounts as juice (Chhikara *et al.*, 2019). One option for replacing synthetic colorants is beetroot (Slavov *et al.*, 2013), and can be used as a marketing strategy in the food sector. Additionally, consumers are gravitating toward green consumerism that uses less synthetic ingredients (Yadav *et al.*, 2014).

Natural colorants are thought to be safe for human ingestion. Natural colorants are therefore more anticipated for commercial use as food additives than synthetic colorants. Synthetic colorants are harmful to human health, cause allergies, and have a long-term carcinogenic effect when consumed (Panghal *et al.*, 2018). Natural colors may be easily incorporated into aqueous food systems since they are water soluble. Additionally, because they include strong antioxidants, natural food colorants are more appealing, have qualities that enhance visual acuity, and may have health effects. Beets are mostly produced as beet juices and ground dehydrated beets by food and beverage industries. Beet liquids can be sprayed dried into powder form, as can dehydrated beets that have been pounded into a powder (Kazimierczak *et al.*, 2014).

Fresh beetroot, beetroot powder, or extracted pigments are used to intensify the red color of tomato pastes, soups, sauces, desserts, jams, jellies, candies, ice cream, and breakfast cereals (Chhikara *et al.*, 2019). Beetroot juice is used to add color to a wide range of dishes, including dairy goods, yogurt, processed cheese, and candies. Only ice cream, sweets, and other confectionery products use it since it changes color when heated. It can be used as a substitute for synthetic antioxidants in the mayonnaise recipe, either in fresh or freeze-dried form (Raikos *et al.*, 2016). Beetroot juice as a dietary supplement improves tolerance for vigorous exercise and physical activity (Satyanand *et al.*, 2014). Sugar is produced from sugar beets, and their byproducts, such as pulp, molasses, and fiber, are utilized as animal feed. The plant's leaves, which are produced when sugar beetroot is cultivated in locations where cattle are raised, can be utilized as fodder. Fluming, flushing, and refinery are the final steps in the extremely complex process of producing sugar, which yields sugar, molasses, and bagasse as its final products. Alcohol production and other types of fermentation both utilize molasses (Wenninger, 2011).

2.15 Drying

A traditional method of food preservation and shelf-life extension is drying. By definition, drying or dehydration is a heat and mass transfer technique used to remove water from a

solid or liquid food in order to produce a solid product with a suitably low water content. where the removal of water is accomplished by a change in osmotic pressure rather than through evaporation. The primary goals of dehydration are: Preservation due to a decrease in water activity; reduced transport and storage costs due to a decrease in weight and volume; transformation of food into a form that is easier to handle, transport, store, and consume, such as turning liquids like milk or coffee extract into a dry powder that can be reconstituted into its original form by adding water (Kumar *et al.*, 2015). The rate of drying is influenced by internal factors such as beginning moisture content, crop type, crop absorptivity, and mass of product per unit exposed area as well as external factors such as sun radiations, ambient temperature, wind velocity, and relative humidity (Sahdev, 2014). By removing the moisture from the food, drying prevents bacteria, yeast, and mold from growing and tainting it. Enzymes, which are naturally occurring molecules that cause foods to ripen, are likewise slowed down by drying, but they are not rendered inactive (Baral, 2019).

2.15.1 Cabinet drying

A cabinet dryer consists of an insulated cabinet with shallow mesh or perforated trays, each of which holds a thin layer of food. The cabinet tray is filled with hot air. To provide equal air distribution either horizontally between the trays of food materials or vertically through the trays and food, air is directed over and/ or through each tray using a system of ducts and baffles. Direct gas burners, steam coil exchangers, and electrical resistance heaters are all examples of air heaters. Heater-heated air is used to dry by blowing the air past the devices. It has a flexible architecture, is relatively inexpensive to construct and maintain, and because of the lack of adequate control, generates products of varying quality. It is mostly used for small-scale production (1-20 ton/day) of dried fruits and vegetables, either alone or in groups (Fellows, 2022).

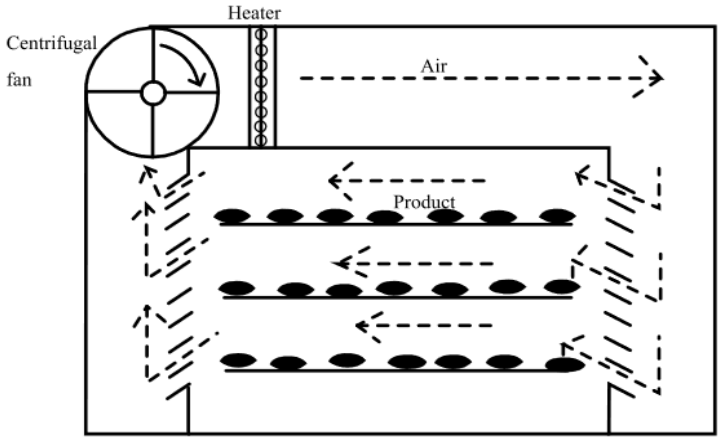


Fig. 2.3 Cabinet dryer

2.16 Effect of temperature on bioactive components

The antioxidant activity and bioaccessibility of phytochemicals are significantly influenced by the processing techniques. Processing methods for beetroot that increase antioxidant capacity and pigment stabilization include vacuum-microwave drying, fermentation, and irradiation, but hot air drying reduces color retention (Gokhale *et al.*, 2011). Typically, thermal processing is employed in the creation of various processed goods. The stability of betalains is influenced by temperature, and rising temperatures cause both betalains and PPO (polyphenol oxidase) enzymes to degrade. However, factors such as temperature range, heating intensity, oxygen content, and pigment concentration also have an impact on thermal degradation (K. M. Herbach *et al.*, 2006).

Part III

Materials and methods

3.1 Materials

3.1.1 Collection of beetroots

Beetroots (*Beta vulgaris*) at the mature stage were bought from the local market of Dharan, Sunsari.

3.1.2 Chemicals, equipments and glass wares

All the required chemicals, equipments and glass wares were provided in the laboratory of Central Campus of Technology, Dharan. The list of chemicals and equipments is placed in Appendix A.

3.2 Methods

3.2.1 Preparation of beetroot powder and extract

The beetroots free from any defects or damages were choosen and subsequently washed with tap water available.

After that, the beetroots were cut into 2 × 1 × 0.5 cm^3 pieces. These peel pieces were spread out on trays and dried at various temperatures, 50°C, 55°C, 60°C, 65°C and 70°C respectively, in a cabinet dryer. The side walls of the cabinet dryer included built-in heaters that produced heat, and a fan moved the heated air around the samples. Using a temperature control dial, the cabinet dryer's temperature was adjusted. Slices were introduced into the drying chamber on trays after being placed in the cabinet dryer after it reached the correct temperature. The drying samples were sampled, allowing for the hourly measurement of mass and moisture loss. The drying was proceeded till the constant weight of the samples was achieved and the point was determined using weighing balance technique. In order to ensure precision and reliability, the experiments were carried out three times.

The samples were dried, allowed to cool in a desicator, and then sealed in bags made of high-density polyethylene (HDPE). To guarantee the samples would be safe until their next use, the bags were heat-sealed. Each 70 g samples of dried beetroot were crushed in an

electric blender after drying at various temperatures. The resultant powder was then sieved for examination via a 40 mesh size. The processed beetroot powder was tightly sealed in plastic bags to avoid moisture absorption. For further analysis, these bags were kept at a temperature of 5±1°C.

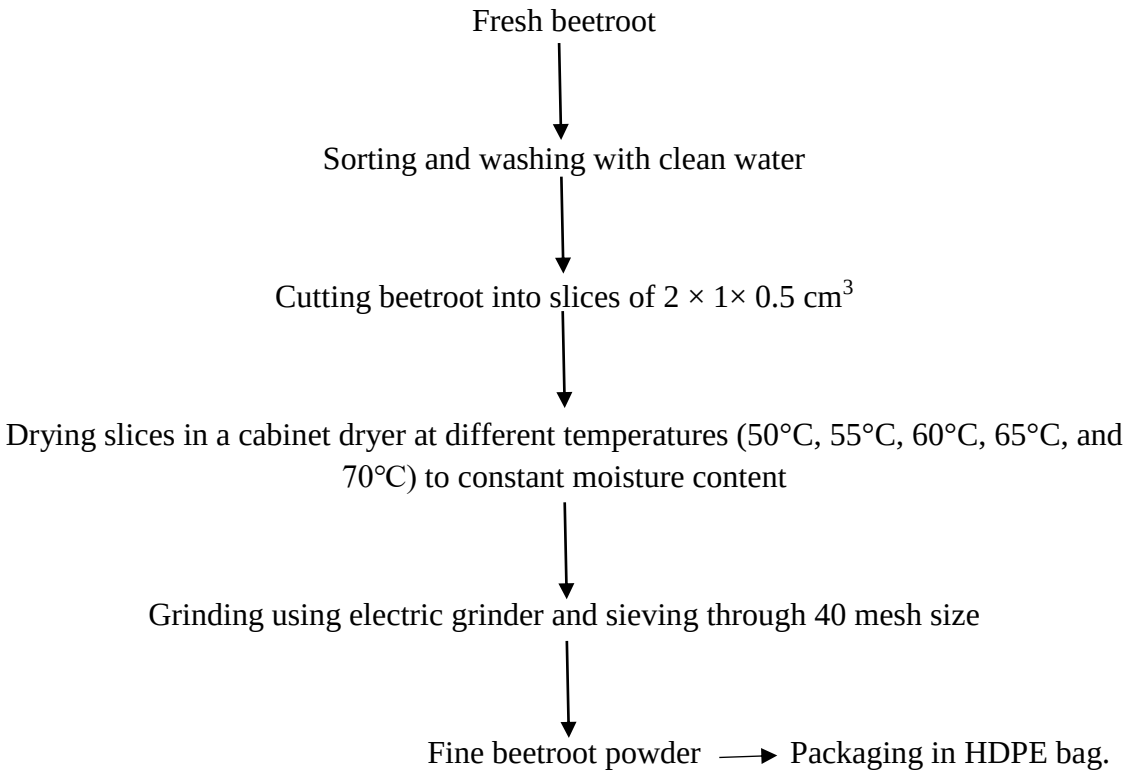


Fig. 3.1 Preparation of beetroot powder

Source: Jabeen *et al.* (2015)

3.2.1.1 Extract preparation

The extracts were made using raw beetroot and beetroot samples that had been dried at various temperatures (50°C, 55°C, 60°C, 65°C and 70°C). Methanolic extraction method was used to extract the phytochemicals with a few minor modifications as described by Samatha *et al.* (2012). 10gm of the material was placed in 100 ml of 80% methanol and left overnight in the dark for extraction. Whatman filter paper of 40 size was used to filter the extract, and the filtrate was then utilized to analyze the extract's bioactive components.

3.2.2 Analytical procedure

3.2.2.1 Bulk density

The method described by Kanpairo *et al.* (2012) was used to measure the bulk density. 25g of the sample was gently poured into a dry, 50 ml graduated cylinder. Then, the cylinder was tapped 25 times to adjust the compactness of the powder. The powder's volume was measured then, and the bulk density was determined using the formula below.

$$\text{Bulk density} = \frac{\text{Weight of the sample}}{\text{Volume of the sample}}$$

3.2.2.2 Solubility

According to the procedure outlined in Onuegbu *et al.* (2013), the solubility was determined. 1 g sample was mixed with 10 ml of distilled water. The mixture was allowed to settle for 15 minutes, and then 2 ml of the liquid (supernatant) over the settled particles was pipetted into a dry Petri dish. The liquid in the Petri dish was evaporated until it was completely dry, and the dish was then weighed once more to ascertain how much weight the dried particles had. Based on this procedure, solubility was computed as the total soluble solids (TSS).

$$\text{TSS\%} = \text{Vs} \times \frac{\text{Me - Md}}{2\text{Ms}}$$

Where, V_s = Total filtrate/supernatant.

M_d = weight of empty petridish

M_s = weight of sample used to prepare dispersion

M_e = weight of petridish plus residual solid after drying

3.2.2.3 Swelling capacity

Swelling capcity was measured according to method described by Jackson (1991). In a centrifuge tube, 1 g of flour sample was combined with 10 ml of distilled water. The mixture was continually shaken for 30 minutes while being heated to 80°C. The suspension was heated, and then centrifuged for 15 minutes at 1000 rpm. After carefully pouring out the liquid component (supernatant) above the settled particles, the weight of the residual paste was calculated. The swelling capacity was calculated by using formula below:

$$\text{Swelling capacity} = \frac{\text{Weight of the paste}}{\text{Weight of dry sample}}$$

3.2.2.4 Oil absorption capacity

The method described by Onuegbu *et al.* (2013), was used to determine the oil absorption capacity. One gram of each flour sample was weighed out and put into separate, clean centrifuge tubes with specified weights. Each tube received 10 ml of sunflower oil in total. The tubes were then centrifuged for 15 minutes at 3500 rpm. After centrifugation, the liquid portion (supernatant) was removed, and the tubes were weighed again. The capacity of the flour to absorb oil is represented by the increase in mass that was noted.

3.2.2.5 Total phenolic content

The total phenolic content was determined by using Folin-Coicalteu method according to AOAC (2005) with slight modifications. An extracted dry sample (10g) was centrifuged at 3500 rpm at room temperature after being extracted with 100 ml of 80% methanol. The residue was centrifuged after being extracted three more times with 80% methanol. Following the addition of 2 ml of methanolic extract, 1 ml of distilled water, and 0.5 ml of the Folin-Ciocalteu reagent, 2 ml of 20% sodium carbonate was added. Gallic acid solution (100-500 mg/L) was used as the standard, and the results will be expressed as mg of gallic acid equivalents (GAE) per 100 g dry basis of samples. After allowing the reaction to occur in the dark for an hour, an absorbance was measured at 765 nm using an UV-visible spectrophotometer.

3.2.2.5 Flavonoid content

Slightly modified aluminum chloride assay technique was used to determine the total flavonoid concentration as described by Barek *et al.* (2015). A volumetric flask with a 10 ml capacity was filled with 2 ml of each extract solution. The flask was filled with 0.2 ml of 5% $NaNO_3$ solution and let to stand for 5 minutes. After that, 0.2 ml of 5% $AlCl_3$ solution was added, and the mixture stood for an additional 5 minutes. Following the addition of 2 ml of 1N NaOH, distilled water (DW) was used to bring the volume to 5 ml in the flask. After 15 minutes, a reagent blank was used to measure the solution's absorbance at 510 nm.

Quercetin standard curves with concentrations of 100, 200, 300, 400, and 500 mg/L were used to compare the test results to. Following that, the amount of flavonoids in total was determined and expressed as milligrams of quercetin equivalents (QE).

3.2.2.6 Tannin content

According to the procedure described by Mythili *et al.* (2014), the Folin-Ciocalteu method was used to determine the tannin concentration. In a 10 ml volumetric flask, 0.1 ml of the sample extract was combined with 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent. The mixture was then diluted with distilled water to 10 ml before 1 ml of a 35% Na_2CO_3 solution was added. After vigorously shaking, the mixture was let to stand for 30 minutes at room temperature. While this was going on, a series of reference standard solutions of Tannic acid (100, 200, 300, 400, and 500 mg/L) were made using the same technique. Using a UV-visible spectrophotometer, the absorbance of the test solution and the standard solution were both measured at 725 nm, with the blank serving as the reference. The tannin content was quantified in milligrams of Tannic acid equivalents (TAE) per gram of extract.

3.2.2.7 Antioxidant activity

With some tiny variations, the DPPH free radical scavenging abilities of extracts were assessed using the techniques described by Upadhyay *et al.* (2012). A 10g sample was extracted over night in 100ml of an 80% methanol solution. The reaction was then fully completed by adding 2 ml of 0.1 mM DPPH solution to 2 ml of extract, which was then kept in the dark for 30 minutes. An UV-visible spectrophotometer was used to evaluate the anti-radial activity at 517 nm. The control for the experiment was produced by mixing 2 ml of DPPH and 2 ml of methanol. The following formula will be used to compute the percentage of DPPH radical scavenging activity:

$$\text{DPPH scavenging activity}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where, $A_{control}$ and A_{sample} are absorbance of control and sample solution.

3.2.2.8 Betalain content

Betalains were measured, with a few minor modifications in the methods, as stated by Ben Haj Koubaier *et al.* (2014). Using UV-visible spectrophotometer, the betacyanin and betaxanthin contents of the methanol extract were measured at 538 nm and 480 nm, respectively. The betalain content(BLC) was calculated usin following formula reported by Singleton *et al.* (1965):

$$\text{BLC } (\frac{\text{mg}}{\text{L}}) = \frac{\text{A}\times\text{DF}\times\text{MW}\times 1000}{\text{e} \times \text{l}}$$

Where A is the absorption value, DF is the dilution factor, and l is the cuvette's path length in centimeters (1cm). The molecular weights and molar extinction coefficients(e) for the measurement of betacyanin are 550 g mol^{-1} and 60000 L mol^{-1} cm^{-1} in water and for betaxanthine are 308 g mol^{-1} and 48000 L mol^{-1} in water respectively.

3.2.2.9 Moisture content

The method outlined in Ranganna (1986) was used to determine the moisture content using a hot air oven.

3.2.2.10 Crude fiber

The crude fiber content of the sample was determined by using methods outlined in Ranganna (1986).

3.2.2.11 Ash content

The total ash content of the sample was determined by using muffle furnance ashing method outlined in Ranganna (1986).

3.2.2.12 Crude fat

The crude fat content of the sample was determined by using solvent extraction (soxhlet) method outlined in Ranganna (1986).

3.2.2.13 Crude protein

The crude protein content of the sample was determined by using Kjeldahl method outlined in Ranganna (1986).

3.2.2.14 Reducing sugar

The reducing sugar content of the sample was determined by using Lane and Enyon method outlined in Ranganna (1986).

3.2.2.15 Iron content

Iron content in the sample was determined by colorimetric method using UV-visible spectrophotometer at 480 nm as per Ranganna (1986). A volume (100 ml) was created by mixing 25 ml of 10% HCl with 1 g of ash. Blank, standard, and sample solutions were created according to the instructions in for the spectrophotometric measurement of iron content.

Table 3.1 Preparation of blank, standard ash solutions

Constituents	Blank	Standard	Sample
Standard iron sol^{n}	0.0	1.0	0.0
Sample ash sol^{n}	0.0	0.0	5.0
Water	5.0	4.0	0.0
Conc. H_2SO_4	0.5	0.5	0.5
Potassium Persulphate(saturated)	1.0	1.0	1.0

3.2.2.16 Yield of beetroot after drying

The percentage yield of beetroot after drying was computed by using weight difference of beetroot slices before drying and after drying usin following formula:

$$\%\ \text{yield of beetroot drying} = \frac{\text{Weight of dried beetroot slices}}{\text{Weight of beetroot slices before drying}} \times 100\%$$

3.2.3 Statistical analysis

IBM SPSS statistics 20 software was used to perform one-way analysis of variance (ANOVA) on the triplicate data from each experimental analysis. To identify significant differences, mean values were compared using Tukey's HSD post hoc test ($P<0.05$).

Part IV

Results and discussion

The purpose of this study was to find out how bioactive components and physicochemical properties of beetroot were affected by drying temperature. The beetroot was prepared by cutting it into slices and drying it in a cabinet dryer at 50°C, 55°C, 60°C, 65°C, and 70°C until it had a constant weight. The dried beetroot was then ground into powder and sieved through a 40 mesh size. The beetroot powder was collected and packaged in HDPE bags and subjected to various physicochemical analyses, including evaluation of bulk density, solubility, oil absorption capacity, and swelling capacity. Similary, the bioactive components were also evaluated, including total phenolic content, total tannin content, total flavonoid content antioxidant activity, and betalain content.

4.1 Analysis of beetroot

4.1.1 Yield of beetroot after drying

The yield of beetroot after completion of drying process was found to be 12.52%.

4.1.2 Proximate analysis of fresh beetroot

Quantitative analysis of the main food ingredients constitutes proximate analysis. In this research proximate analysis of fresh beetroot was done and estimated values are shown in Table 4.1.

Table 4.1 Proximate and mineral composition of fresh beetroot

Parameters	Value
Moisture content (%)	89.03 ± 0.0076
Crude protein (% db)	11 ± 0.0092
Crude fat (% db)	1.3 ± 0.0012
Crude fiber (% db)	9.6 ± 0.0017
Total ash (% db)	12.42 ± 1.2323
Reducing sugar (% db)	36.28 ± 0.0014
Other carbohydrates (% db)	29.4 ± 0.1652
Iron content (mg/100g db)	16.39 ± 0.0047
Moisture content of beetroot powder (% db)	5 ± 0.1528

Note: the total iron content is basically the ultimate analysis parameter but due to its very lesser amount it is shown in the table above.

The moisture content of beetroot was found to be 89.03%, which is consistent with a study conducted by Niroula *et al.* (2022) where the moisture content of the fresh beetroot was reported to be 89.49%. Similar results were found for protein, crude fiber, crude fat, total ash, and iron content which were 10.99%, 9.8%, 1.32%, 11.08%, and 16.66 (mg/100 g) respectively in db as conducted by Niroula *et al.* (2022) .

4.1.3 Effect of drying temperature on physicochemical properties of beetroot

The physicochemical characteristics, including bulk density, solubility, oil absorption capacity, and swelling capacity of beetroot powder were assessed. These features are built-in to the material and can be seen or measured without changing the substance's identity. All the parameters were examined in dry basis.

4.1.3.1 Bulk density

According to Table 4.2, the bulk density at 70℃ was the highest i.e 0.78 ± 0.01 g/ml , while the bulk density of fresh beetroot was 0.46 ± 0.01 g/ml. Kerr and Varner (2020) carried out a similar study and discovered that beetroot powder at temperature of 53℃ had a bulk density of 0.548 ± 0.0041 g/ml. According to the findings, bulk density rises in direct

proportion to temperature. The increase in bulk density on increasing temperature was because of smaller particle size powder formation resulted by cell destruction phenomena due to thermal shock impacted during drying period as reported by Kerr and Varner (2020).

Table 4.2 Bulk density (g/ml)of fresh and different temperature beetroot powder

Samples	Bulk density (g/ml)
Fresh	0.46 ± 0.01^{a}
50℃	0.48 ± 0.01^{a}
55℃	0.55 ± 0.01^{b}
60℃	0.63 ± 0.0058^{c}
65℃	0.67 ± 0.0058^{d}
70℃	0.78 ± 0.01^{e}

The data displayed here are the mean value ± standard deviation of three independent measurements. The meaning of different superscripts is that they are significantly different.

Fresh and 50℃ samples do not significantly differ from one another. Other samples, however, considerably differed from fresh sample for bulk density. Same superscript in the same column of the table indicates no significance difference. The increment in bulk density implies the increament in space utilization of the powder inside the vessel.

4.1.3.2 Solubility

The highest solubility, interpreted as TSS (%), was found in the sample of at 70℃, which had a value of 49.67 ± 0.577 %, which was significantly higher than that of the other samples. A similar study was conducted by Kaur and Singh (2014) where solubility was found to be 24% at 55℃ which is similar with the result of this study at 55℃ i.e. 34.33 ± 1.1547 in percentage. Solubility, temperature and solubility indices are directly related, according to the overall trend seen in Table 4.3. Solubility coefficients increased as treatment temperatures increased for beetroot. Over the course of the drying process, the majority of the free water evaporated and the solids stuck to the food's pores, increasing the amount of soluble solids in the food overall as reported by Kaur and Singh (2014). Greater the temperature of drying greater will be the evaporation rate but cells of food material will be damaged more.

Table 4.3 Solubility(%) of fresh and different temperature beetroot

Sample	Solubility(%)
Fresh	10.5 ± 0.5^{a}
50℃	30 ± 1^{b}
55℃	34.33 ± 1.1547^{c}
60℃	35 ± 1^{c}
65℃	39.67 ± 0.577^{d}
70℃	49.67 ± 0.577^{e}

The data displayed here are the mean value ± standard deviation of three independent measurements. The meaning of different superscripts is that they are significantly different.

Samples at 55℃ and 60℃ are not significantly different in terms of solubility. But fresh sample is significantly different from rest of the samples for solubility.

4.1.3.3 Oil absorption capacity

The maximum oil absorption capacity was found in the sample dried at 70°C, which measured as 2.69 ± 0.0058 ml/g. Similar study was conducted by Sahni *et al.* (2017) where OAC (oil absorption capacity) was found to be 2.206 ± 0.064 ml/g at 65℃. Oil absorption capacity (OAC) is influenced by the polysaccharide composition of the plant, its surface characteristics, overall charge density, thickness, hydrophobicity of the fiber particle, particle size, and drying (Carme *et al.*, 2007).

Table 4.4 Oil absorption capacity (ml/g) of fresh and different temperature beetroot

Sample	Oil absorption capacity(ml/g)
Fresh	0.87 ± 0.1082^{a}
50℃	1.45 ± 0.0058^{b}
55℃	1.48 ± 0.0058^{c}
60℃	1.51 ± 0.0058^{c}
65℃	1.53 ± 0.0058^{d}
70℃	1.69 ± 0.1589^{e}

The data displayed here are the mean value ± standard deviation of three independent measurements. The meaning of different superscripts is that they are significantly different.

Samples dried at 55°C and 60°C do not significantly differ from one another in terms of their oil absorption capacity. Where as all of the samples are significantly different from fresh sample.

4.1.3.4 Swelling capacity

The sample dried at 70°C displayed a higher swelling capacity of 9.53 ± 0.0416. The information in Table 4.5 shows a positive association between temperature and swelling capacity, with higher temperatures leading to more swelling. Similar study conducted by Sahni *et al.* (2017) had similar results which was of 7.8 g/g at 65°C. The chemical and structural characteristics of fiber, particularly its affinity for water, have a substantial impact on how quickly water is absorbed.

Table 4.5 Swelling capacity (g/g) of fresh and different temperature beetroot

Sample	Swelling capacity (g/g)
Fresh	2.43 ± 0.0577^{a}
50℃	8.51 ± 0.2517^{b}
55℃	8.88 ± 0.1097^{c}
60℃	9.07 ± 0.0503^{cd}
65℃	9.24 ± 0.0603^{de}
70℃	9.53 ± 0.0416^{e}

The data displayed here are the mean value ± standard deviation of three independent measurements. The meaning of different superscripts is that they are significantly different.

Samples of 55°C and 60°C, of 60°C and 65°C and, of 65°C and 70°C are not significantly different in terms of their swelling capacity respectively. But all of the samples are significantly different from fresh sample.

4.1.4 Optimum drying temperature for physicochemical properties

From one way ANOVA analysis within level of confidence ($P<0.05$) of above data of different physicochemical properties at different drying temperatures, the optimum drying temperature for beetroot was found to be 70℃. Above tables show the values of parameters such as bulk density, solubility, oil absorption capacity and swelling capacity of at 70℃ are the highest as compared to values of at other temperatures. There is no significance differences in overall parameters' mean value for all the temperatures.

4.1.5 Effect of drying temperature on bioactive components of beetroot

In this study, the total phenolic content (TPC), total tannin content (TTC), total flavonoid content (TFC), antioxidant activity, and betalain content (BLC) were all examined as bioactive components. All the parameters mentioned here were measured in dry basis (db).

4.1.5.1 Total phenolic content (TPC)

There are two reasons for the reduction in phenolic compounds after drying. First of all, using various drying temperatures may cause some phenolic chemicals to degrade. Second,

as the material dries, the parts of the cells tend to stick together in the absence of water, making solvent extraction more difficult and lowering the overall recovery of phenolic chemicals (Ghanem *et al.*, 2012). Fresh beetroot was found to have a greater total phenolic content 1912.39 ± 0.5472 mg GAE/100g than the other samples shown in Figure 4.1. The figure demonstrates that the amount of polyphenols reduces as temperature rises from 50°C to 70°C. There was larger difference in terms of TPC between samples taken at from 50°C and 70°C as compare to difference between of fresh and other samples. Similar study was conducted by Niroula *et al.* (2022) which reports similar result of the total phenolic content of beetroot to be 1966.46 ± 30.92 mg GAE/100g db. Another study conducted by Liu *et al.* (2020) had found similar data of TPC at 70°C i.e. 817 ± 0.2 mg GAE/100g db while the finding of this study was of 705 ± 1.004 mg GAE/100g db. The difference in results may be due to difference in variety and cultivation of the beetroot.

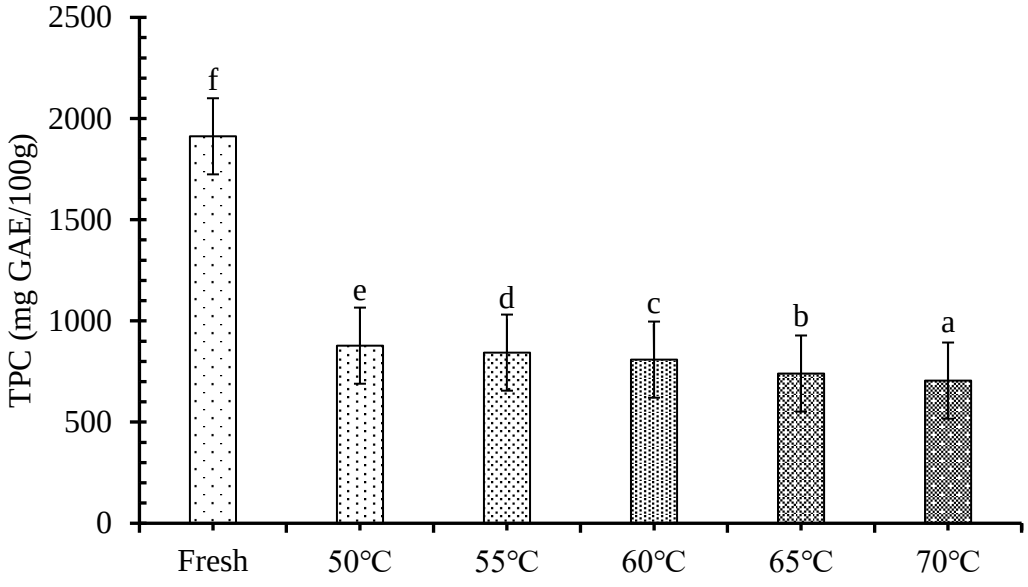


Fig. 4.1 Total phenolic content (TPC) of beetroot

There is significance difference among all the samples shown in Fig. 4.1.

4.1.5.2 Total tannin content (TTC)

Tannins are a group of intricate polyphenolic macromolecules produced by a wide range of plants, and they are employed as pesticides and antipredators (Mathias *et al.*, 2016). Tannin content of fresh sample was found to be higher than other samples i.e. 358.49 ± 0.4644 mg TAE/100g as shown in Figure 4.2. Similar study was conducted by El-Beltagi *et al.* (2018)

in which tannin content of fresh beetroot was of 513 mg TAE/100g. Figure 4.2 shows that there are significance differences among tannin content of all the samples. The order of the data is in decreasing manner from 50-70℃ in the range from 292.29-92.43 mg TAE/100g. This difference in tannin content may be resulted from being different varieties. The increase in thermal degradation and tannin condensation that occurs at higher temperatures, may be the cause of the decrease in tannin concentration.

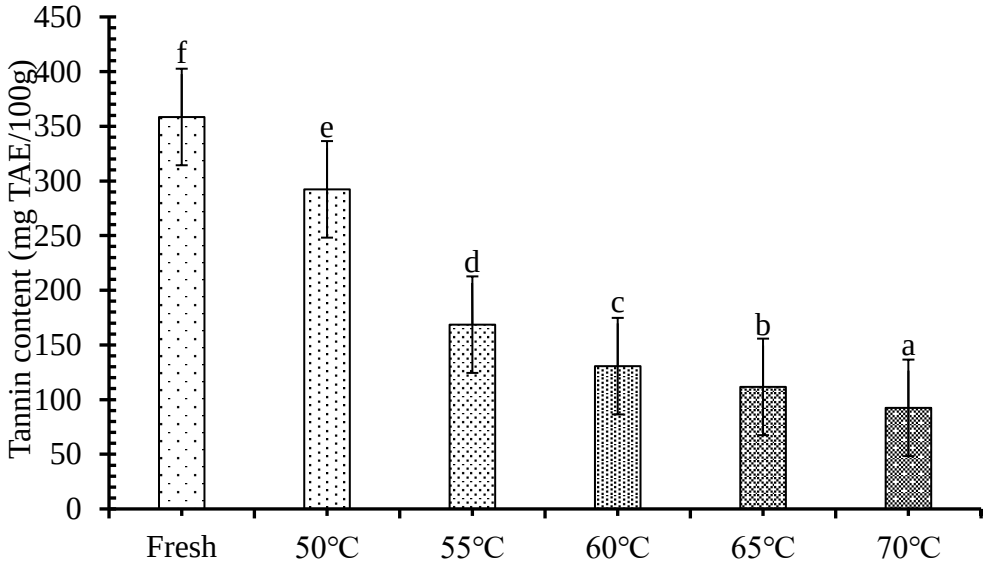


Fig. 4.2 Total tannin content (TTC) of beetroot

There is significance difference among all the samples shown in Fig. 4.2.

4.1.5.3 Total flavonoid content (TFC)

Figure 4.3 displays the total amount of flavonoids in both fresh and dried beetroot. In comparison to the other samples, the fresh beetroot sample was much higher. TFC of fresh beetroot was found to be 338.01 mg QE/100g which is similar with the result of the study conducted by Lin and Tang (2007). The decrease in TFC with increase in drying temperature may be due to increasing degradation of flavonoid at higher temperature. The decrease in flavonoid content of beetroot during drying might be minimized prefering lower drying temperature.

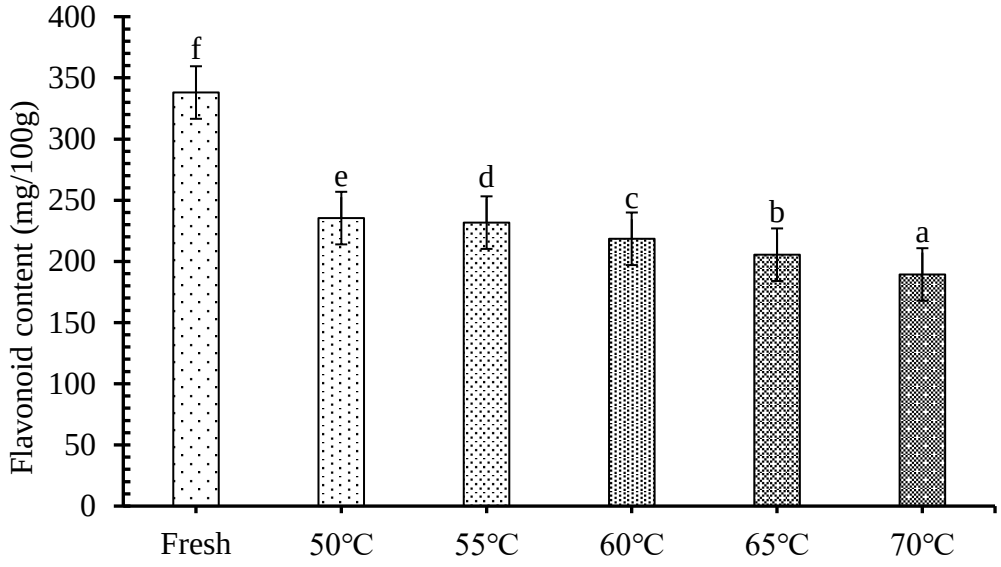


Fig. 4.3 Total flavonoid content (TFC) of beetroot

Figure 4.3 shows that there is significance difference among samples fresh, 50℃, 55℃, 60℃, 65℃, and 70℃.

4.1.5.4 Antioxidant activity (AA)

The antioxidant activity was measured in terms of DPPH radical scavanging activity %. Figure 4.4 shows that there is significance difference ($P<0.005$) among samples fresh, 50℃, 55℃, 60℃, 65℃, and 70℃. AA of fresh beetroot was found to be 94.94 ± 0.05% inhibition which similar with the findings of a study conducted by Georgiev *et al.* (2010) in which AA was of 90.7% inhibition. The value of AA was decreasing along the increasing drying temperature. In most cases, changes in TPC come after changes in the antioxidant capacities of the samples. An increase in drying temperature may affect the molecular makeup of phenols, which could lead to a decrease in TPC (Mella *et al.*, 2022).

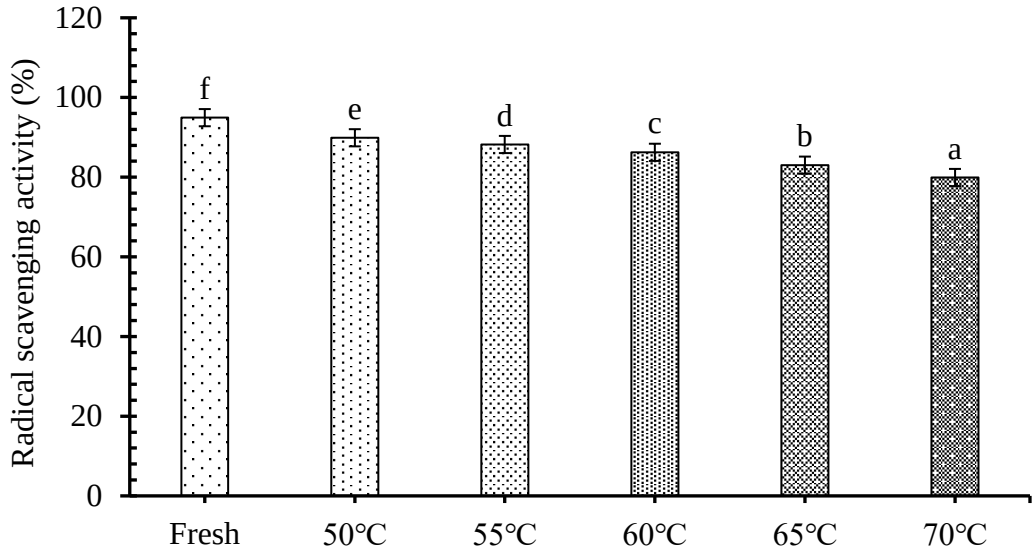


Fig. 4.4 Antioxidant activity (AA) of beetroot

4.1.5.5 Betalain content (BLC)

The BLC of fresh beetroot was found to be 668.46 ± 0.4952 mg/100g as shown in Figure 4.5 which is similar with the outcome of a study conducted by Niroula *et al.* (2022) in which BLC was reported as 676.03 mg/100g. Figure 4.5 shows the significantly decreasing pattern of BLC with the increase in drying temperature. There is significance difference among samples fresh, 50℃, 55℃, 60℃, 65℃, and 70℃. BLC at temperature 50℃ was of 477.1 ± 0.5272 mg/100g which is similar with the result of an another study conducted by Liu *et al.* (2020) reported as 441.51 mg/100g. According to K. Herbach *et al.* (2004), betalain in beetroots can experience a variety of degradation processes during heat treatment, including isomerization, decarboxylation, and cleavage by heat and acids. It is possible for betanin to degrade in a number of ways after thermal treatment.

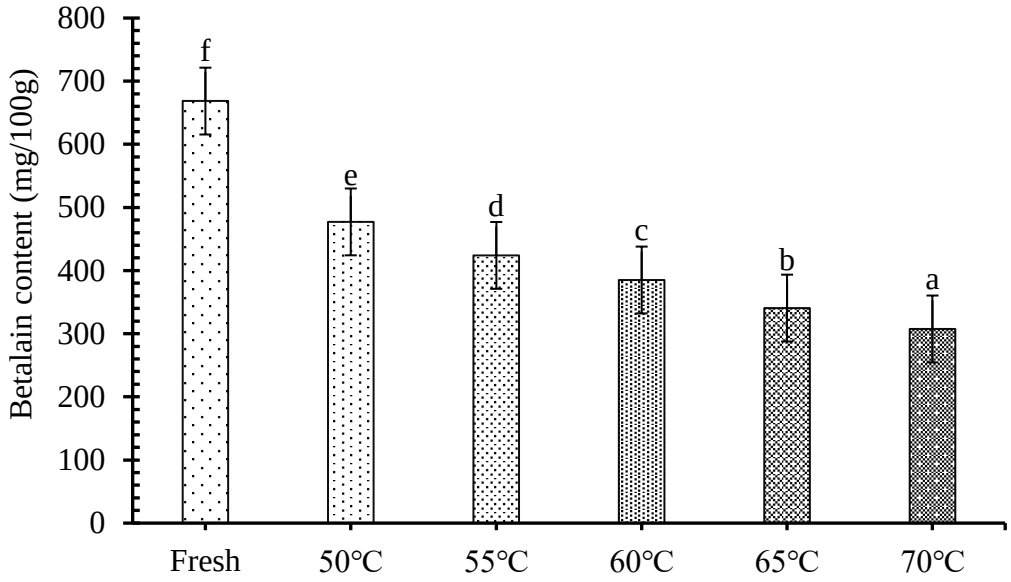


Fig. 4.5 Betalain content (BLC) of beetroot

4.1.6 Optimum drying temperature for bioactive components

From one way ANOVA analysis within level of confidence ($P<0.05$) of above data of different physicochemical properties at different drying temperatures, the optimum drying temperature for beetroot was found to be 50℃. Figure 4.1, Figure 4.2, Figure 4.3, Figure 4.4 and Figure 4.5 show the values of parameters at 50℃ are highest than of at other temperatures. There is no significance differences in overall parameters' mean value for all the temperatures.

Part V

Conclusions and recomendations

5.1 Conclusions

The key conclusions from this study's findings can be summed up as follows:

1. The physicochemical properties including bulk density, oil absorption capacity (OAC), solubility and swelling capacity of beetroot showed increament by 70%, 94%, 373% and 292%, respectively, within the range of fresh to 70°C.
2. The bioactive components of beetroot decreased as the temperature increased. When compared to a fresh sample with sample dried to 70°C, the TPC, TFC, TTC, AA, and BLC decreased by 63%, 44%, 74%, 16% and 54% respectively.
3. The optimum drying temperature for beetroot considering physicochemical properties and bioactive components were found to be 70°C and 50°C respectively.

5.2 Recommendations

The following recommendations can be used to further the research:

1. Study at further temperature range can be done to optimize drying temperature.
2. Study including pre-treatment such as blanching, can be done to know the effects on physicochemical properties and bioactive components of beeetroot.
3. Similar study on other varities of beetroot can be done.
4. Study below 50°C temperature range can be done to optimize the higher retention of functional components.
5. Further study can be done by varying drying methods such as sun drying.

Part VI

Summary

Although beetroots are nowadays grown economically in many places of Nepal, they aren't being used to their full potential in the market. Beetroot is a nutrient-rich underrated crop that has a unique combination of vitamins, minerals, and antioxidants.

The bioactive components of five different beetroot powder samples, including fresh beetroot and beetrrot slices that had been dried at temperatures of 50°C, 55°C, 60°C, 65°C, and 70°C, were examined during the analysis. The mass balance method was used to continue drying the samples until they had a constant moisture content. The bioactive components were evaluated in dry basis, for the fresh and dried beetroot powders, including antioxidant activity (IC_{50} mg/100g), polyphenol content (mg GAE/100g), flavonoids content (mg QE/100g), tannin content (mg TAE/100g), and betalain content (mg/100g). Also examined were the physical characteristics of the identical powder samples, including bulk density (g/ml), solubility (%), oil absorption capacity (ml/g), and swelling capacity (g/g).

According to the results, employing the oven drying method at 50°C led to a less significant loss of bioactive components than that did using other drying temperatures. The physicochemical characteristics of the identical samples were also evaluated in this study. As compared to fresh and 70°C, the results showed that raising the drying temperature increased physicochemical attributes such as bulk density by 70%, oil absorption capacity by 94%, solubility by 373% and swelling capacity by 292%.

Similarly, it was found that a rise in temperature was accompanied with a fall in the concentrations of the bioactive components. For example, when comparing a fresh sample to a sample that had been dried at 70°C, the TPC, TFC, and antioxidant scavenging activity all decreased by 63%, 44%, and 16%, respectively, while the concentrations of tannin and betalain contents were decreased by 74% and 54%, respectively. The study of all the samples revealed that the low-temperature drying process is the best technique for obtaining larger retention of bioactive components.

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Appendices

Appendix A

Equipments and utensils

i. Grinder
ii. Water bath (Intake Serological Water Bath)
iii. Weighing balance
iv. Heating mantle
v. Muffle furnace
vi. Standard sieve (40 mesh size)
vii. Cabinet dryer
viii. Refrigerator
ix. Hot air oven
x. Spectrophotometer

Chemicals used

i. NaOH (HIMEDIA- GRM1183, Assay 97.00-103.50 %)
ii. Distilled water
iii. Indicators (Methyl blue, Phenolphthalein)
iv. Absolute Alcohol (Bengal Chemicals and pharmaceuticals)
v. Na_2CO_3 (Qualigens, Assay 99-101%)
vi. HCl
vii. Folin-Ciocalteuphenol reagent (FC reagent)
viii. $AlCl_3$
ix. Con. H_2SO_4
x. 2, 2-Diphenyl-1- picrylhydrazyl (DPPH)
xi. Potassium persulphate
xii. Standard iron solution
xiii. Boric acid, etc.

Appendix B

ANOVA results for Analysis of different parameters of beetroot

Table B.1 One way ANOVA (no blocking) for bulk density

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	0.221	5	0.044	569.557	0.000
Residual	0.001	12	0.000		
Total	0.222	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.2 Homogeneous subsets for bulk density

Bulk density						
Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fresh	3	0.4600				
50	3	0.4800				
55	3		0.5500			
60	3			0.6267		
65	3				0.6667	
70	3					0.7800
Sig.		0.129	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Table B.3 One way ANOVA (no blocking) for solubility

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	2529.069	5	505.814	714.090	0.000
Residual	8.500	12	0.708		
Total	2537.569	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.4 Homogeneous subsets for solubility

Solubility						
Tukey HSD						
Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fresh	3	10.5000				
50	3		30.0000			
55	3			34.3333		
60	3			35.0000		
65	3				39.6667	
70	3					49.6667
Sig.		1.000	1.000	0.919	1.000	1.000
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Table B.5 One way ANOVA (no blocking) for oil absorption capacity

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	2529.069	5	505.814	714.090	0.000
Residual	8.500	12	0.708		
Total	2537.569	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.6 Homogeneous subsets for oil absorption capacity

Oil absorption capacity						
Tukey HSD						
Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fresh	3	10.5000				
50	3		30.0000			
55	3			34.3333		
60	3			35.0000		
65	3				39.6667	
70	3					49.6667
Sig.		1.000	1.000	0.919	1.000	1.000
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Table B.7 One way ANOVA (no blocking) for swelling capacity

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	111.053	5	22.211	1538.838	0.000
Residual	0.173	12	0.014		
Total	111.226	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.8 Homogeneous subsets for swelling capacity

Swelling capacity						
Tukey HSD						
Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
Fresh	3	2.4333				
50	3		8.5067			
55	3			8.8833		
60	3			9.0733	9.0733	
65	3				9.2367	9.2367
70	3					9.5267
Sig.		1.000	1.000	0.427	0.576	0.097
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Table B.9 Mean comparison of overall physicochemical properties

Descriptives								
Value								
	N	Mean	S.D	S. Error	95%Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
50	4	10.11	13.7344	6.8672	-11.7446	31.9646	0.48	30.00
55	4	11.31	15.7927	7.8964	-13.8198	36.4398	0.55	34.33
60	4	11.55	16.0842	8.0421	-14.0410	37.1460	0.63	35.00
65	4	12.78	18.3377	9.1689	-16.4019	41.9569	0.67	39.67
70	4	15.42	23.1704	11.5852	-21.4517	52.2867	0.78	49.67
Total	20	12.23	15.8524	3.5447	4.8143	19.6527	0.48	49.67

Table B.10 Homogeneous subsets for overall physicochemical properties

Value		
Tukey HSD		
Temperature	N	Subset for alpha = 0.05
		1
50	4	10.1100
55	4	11.3100
60	4	11.5525
65	4	12.7775
70	4	15.4175
Sig.		0.993
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 4.000.		

Table B.11 Mean comparison of overall bioactive components

Descriptives								
Value								
	N	Mean	Std. D	Std. Error	95%Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
50	5	466.09	251.6321	112.5333	153.6515	778.5365	235.39	877.67
55	5	421.67	263.7524	117.9537	94.1781	749.1619	168.55	843.24
60	5	394.83	261.5692	116.9773	70.0449	719.6071	130.60	808.75
65	5	352.63	255.0111	114.0444	35.9899	669.2661	62.00	739.84
70	5	338.73	235.4976	105.3177	46.3212	631.1388	92.43	705.05
Total	25	394.79	236.3617	47.2723	297.2243	492.3549	62.00	877.67

Table B.12 Homogeneous subsets for overall bioactive components

Value		
Tukey HSD		
Temperature	N	Subset for alpha = 0.05
		1
70	5	338.7300
65	5	352.6280
60	5	394.8260
55	5	421.6700
50	5	466.0940
Sig.		0.929
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 5.000.		

Table B.13 One way ANOVA (no blocking) for total phenolic content

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	3183365.760	5	636673.152	2337129.956	0.000
Residual	3.269	12	0.272		
Total	3183369.029	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.14 Homogeneous subsets for total phenolic content

TPC							
Tukey HSD							
Sample	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
70	3	705.0533					
65	3		739.8400				
60	3			808.7533			
55	3				843.2400		
50	3					877.6700	
fresh	3						1912.3933
Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.							
a. Uses Harmonic Mean Sample Size = 3.000.							

Table B.15 One way ANOVA (no blocking) for total tannin content

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	175408.803	5	35081.761	258312.890	0.000
Residual	1.630	12	0.136		
Total	175410.432	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.16 Homogeneous subsets for total tannin content

TTC							
Tukey HSD							
Sample	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
70	3	92.4333					
65	3		111.6233				
60	3			130.5967			
55	3				168.5467		
50	3					292.2900	
Fresh	3						358.4867
Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.							
a. Uses Harmonic Mean Sample Size = 3.000.							

Table B.17 One way ANOVA (no blocking) for total flavonoid content

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	41539.349	5	8307.870	39624.180	0.000
Residual	2.516	12	0.210		
Total	41541.865	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.18 Homogeneous subsets for total flavonoid content

TFC							
Tukey HSD							
Sample	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
70	3	189.2867					
65	3		205.4600				
60	3			218.4667			
55	3				231.6800		
50	3					235.3867	
Fresh	3						338.0100
Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.							
a. Uses Harmonic Mean Sample Size = 3.000.							

Table B.19 One way ANOVA (no blocking) for antioxidant activity

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	9972.058	5	1994.412	1739.725	0.000
Residual	13.757	12	1.146		
Total	9985.815	17			

Since, $P < 0.05$, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.20 Homogeneous subsets for antioxidant activity

Antioxidant activity							
Tukey HSD							
Sample	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
70	3	399.3567					
65	3		415.2500				
60	3			431.2667			
55	3				440.8667		
50	3					448.0167	
Fresh	3						473.0333
Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.							
a. Uses Harmonic Mean Sample Size = 3.000.							

Table B.21 One way ANOVA (no blocking) for betalain content

Source of variation	Sum of Squares	df	Mean Square	F	Significance
Temperature	252148.705	5	50429.741	250154.418	.000
Residual	2.419	12	0.202		
Total	252151.124	17			

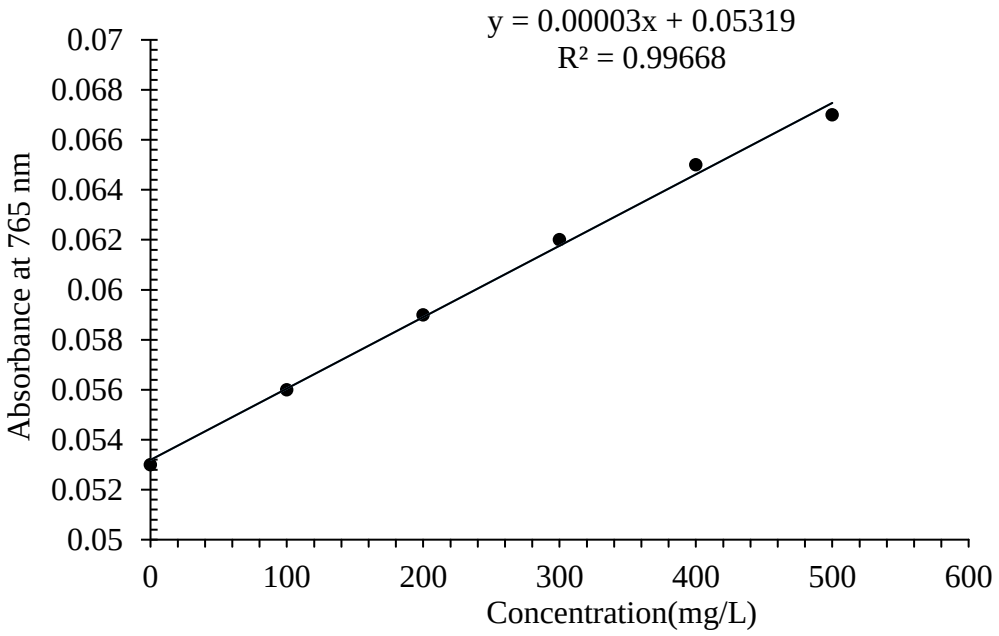
Since, P < 0.05, there is significant difference between the sample at 5% level of significance so LSD testing is necessary.

Table B.22 Homogeneous subsets for betalain content

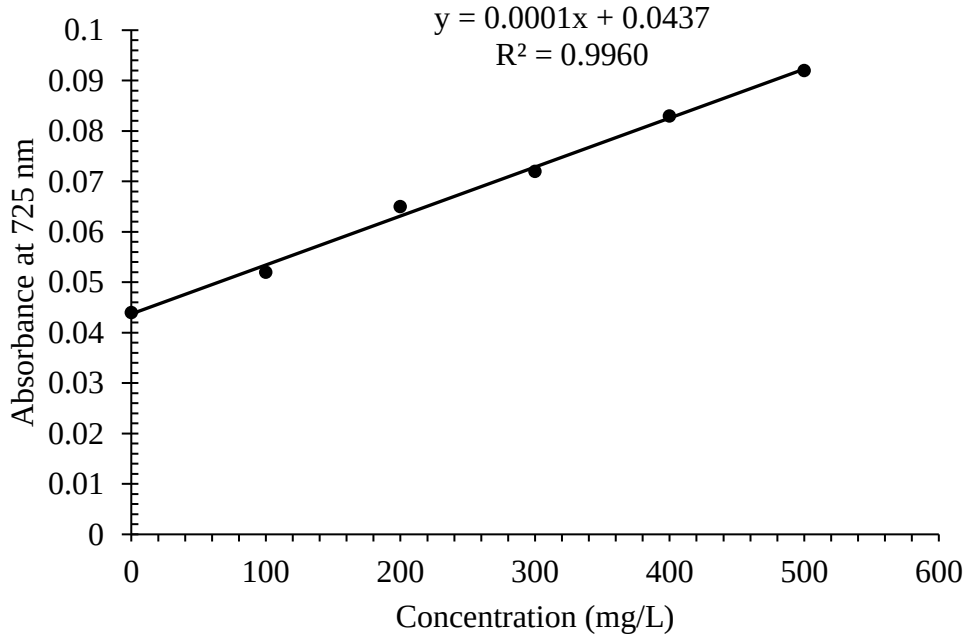
Betalain content							
Tukey HSD							
Sample	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
70	3	307.5200					
65	3		340.5900				
60	3			385.0433			
55	3				424.0100		
50	3					477.1000	
Fresh	3						668.4633
Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.							
a. Uses Harmonic Mean Sample Size = 3.000.							

Apendix C

1. Standard curve for total phenolic content determination



2. Standard curve for total tannin content determination



3. Standard curve for total flavonoid content determination

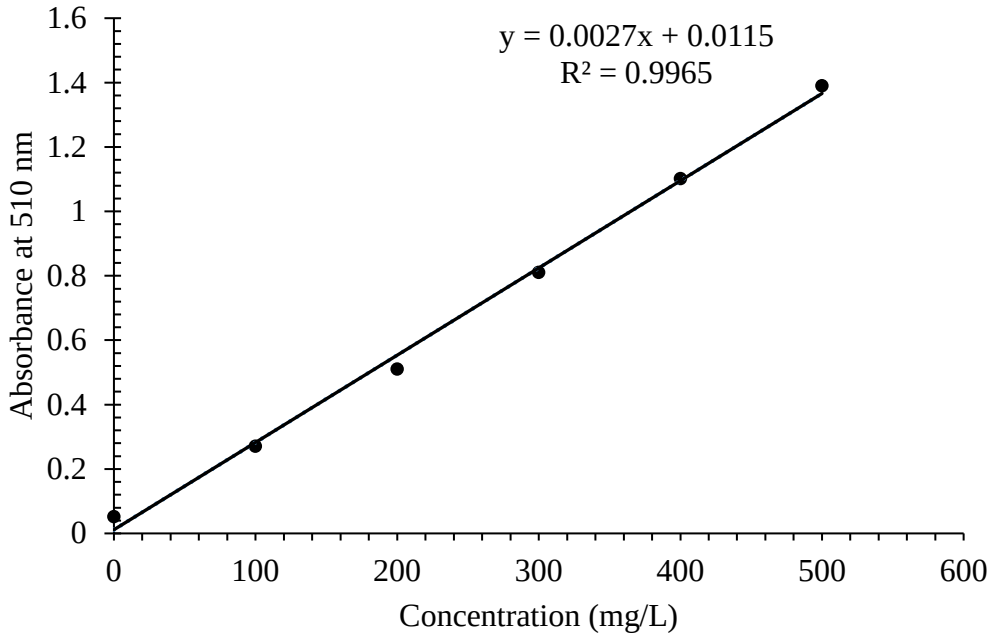


Photo gallery

Plate 1 Cabinet drying of beetroot

Plate 2 Beetroot dried

Plate 3 Fiber content determining

Plate 4 Extract prepared

Plate 5 Absorbance determining