EFFECT OF EVAPORATION TEMPERATURE ON BIOACTIVE COMPONENT AND SENSORY QUALITY OF LEMON JUICE CONCENTRATE

By

Chiranjivi Belbase

Department of Food Technology

Central Campus of Technology

Institute of science and Technology

Tribhuvan University, Nepal

2022

Effect of evaporation temperature on bioactive component and sensory quality of lemon juice concentrate

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

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Chiranjivi Belbase

Department of Food Technology

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Institute of science and Technology

Tribhuvan University, Nepal

March, 2022

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Effect of Evaporation Temperature on Bioactive Component and Sensory Quality of Lemon Juice Concentrate*** presented by **Chiranjivi Belbase** 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, FTQC, Division Head, Dhankuta)
3. **Supervisor** _____
   (Mr. Kabindra Bhattarai, Asst. Prof.)
4. **Internal Examiner** _____
   (Mrs. Geeta Bhattarai, Prof.)
5. **RMC Member** _____
   (Mr. Basanta Kumar Rai, Prof.)

March, 2022

Acknowledgements

I would like to express deep gratitude to my respected supervisor Asst. Prof. Kabindra Bhattarai Head of Nutrition and Dietetics Department, 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 Central Campus of Technology for his continuous support and thankful to Prof. Basanta Kumar Rai, Head of central department of food technology, for his generosity and co-operation.

Many thanks and gratitude is expressed to all the teachers and staff members, librarian for their support. My special thanks goes to Mr. Prajwal Bhandari, Head Technical Assistant, CCT, Hattisar, Dharan and Mr. Sachin Basnet for making my entire study in an enthusiastic and passionate environment. Also, I would like to thank all my teachers, classmates, seniors and juniors who helped me directly or indirectly during my work.

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: …….

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Chiranjivi Belbase

Abstract

The objective is to study the effects of processing temperatures on bioactive components of lemon juice concentrates. Lemon was collected from a farm in Budhiganga Rural Municipality, Morang. The juice was extracted and concentration was done to 50°Bx TSS in rotary vacuum evaporator at different temperature (50° C, 60° C, 70° C) and open pan boiling coded as A, B, C, D and market sample was coded as E. Time taken for concentrating was approx. 8.5 hrs. for sample A, 5.5 hrs. for sample B, 2.5 hrs. for sample C and 45 minutes for sample D. Physicochemical parameters (acidity, ascorbic acid, browning index) and bioactive components (total phenolic content, total tannin content, total flavonoid content and antioxidant activity DPPH free radical scavenging activity) was studied.

The mean values of TSS, acidity, pH, ascorbic acid, browning index (BI), total phenolic content, total tannin content, total flavonoid content and antioxidant activity of fresh lemon juice was found to be 5.0° Bx, 5.02%, 2.2, 455 mg/L, 0.268, 926.63 mg GAE/L, 510.61 mg TAE/L, 320.22 mg QE/L and 81.01% respectively. All the bioactive components were decreased while increasing evaporation temperature. From concentrated sample, sample concentrated at 60°C was found to be most effective on preserving bioactive components of juice concentrates in terms of higher polyphenol retention, flavonoid retention, tannin retention and higher antioxidant activity. For all bioactive properties there was significant different ($p<0.05$) along with difference in processing temperatures. Sensory evaluation was conducted and statistical analysis of sensory data showed that sample C i.e. concentrated at 70°C was found to be best sample among all the concentrated sample and market sample. The present findings indicates that it is possible to improve nutritional and sensory quality of concentrates by optimizing temperatures and reducing thermal processing time.

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List of Abbreviation

Abbreviation	Full form
°Bx	Degree brix
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
BI	Browning Index
CCT	Central Campus Technology
LSD	Least Significant Difference
FAO	Food and Agriculture Organization
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
TAE	Tannic Acid Equivalent

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Part I

Introduction

1.1 General Introduction

Citrus fruit is very popular in many parts of the world due to its distinctive flavour, taste, and aroma as well as multiple health benefits associated with it. The consumption of citrus fruits or their products is believed to have beneficial effects against different diseases, the main reason being the presence of important bioactive compounds (Al Juhaimi and Ghafoor, 2013). Bioactive compounds are capable of modulating metabolic processes and demonstrate positive properties such as antioxidant effect, inhibition of receptor activities, inhibition or induction of enzymes, and induction and inhibition of gene expression (Carbonell-Capella *et al.*, 2014). The diversity of chemical structures of bioactive compounds influences bioavailability and biologic properties, while antinutritional factors can decrease the bioavailability of certain compounds or inhibit digestion enzymes (Septembre-Malaterre *et al.*, 2018).

During heat processing, food can be subjected to some chemical change. Generally can decrease significantly the concentration of nutriments and their biological activity (Tiwari *et al.*, 2008). Many studies have shown that the heat processed fruits and vegetables are expected to have lower health-protecting capacity than fresh ones. This is because the most of the bioactive compounds are relatively unstable to heat. However, recent studies have shown that heat treated foods, especially fruits, vegetables and honey, have higher bioactive compounds and more biological activities (Abbès *et al.*, 2013).

Citrus juices contain 86–90% water. The conventional mode in which fruits are processed and preserved is the form of fruit juices/pulps. However, preservation of juices is not economical, since the water content of fruit juices is very high, i.e. 75 to 90% (Ramteke *et al.*, 1993). Evaporation is the leading method of water removal in the citrus industry. Evaporation is a thermal process where juice is heated to its boiling point. Thermal damage to quality (cooked taste, degradation of vitamins, browning) is to be expected. Water is not the only volatile constituent of lemon juice. Most aroma compounds are even more volatile. They are the first ones to be lost in evaporation (Berk, 2013). The composition of fresh citrus juice is adversely affected by industrial processing and/or storage conditions. Industrial

processing involves a number of different stages that result in some alterations from the original composition of fresh citrus juice (Jordan *et al.*, 2003).

Citrus fruits and juices are the important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids phenolic compounds and pectin that are important to human nutrition. The main flavonoids present in citrus species are Hesperidine, Narirutin, Naringin and Eriocitrin. Besides sugars, acids, and polysaccharides, citrus are an important source of phytochemicals such as phenolics, vitamin C and carotenoids. These compounds also known as nutraceuticals, provides health benefits due to a risk reduction of chronic illness such as cancer and cardiovascular disease (WHO, 1998).

The vacuum evaporation is a unit operation in fruit juice processing. By this process, the fruit juice product is evaporated with higher quality than by the conventional process at atmospheric pressure under sub-atmospheric pressures because it can be operated at low temperatures. Vacuum evaporation process has the advantage of highly efficient technology and provides high product quality (Gerard and Roberts, 2004).

1.2 Statement of the problems

Lemon juice is the source of antioxidants such as phenolic acids, flavonoids, and vitamin C (Luzia and Jorge, 2009). Bioactive compounds in plants are produced as secondary metabolites that are not necessary for the daily functioning of the plant (such as growth); however, they play an important role in the competition, defense, attraction, and signaling. These compounds can then be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in humans and animals (Bernhoft, 2010) . At the present scenario, evaporated lime juice and other products are not prepared considering the content of bioactive components. This study specifically determines bioactive components of raw lemon juice and the effect of various controlled processing temperature on bioactive components which in turn provides consistent product quality which is lacking at present scenario. Lemon is frequently used in kitchen as acidulants, during off season it can be used as concentrate but if not properly prepared the bioactive components might be lost due to heat so i have done this work to find out the optimized temperature for processing of lemon juice to prepare concentrate.

Many research has been carried out in lemon like functional properties of juice, bioactive components present and health benefits of bioactive compounds presents in lemon juice,

different products are made from lemon juice, many research on lemon peel. Research on physiochemical changes in lemon juice (Change in acidity, ascorbic acid degradation, decrease in reducing sugar, bioactive components change, etc.) on storage has not been carried out. The changes on bioactive components due to the processing temperature has not studied as others things are studied. Citrus juice is rich in different bioactive components and health benefit of bioactive components has very important in human life. On processing lemon juice bioactive components degradation is seen which is not good. This dissertation is important to know the minimal loss in bioactive components while processing on different temperature and to find out best temperature to have minimal loss.

1.3 Objectives

1.3.1 General objectives

The general objective of the work is to study the effect of evaporation temperature on bioactive component and sensory quality of lemon juice concentrate

1.3.2 Specific objectives

i. To determine the bioactive components (tannin, polyphenol, flavonoid and antioxidant activity) and browning index of fresh lemon juice.
ii. To prepare the concentrate at 50°C, 60°C, 70°C and open pan boiling.
iii. To determine the bioactive components (tannin, polyphenol, flavonoid and antioxidant activity) and browning index of lemon juice concentrate.
iv. To carry out proximate analysis of lemon juice concentrate.
v. To carry out sensory analysis of lemon juice concentrate prepared in lab and market sample.

1.4 Significance of the study

Once the study is completed, it will lets us know the pattern of loss of bioactive components of the lemon juice from different evaporating temperature so that the maximum retention of bioactive components is achieved. Moreover, it will be largely beneficial to commercial industries that are working on product making from lemon juice and it will be automatically beneficial to the people who use those products. It may be beneficial for the lemon producer and product developer with new product having distinct flavor and having more bioactive components.

1.5 Limitations of study

The hurdle that came during the thesis is listed below:

1. Only change in bioactive components (polyphenol, anti-oxidant, tannin, and flavonoid) was studied.

Part II

Literature review

Citrus is a common term and genus (Citrus) of flowering plants of the rue family, Rutaceae. Citrus is believed to have originated in the part of Southeast Asia bordered by North-eastern India, Myanmar (Burma) and the Yunnan province of China (Gmitter and Hu, 1990). Citrus juices are consumed majorly because of their Nutritional value and special flavor. The positive health benefits of juices have been ascribed in part to vitamin-C (ascorbic acid), the major vitamin found in fruits and vegetables (Rekha et al., 2012). Lemon is one of the non-climacteric citrus fruits containing higher amount of vitamin-C and acidity. Its juice is an excellent and economical acidulants to enhance taste and flavor of different fruit products (Barwal and Shrera, 2009). Citrus fruits like lemon are widely consumed universal since they can help to improve the health of people. It was recognized that lemon not only prohibits the different diseases such as hypertension and tumor, but also can be even applied as a fuel or catalyst (Sharif *et al.*, 2021).

Citrus has been cultivated in an ever-widening space since ancient times; the known examples are oranges, lemons, grapefruit, and limes Citrus is one of the foremost necessary industrial fruit crops grown in all continents of the planet (Scora, 1975). Currently, citrus is cultivated within the subtropic and tropical regions of the planet between 40° north and south latitude in over 137 countries on six continents and generates concerning one hundred and five billion US dollars per annum within the world fruit market (Ismail and Zhang, 2004).

2.1 Introduction and history of lemon

Lemon is a kind of plant with the characteristic evergreen leaves that hails from Asia and has yellow edible fruit. The distinctive traits of this kind of plant area unit its thorny branch, white flower, and its oval-shaped fruit with a robust bitter taste (Chaturvedi *et al.*, 2016). The origins of the Lemon aren't well-known though it's believed that the primary lemons were cultivated in Assam (in north-eastern India), northern Myanmar or China. A lemon genetic origin analysis has shown that it's hybrid from bitter orange to lemon (sour orange) (Al-Qudah *et al.*, 2018).

C. limonum is a wealthy source of ascorbic acid, that is employed as folks drugs for the treatment of stomach ache, carminative, as antipneumonia, and additionally for the treatment of infectious disease and diarrhoea (Kulkarni *et al.*, 2005). Juice contains slightly a lot of acid than lime juice (about 47 g/l), nearly double the acid of grapefruit juice, and regarding five times the number of citric acid found in orange juice (Penniston *et al.*, 2008). Because of its versatility, Lemon may be employed in a spread of preparation, medicinal, cosmetic, and aromatherapy. Historically, lemon juice was wont to treat fever, high blood pressure, and menstrual irregularities. Meanwhile, a lemon oil is employed to alleviate coughs. In many studies, it's explained that extracts, essential oils, and juice have pharmacological effects like antioxidants, anti-inflammatory, antibacterial, neuroprotective, anticancer, and antihyperlipi-demic agents. These pharmacological effects resulted from the fact that lemon may be a plant rich in varied active chemical components, particularly acid, water-soluble vitamin, flavonoid, and different essential oils (Hartati *et al.*, 2021).

Massive acid and juice content is present within the Omani lemon variety. The foremost popular varieties are offered within the Batinah region, for the Sohar and Saham mandate, etc. (Silalahi, 2002). Citrus fruits area unit of the foremost vital husbandry crops, and lemon (*Citrus limon*) is that the third most vital citrus crops. Lemon fruit contains a robust industrial worth for the recent product market and food trade (González-Molina *et al.*, 2010). Lemons ranked the third among the citrus trade within the world, with a complete annual production of regarding nine 9% the citrus production (Mu *et al.*, 2012).

2.2 Taxonomic classification of lemon

The lemon, *Citrus lemon* Burm. F. (syns. *C. limonium* Risso, *C. limonia* Osbeck, *C. medicavar*. Limonium Brandis), is known as "*Limone"* in Italy, *Limon* real in Spanish, *Limonene* in German, *Citronnier* in French, *Citroen* in Dutch, *Limon Amarillo* in Haiti, *Lamoentsji, or Lamunchi* in Netherland, '*Elumitchai*' in Tamil, '*Nimbu*' Hindi,'*Guaranga*'in Malayalam (Obreza, 1993).

Lemon is members of the rutaceae family, and its taxonomic hierarchy according to USDA is:

Kingdom	Plantae
Order	Geraniales
Suborder	Geraniineae
Class	Dicoty ledoneae
Subclass	Archichalmydeae
Division	Embryophyta
Subdivision	Angiospermeae
Family	Rutaceae
Subfamily	Aurantiodeae
Tribe	Citreae
Subtribe	Citrinae
Genus	Citus L.
Subgenus	*Citrus*
Species	*Citrus sinensis (lemon)*

Source: (USDAa, 2018)

2.3 Main varieties of *Citrus limon*

The lemon, *Citrus limon (L.) Osbeck*, is a species of small evergreen tree in the flowering plant family Rutaceae, native to South Asia, primarily north eastern India. The tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, which has both culinary and cleaning uses. The pulp and rind are also used in cooking and baking. The juice of the lemon is about 5% to 6% citric acid, with a pH of around 2.2, giving it a sour taste. The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods such as lemonade and lemon meringue pie (Osbeck, 2012).

2.4 Worldwide Lemon and lime production

In 2018, world production of lemons (combined with limes for reporting) was 19.4 million tonnes. The top producers – India, Mexico, China, Argentina, Brazil, and Turkey – collectively accounted for 65% of global production table (FAOSTAT, 2019). The worldwide production of lemon and lime is shown in table 2.1.

Table 2.1: Worldwide lemon and lime production

Lemon (and lime) production, 2018	(in millions of tonnes)
Country	2018
India	3.1
Mexico	2.5
China	2.5
Argentina	2.0
Brazil	1.5
Turkey	1.1
Nepal	0.274
World	19.4

Source: (FAOSTAT, 2019)

2.5 Production of lemons and limes in Nepal

It is found that lemons and limes production is increasing rapidly in recent years. From analyzing the data of last ten years 2007 to 2017 it is found that the yearly production of lemon and lime increased form 24,835 tons to 36,839 tons (FAOSTAT, 2019).

2.6 Morphology of lemon

A schematic section of a citrus fruit illustrating different structures is presented below in figure 2.1.

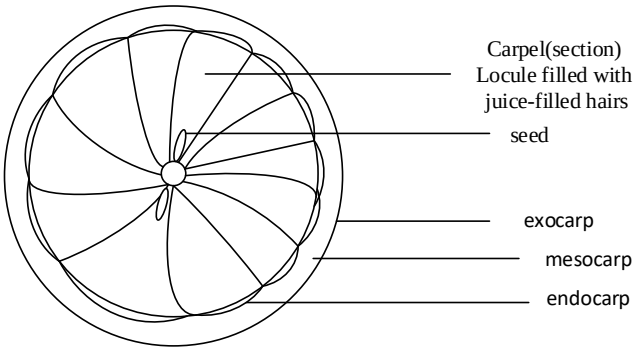

Fig. 2.1 Structure of Lemon fruit

Source: Ranganna *et al.* (1983b)

Fruits are ovoid or spherical, berry, hesperidium, and yellow once ripe. Fruits belonging to the citrus cluster are referred to as “hesperidium”. Fruits form can change as the fruit matures or the trees become old and are also ruled by variety choice. Fruit size is influenced by variety, crop load, rootstock and irrigation practices. Mature lemons flip green to yellow, weigh concerning 50-80 g in weight and 5-8 cm in diameter (Sadat *et al.*, 2012).

2.6.1 The outer peel

The outer peel of citrus fruits is additionally referred to as flavedo because of the presence of flavonoid compounds (Ortiz, 2002). It consists of the cells containing the carotenoids, which provide the characteristic color to the fruits according to the species or variety. The color ranges from deep orange or cherry-red to light-weight orange, yellow or chromatic. The oil glands, that contain the citrus essential oils, also are found within the flavedo. The glands are spherical in form and have totally different sizes. The carotenoid pigments are settled within the chromoplasts in the flavedo (Kefford, 1966).

2.6.2 The inner peel

Also referred to as albedo, the inner peel is found beneath the flavedo. It’s usually a layer of spongy and white parenchyma tissue that's made in sugars, pectic substances, celluloses, hemicelluloses and pentosans. The thickness of the albedo varies with the species. For instance, mandarins usually have terribly skinny albedo whereas the one in citrons is extremely thick. Each flavedo and albedo type the non-edible a part of the fruit referred to as the pericarp, and that they are normally referred to as the rind or peel (Ranganna *et al.*, 1986).

2.6.3 The endocarp

Beneath the albedo of citrus fruits is that the edible portion or conjointly referred to as endocarp. It’s composed of the many segments or carpels, typically around 8-12 in most citrus. Every segment is enclosed by a reasonably powerful, continuous membrane and lined by vascular bundles that transfer nutrients for growing of the fruit. The inside of a segment consists of two major components, the juice vesicles and therefore the seeds (Soule and Grierson, 1986). little and densely packed sacs containing juice and seeds in most varieties fill the segments, and therefore the citric acid contained within the juice along with a complex mixture of other acids, oils, and sugars, provide the characteristic flavor (Ranganna *et al.*, 1983a).

2.7 Chemical and nutritional composition of lemon

Lemon fruit ranks very high in its medicinal value in this way lemon are favorite all over the world. It is a good source of vitamin-C and other nutrients like potassium, iron, calcium, fiber, thiamine, riboflavin and vitamin B_6. Natural foods, especially citrus products, have always been highly regarded as excellent sources of human nutrition. Lemon juice has many health benefits can be used for different purpose like therapeutically uses, natural antiseptic, to control asthma, headaches, pneumonia and arthritis etc.

2.7.1 Nutritional Composition

There are valuable natural chemicals in citrus fruits, it is an excellent source of different elements and vitamins required for the human body, including C vitamins, B vitamins, potassium, phosphorous, and other elements (Abobatta, 2019). Chemical composition of citrus fruits (per 100g of edible portion) is shown in table 2.2

Table. 2.2 Nutritional composition of *Citrus limon* fruits per 100g

Components	***C.limon***
Moisture (g)	85
Protein (g)	1
Fat (g)	0.9
Fibre (g)	1.7
Carbohydrate (g)	11.1
Minerals (g)	0.3
Calcium (mg)	1.7
Phosphrous (mg)	10
Iron (mg)	2.3
Thiamine (mg)	0.02 (in juice)
Vitamin C (mg)	39 (in juice)
Carotene μg	-
Energy, K cal	57

Source: (Abobatta, 2019)

2.7.2 Physiochemical parameters of lemon

The physiochemical parameters found by (Ghimire, 2020) while carrying research on comparative study effect of evaporation temperatures on chemical characteristics of lemon juice concentrates is shown in table 2.3.

Table 2.3 Physiochemical parameters of lemon

Parameters	**Lemon**
Weight (g)	104.60±10.13
Diameter (cm)	7.36±0.65
Length (cm)	8.32±2.25
Acidity % (as citric acid)	4.92 ±0.10
pH	2.3±0
TSS (°Bx)	5.5±0.2
Ascorbic acid (mg/100g)	23.75±3.1

Source:(Ghimire, 2020)

2.7.2.1 Total Soluble Solids (TSS)

The TSS content of the fruit is usually obtained from assessing the ^{o}Brix of the fruit. The TSS or sugar content measures and includes the carbohydrates, organic acids, proteins, fats and minerals of the fruit. It represents from 10-20% of the fruit's fresh weight and increases as fruit matures to produce a less acidic, sweeter fruit. Citrus fruits are considered as acid fruits, since their content of soluble solids are composed mainly of organic acids and sugar (Kale and Adsule, 1998). Brix scale was being used by the fruit juice industry in determining the sucrose equivalent of soluble solids; the term "Brix" or "degrees Brix" was being used interchangeably with the % sucrose or the % soluble solids by weight in fruit juices and was determined by using density measurements. This usage led to the Brix scale's becoming the standard for the measurement of juice concentration in the citrus and related industries. Necessary. Brix measurements can be performed much faster, and the Brix range of refractometers can be much broader, up to 0 to 70°Brix, which is important in processing plants that manufacture concentrated juices (Chen, 1983).

°Brix level (°Brix is a measure of soluble solids, which is correlated to sugar content or sucrose equivalent). Since the major soluble solid in high-acid fruit (lemons and limes) is citric acid, not sugar, lemon and lime concentrates are produced and sold on the basis of grams of equivalent citric acid per liter of solution (GPL). Lemon concentrate of 400 ± 5 GPL is most commonly produced which was stated by Subramanyaiah *et al.* (2019) which is equivalent to about 40.5% acidity as anhydrous citric acid. TSS of lemon and lime juice varies from 6-11^{o}Bx.

2.7.2.2 Acidity

Acids plays a vital role in the quality of citrus juices, second only to the Brix in importance. The acid content in juice has an important role in determining the quality of a variety as well as maturity indices of fruit (Koehler *et al.*, 2003). They give the characteristic tartness or sourness of citrus products and have been acclaimed for their effectiveness as thirst quenchers. These acids and their salts replace many of the acids and salts lost by the body through vigorous exercise (Kimball, 1991). The structure of citric acid is shown in Fig. 2.2.

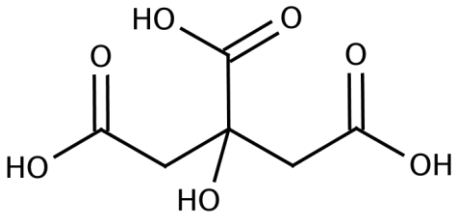

Fig. 2.2 Molecular structure of Citric acid

Organic acids significantly contribute to juice acidity, citric acid being primary organic acid (70–90% of total). Organic acids comprise a significant portion of the soluble solids in citrus juices. The primary acid found in these juices is the triprotic citric acid or tricarboxylic acid. Malic acid also is present, comprising about 10% of the total acid content. Unlike citric acid, malic acid levels remain fairly constant in the juice throughout fruit maturation. Potassium and sodium salts of citric acid comprise about 20% of the total acid salt composition. These salts help buffer the acid, thus preventing sudden changes in pH during a harvesting season. These salts generally are ignored by the citrus industry, as are the differences between citric and malic acids. A single acid titration generally is performed, and the results are calculated as if all the acid were undissociated citric acid. In reality, all the acid is not citric. Because of the presence of the salts, the real structure of the citric acid

in solution may be more that of a partially dissociated form, such as dihydrogen citric acid (Kimball, 1991).

Citric acid content in juice of different lemon cultivars at harvest varies from 5% to 7% (Al-Mouei, 2014). Acidity content of *kagzi* lime juice was found to be 5.92 % during analysis performed by Kaul and Saini (2000a) where, they concentrated that juice to 28.17 % acidity.

2.7.2.3 Vitamin-C (L-ascorbic acid)

L-Ascorbic acid, also known as L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone, antiscorbutic vitamin and vitamin-C, has the chemical formula $C_6H_8O_6$ and a molecular weight of 176.12. This water-soluble vitamin-Contributes to: iron absorption; cold tolerance; maintenance of the adrenal cortex; antioxidizing activity; metabolism of tryptophan, phenylalanine, and tyrosine; body growth; wound healing; synthesis of polysaccharides and collagen; formation of cartilage, dentine, bones, and teeth; and capillary maintenance. Humans cannot synthesize vitamin-C, and must be solely obtained from the diet, mainly through fruits and vegetables to maintain a normal metabolic functioning of the body (Daud *et al.*, 2016). Vitamin-C is very stable in citrus juices and degrades very little with storage, another nutritional advantage. L-Ascorbic acid has the following chemical structure as shown in Fig. 2.3.

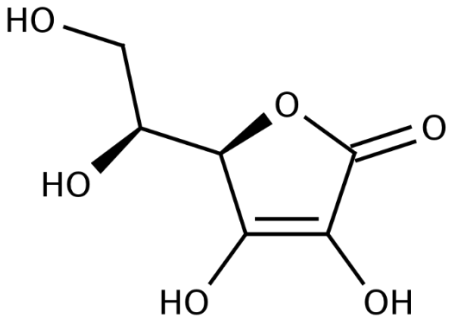

Fig. 2.3 Molecular structure of L-Ascorbic acid

Njoku *et al.* (2011) found lemons to contain vitamin-C, also known as ascorbic acid, only behind oranges and grapes but more than in lime fruits at varying storage temperature. The ascorbic acid content of food is strongly influenced by season, transportation to market, shelf life, time of storage, cooking practices and chlorination of water. The concentration of ascorbic acid has been reported to decrease with maturity or remain constant until late in the season and then decline (Baldwin, 1993). Only 25% of ascorbic acid in the fruit is in the juice, the remainder is found in the peel, especially in the flavedo (Kefford and Chandler,

1970). Vitamin-C content varies with variation in the variety and maturity level. Its range varies from 20 mg/100 g to 60 mg/100 g (Al-Mouei, 2014). Vitamin-C contributes to: iron absorption; cold tolerance; maintenance of the adrenal cortex; antioxidizing activity; metabolism of tryptophan, phenylalanine, and tyrosine; body growth; wound healing; synthesis of polysaccharides and collagen; formation of cartilage, dentine, bones, and teeth; and capillary maintenance. Humans cannot synthesize vitamin-C, and must depend on an external source for its supply.

The vitamin-C level in citrus decreases with maturity. Florida oranges go from about 50 mg /100 ml of single-strength juice at the beginning of the season to about 30 mg/100 ml by the end of the season (Harding *et al.*, 1940). During an average marketing period, the loss of vitamin-C amounts to less than 10%, which is indicative of the stability of vitamin-C in citrus juices. Atmospheric oxygen· is responsible for most vitamin-C loss during long-term storage. Many polymeric containers readily admit oxygen, which degrades vitamin-C as well as contributing to the development of off colors and flavors. Grapefruit juice generally contains slightly less vitamin-C (45 mg /100 ml) than orange juice (50 mg/100 ml) and lemons (60 mg/100 ml). Tangerines and limes contain even less vitamin-C (about 30 mg/100 ml). These vitamin-C levels represent approximate averages and indicate general relative comparisons. Actual vitamin-C levels may vary widely, by as much as 50%.

2.7.2.4 Sugars

Citrus juices contain a wide variety of chemical compounds, but none as prevalent as sugars or carbohydrates. Carbohydrates make up better than 80% of the soluble material in citrus juices, and of these soluble carbohydrates, half are in the form of sucrose. The sucrose molecule consists of one molecule of glucose and one molecule of fructose. The other half of the carbohydrates in citrus juices consist of relatively even amounts of glucose and fructose, which result from natural enzymatic breakdown of the sucrose.

The sweetness of citrus fruits is due to the presence of glucose, fructose, and sucrose. Total sugars may vary from less than 1% in certain limes to nearly 15%in some oranges. (Kimball, 1991). Reducing Sugar content was found to be 0.79% in *kagzi* lime juice during analysis by Kaul and Saini (2000a).

2.8 Bioactive components

A bioactive compound is a compound having some biological activity. As the name suggests (Greek 'bios' means life and Latin 'activus' means dynamic or full of energy), a bioactive compound (or substance) has its direct physiological or cellular effects on a living organism. Such effects may be positive or negative depending on the nature of the substance, its dose, and its bioavailability (Oh and Jun, 2014). Bioactive compounds are present in small quantities in foods, mainly in fruits, vegetables, and whole grains, and provide health benefits beyond the basic nutritional value (Gökmen, 2016). In plants, nutrients are generally not included in the term "plant bioactive compound". Typically, bioactive compounds in plants are produced as secondary metabolites that are not necessary for the daily functioning of the plant (such as growth); however, they play an important role in the competition, defense, attraction, and signaling. These compounds can then be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in humans and animals (Bernhoft, 2010) .

Potential health effects of bioactive compounds and nutrients are dependent on the digestion process, as this affects bioactive compounds and their stability and as a consequence affects the bioavailability and potential beneficial effects on health (Carbonell-Capella *et al.*, 2014). The main raw material of *C. limon* is the fruit, particularly the essential oil and juice obtained from it. The *C. limon* fruit stands out as having well-known nutritional properties, but it is worth remarking that its valuable biological activities are underestimated in modern phytotherapy and cosmetology (Goetz, 2014). *C. limon* fruit juice (lemon juice) has traditionally been used as a remedy for scurvy before the discovery of vitamin C (Mabberley, 2004).

2.8.1 Phenol

Phenolic compounds are the largest category of phytochemicals and the most widely distributed in the plant kingdom. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group (Altiok, 2010). Phenolic composition of Citrus juice comprises flavanones (major group), flavones and flavonols which usually occur as glycosides. (Abad-García *et al.*, 2014). Phenolic compounds constitute a large group of phenyl-propanoids produced by plants as secondary metabolites. They are involved in different functions in the ecology, physiology,

and biochemistry of plants such as chemical defense against predators, reproduction, and in plant–plant interference. So far, 8000 PCs have been identified in 16 different classes with very diverse chemical structures and molecular masses (Velderrain-Rodríguez *et al.*, 2014). Based on the numbers of carbon atoms present in its structure, phenolic are categorized as Table 2.4.

Table 2.4: The major classes of phenolic compound

S.N.	Number of carbon atom	Basic skeleton	Class
1	6	C_6	Simple phenols, Benzoquinones
2	7	C_6-C_1	Phenolic acids
3	8	C_6-C_2	Acetophenones, Tyrosine derivatives
4	9	C_6-C_3	Hydroxycinnamic acid, Coumarins
5	10	C_6-C_4	Naphthoquinones
6	13	C_6-C_1-C_6	Xanrhones
7	14	C_6-C_2-C_6	Stilbenes
8	15	C_6-C_3-C_6	Flavonoids
9	18	$(C_6$-$C_3)_2$	Lignans
10	30	$(C_6$-C_3-$C_6)_2$	Biofalvonoids
11	N	$(C_6$-C_3-$C_6)_n$	Condensed tannins

Source: (Saxena *et al.*, 2013)

The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins (Siddhuraju and Becker, 2007).

2.8.2 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. These appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. These are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives. The flavonoid polymers are also known as proanthocyanidins. They occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, antistressors, and UV irradiation protection (Vaya and Aviram, 2001). More than 5000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known. They are the largest group of phenolic compounds and have the basic skeleton composed of the three rings (C6-C3- C6) (Harborne and Baxter, 2001).

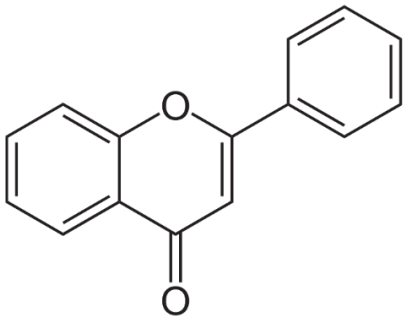

Fig. 2.4 Basic structure of flavonoid

They have been classified into six subgroups (Ghasemzadeh and Ghasemzadeh, 2011):

1. Flavones (luteonin, apigenin, tangeritin).
2. Flavonols (quercetin, kaemferol, myricetin, isorhamnetin, pachypodol).
3. Flavanones (hesteretin, naringenin, eriodictyol).
4. Flavan-3-ols: catechins and epicatechins.
5. Isoflavones (genistein, daidzein, glycitein).
6. Anthocyanidins compounds (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin).

2.8.3 Tannins

Tannins are water soluble phenolic compounds having molecular weights between 500 to 3000 Da giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and proteins. (Arias *et al.*, 2004). Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (Ashok and Upadhyaya, 2012).

They are plant secondary metabolites which have a role in plant defense mechanism against microbial infection, insect and animal herbivores. Tannins are classified into 2 groups: hydrolysable and condensed tannins. The latter is the major tannins in the plant and are flavonoid polymers (Prommajak *et al.*, 2020). There are three major classes of tannins: Shown below are the base unit or monomer of the tannin.

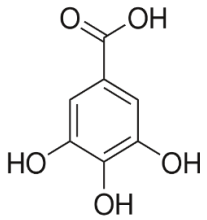

Fig: Gallic acid

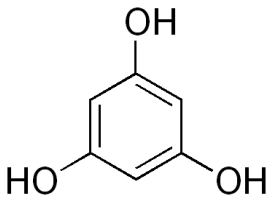

Fig: phloroglucinol

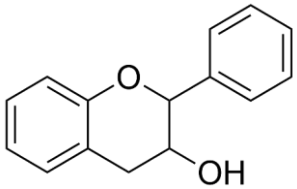

Fig: flavan-3-ol's scaffold

Fig. 2.5 Three major classes of tannin

Hydrolysable tannins are distinguished by having a polyhydric alcohol, such as glucose, as the core. The hydroxyl groups of this alcohol are esterified either partially or wholly by Gallic acid or its congener. Such tannins are readily hydrolyzed by acids, bases or enzymes, creating carbohydrates and a number of isolable crystalline phenolic acids. Tannins of this class contain only phenolic nuclei. When treated with a hydrolytic reagent, particularly in an acid solution, they show a tendency to polymerise into insoluble, amorphous red coloured compounds known as phlobaphenes. These tannins are mostly formed by the condensation of two or more molecules of flavon-3-ols, specifically catechin (Patel, 2011).

Tannins have capability to form complex with proteins by nonspecific bonding, viz. hydrophobic and hydrogen bonding and also with the help of covalent binding. The antimicrobial activity of tannins may therefore be associated with their potential to denature microbial enzymes, adhesins, plasma membrane transport proteins, etc. Because of their property to bind with metals and proteins, tannins also prevent the growth of microbes through the deprivation of metal ion and substrate (Gupta and Pandey, 2020). Hydrolyzable and condensed tannins have been suggested to show similar antibacterial and antifungal potency, although the hydrolyzable tannins seemed to be more significant against yeasts. The occurrence of a hexahydroxydiphenoyl group or its oxidatively altered entities was an essential factor for the anticryptococcal action of the ellagitannins corilagin, phyllanthusiin, and pelargoniin B. It has also been recommended that lethality of tannin would be associated

with molecular size, as larger molecule would more efficiently bind to proteins (Gupta and Pandey, 2020).

The anticarcinogenic and antimutagenic potential of tannins may be related to their antioxidative property which is important in protecting cellular damage including lipid peroxidation. Tannins are reported to exert physiological effects such as accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis and modulate immune responses (Chung *et al.*, 1998).

2.8.4 Antioxidant activity (DPPH free radical scavenging activity)

Antioxidants may be defined as substances that, when present in food, delay, control, or inhibit oxidation and deterioration of food quality. In the body, antioxidants reduce the risk of degenerative diseases arising from oxidative stress (Halliwell, 1999). Antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers (Shahidi *et al.*, 1992).

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. Recently there has been a rise of attention in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well identified and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially also as antioxidant additives or a nutritional supplements (Schuler, 1990). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.*, 1987). Polyphenols are excellent antioxidants due to 3, 4 dihydroxy group in their B ring and the galloyl ester in the C ring of flavonoids (Chu and Chen, 2006).

The antioxidant activity of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and the removal of molecular oxygen (Lee *et al.*, 2004). (Chu and Chen, 2006) reported that flavonoids have the most potent antioxidant activity because their chemical structure contains an O-diphenolic group, a 2-3 double bond

conjugated with 4-oxo function and hydroxyl groups in the position 3 and 5. Flavonoids effectively scavange hydroxyl and peroxyl radicals, form complexes with metals and inhibit metal initiating lipid oxidation. The antioxidant activity of phenolic acid also depend on the number of orientation of hydroxyl groups relative to the electron withdrawing CO_2H, CH_2CO_2H, or $(CH)_2CO_2CH$ functional groups (Rice-Evans *et al.*, 1996).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Tailor and Goyal, 2014). The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract. 24 The scavenging of DPPH by radical scavengers can be summarized as:

DPPH • + FE ⟶ DPPH-H + A• (1)

DPPH + A• ⟶ DPPH – A (2)

A• + A• ⟶ A – A (3)

Where FE is a scavenger of the extract and A• is a radical. The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Tailor and Goyal, 2014).

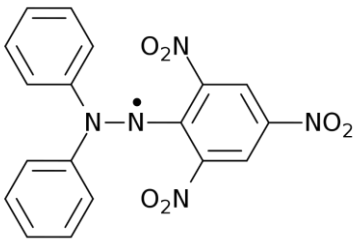

Fig. 2.6 DPPH molecule

2.8.5 Effect of temperature on browning index

Browning in food products is the well-known phenomena that take place during processing and storage leading to brown coloration of juice due to chemical reactions such as

caramelization, ascorbic acid degradation and the Maillard reaction. It is the most common quality problem of many concentrated fruit juices and causes loss of nutrients and the formation of intermediate undesirable compounds like furfural and 5-hydroxymethylfurfural (5-HMF) (Clegg, 1964). Due to tremendous health benefits and their wide use as health drinks, quality of the juice should be monitored and maintained with great care. Non-enzymatic browning (NEB) is one of the most detrimental chemical reactions responsible for quality problems during the heating or prolonged storage of citrus juice. Off colors due to Maillard oxidation (as a result of scorching or excessive heat applied to concentrates over too long a time detract from the color quality of the juice and can result in lower color scores. Such browning reactions dull the natural citrus color. However, off flavors from these reactions develop before the visible effect occurs and further render the juice inferior. Also, hesperidin flakes or brown or black flakes from evaporator bum are readily visible in citrus juices and ruin their appearance (Perez-Cacho and Rouseff, 2008).

At the high evaporation temperatures, sucrose, glucose, fructose, and sometimes maltose, undergo changes. For example, in highly acidic medium (pH < 3), transformation of fructose to hydroxymethyl furfural can occur. Hydroxymethyl furfural polymerizes or polycondenses with other compounds, forming dark-colored substances. This type of browning reaction is important mainly in fruit juices (Kus *et al.*, 2005). In less acidic medium (pH > 3.5), glucose and fructose can form brown-colored substances by reacting with free amino acids present in the juices through non-enzymatic browning or Maillard-reaction.

2.9 Effect of evaporation temperature on lemon juice concentrate

Prolonged exposure or treatment with high temperatures could also lead to active compounds degradation (Wang *et al.*, 2007). Vitamin C and phenolic compounds decreased steadily with longer drying time, while nomilin and limonin contents increased during an early period of drying and decreased thereafter. The phenolic compounds are found to be a major contributor to antioxidant activity of the final product and drying of lime residues at 60 C could retain the highest antioxidant activity (Kuljarachanan *et al.*, 2009). Nicoli *et al.* (1999) indicated that the decrease in antioxidant activity due to drying was related to the degradation of biologically active compounds at high temperatures from chemical, enzymatic, or thermal decomposition. the decrease in total phenolic contents after drying might be due to the binding of polyphenols with other compounds (proteins) or the alterations in the chemical structures of polyphenols (Qu *et al.*, 2010).

According to Schieber *et al.* (2001), the loss of macromolecules like flavonoid during heat treatment might be due to the harsh drying conditions, in particular, the temperature and duration used. Davey *et al.* (2000) reported that wet thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which then resulted in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen.

2.10 Lemon juice concentrate

Concentrated Lemon Juice is the unfermented but fermentable product obtained from the endocarp of mature and sound fruits of the Citrus Limon variety. The product has the characteristic appearance, aroma and clean taste of crystal clear lemon juice. Not less than 2 years when stored at -15° / -18 (Citromax, 2016b). The composition of lemon juice concentrate is shown in table 2.5.

Table 2.5 Composition of Lemon juice concentrate

Parameters	**Value**
TSS (°Bx)	38-70
Citric acid (gm/L)	300-600
Acidity (%)	31.5-44
pH	1.7-2.5
Ascorbic acid (mg/100 ml)	34

Source: Citromax (2016a)

2.11 Importance of concentrates

The main importance of concentration in the case of citrus juices are (Berk, 2013):

i. Reduction of mass and volume, resulting in reduction of the cost of packaging, storage, and transportation.

ii. Availability of raw material for production of lemon/lime related products in off-season also.

iii. Better stability of the concentrate due to the reduction of water activity. High concentration of soluble solids is an efficient barrier against microbial growth and enzyme activity. On the other hand, chemical reactions such as non-enzymatic

browning are accelerated by high concentration. Thus, concentrated juices are more resistant to microbial spoilage than single strength juices.

2.12 Uses of concentrates

i. Used as Drink

Lemon juice and concentrate are most often used throughout the world not as pure juice but as an ingredient in juice beverages. In many parts of the world, a sweetened, diluted lemon drink, known as lemonade, is the most popular citrus drink and other soft drink industry.

ii. Used as Medicine

Lemon juice is widely known as a diuretic, antiscorbutic, astringent, and febrifuge. In Italy, the sweetened juice is given to relieve gingivitis, stomatitis, and inflammation of the tongue. Lemon juice in hot water has been widely advocated as a daily laxative and preventive of the common cold, but daily doses have been found to erode the enamel of the teeth. Prolonged use will reduce the teeth to the level of the gums. Lemon juice and honey, or lemon juice with salt or ginger, is taken when needed as a cold remedy (Obreza, 1993).

iii. Used as acidulants and flavorings

Only plenty of limes is available in peak season , so at off season used in cooking, sweets, jams, soft *drinks*, ice creams, baking, making salad dressings and marinades as source of acidulants and flavorings (Ting, 1980).

2.13 Methods for juice concentration

Concentration of fruit juices permits economic advantages in packaging, storage, and distribution. It also helps in the economic utilization of perishable fruits during the peak harvest periods, thus stabilizing the market prices of fresh produce. Lemon juice contains about 85–90% water; during the concentration step, their bulk is considerably reduced by removing most of water (Berk, 2013).

Several physically different methods are available for water removal. These includes:

i. Evaporation,
ii. Freeze Concentration,

iii. Membrane Concentration (Ultrafiltration, Reverse Osmosis, Microfiltration)
iv. Reverse Osmosis,
v. Osmotic Water Transfer,
vi. Chemical Complexation etc**.**

2.14 Evaporation, its problems and requirements for evaporators

Evaporation is defined as the vaporization by boiling of a volatile solvent (water, in our case) to increase the concentration of a solution or a suspension. The major equipment used in the concentration process is the evaporator. Liquids may become steam at atmospheric pressure or under vacuum.

i. At temperature of about 100˚C, evaporation can take a long time; therefore, browning and degradation in taste and food value may occur. This method is used when caramel-like taste is required for the products (e.g., plum jam).
ii. Vacuum evaporation reduces the detrimental changes in quality. By decreasing the pressure on the surface of the liquid in the evaporator, its boiling point is lowered. At the same time, the evaporation temperature is also decreased (e.g., at 600 Hg mm vacuum, the boiling point of the water is 61.6◦C). This law operates during vacuum evaporation.

2.15 Vacuum evaporators

The rotary evaporator is an essential piece of equipment in most organic laboratories. Commonly vacuum is provided by a water aspirator (or by other mild vacuum sources), and the flask is rotated by an electric motor. This provides a thin film of solution for evaporation, hopefully preventing the solution from bumping. The flask rotates in a temperature-controlled water bath, which provides the heat of vaporization. Little heat may be needed to evaporate highly volatile solvents like ether, whereas heat is definitely required for solvents like toluene (Pirrung, 2007).

It is essential that a glassware bulb be inserted between the flask and the evaporator to make provision for bumping of the solution being evaporated. If a bump does occur, the solution is trapped in the bulb, enabling it to be transferred back into the flask. Without the bulb, the solution would be drawn up into the evaporator itself, where it could mix with condensed solvents, be contaminated by other compounds that have been earlier evaporated in the equipment, or be lost altogether. This bulb naturally must be rinsed with solvent after

evaporation of each sample. The flasks used on a rotary evaporator may be of many sizes and types, but typically round-bottom or pear-shaped flasks are used (Pirrung, 2007). Schematic diagram of one of the Rotary vacuum evaporators with its working parts is given below in Fig. 2.7.

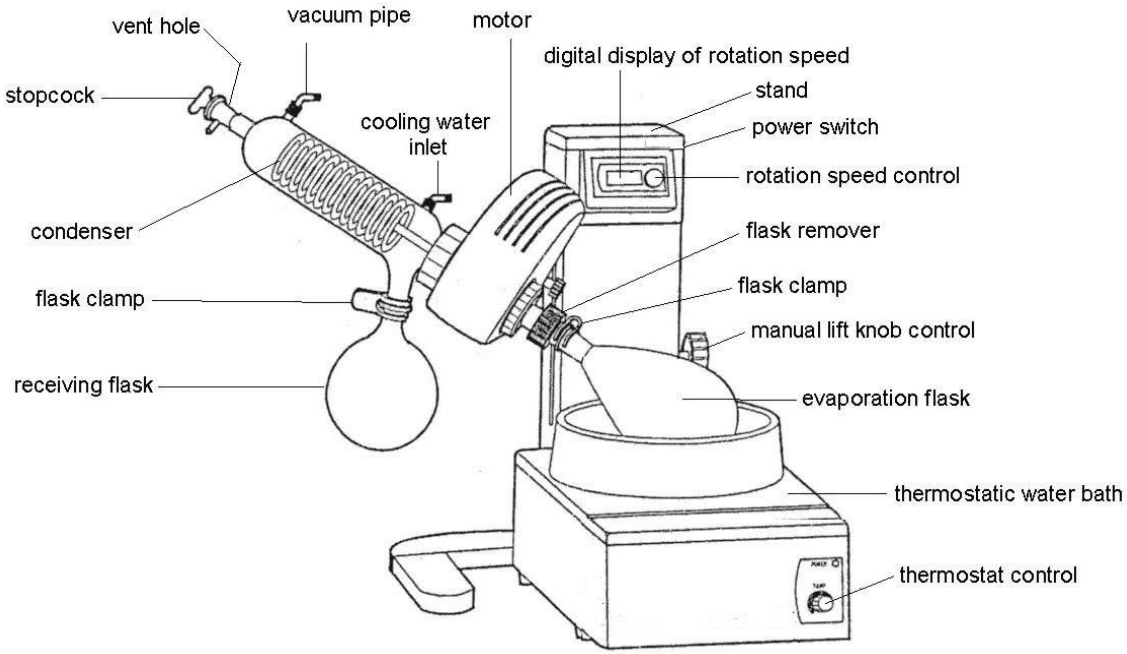

Fig. 2.7 Labelled figure of Rotary vacuum evaporator

Source: (Anon, 2010)

2.16 Evaporation of citrus juices poses a number of specific problems:

i. Evaporation is a thermal process. The juice is heated to its boiling point. Thermal damage to quality (cooked taste, degradation of vitamins, browning) is to be expected.

ii. The viscosity of citrus juices increases rapidly as the juices are concentrated (Berk, 2013). High viscosity has a strong negative effect on the rate of heat transfer in the evaporator. The high viscosity of the concentrated juice is one of the factors limiting the upper limit of concentration.

iii. Water is not the only volatile constituent of lemon juice. Most aroma compounds are even more volatile. They are the first ones to be lost in evaporation.

2.17 Common practice in Nepal for preparation of citrus concentrates

There is no cheap availability of citrus fruits during all season. At peak production season there is easy and cheap availability of citrus fruits. So, for the further need of these fruits they are preserved in different form and stored until it is needed. *Chook* preparation is mostly common during citrus production peak season.

Chook is a tart, concentrated, black juice prepared sour fruits (mainly citrus such as lemon, lime, etc.). It is used for acidulating locally made pickles (*achar*). It also reportedly has certain medicinal values. When properly bottled, chook remains in good condition for 1-2 years, even without chemical preservatives. The product's marked stability can be attributed to the natural preservative (citric acid) present in the juice and the low moisture resulting from concentration. The production method varies from locality to locality, mainly depending on the availability and type of raw materials (Kharel *et al.*, 2009).
The traditional method of *chook* preparation is outlined in Fig. 2.8.

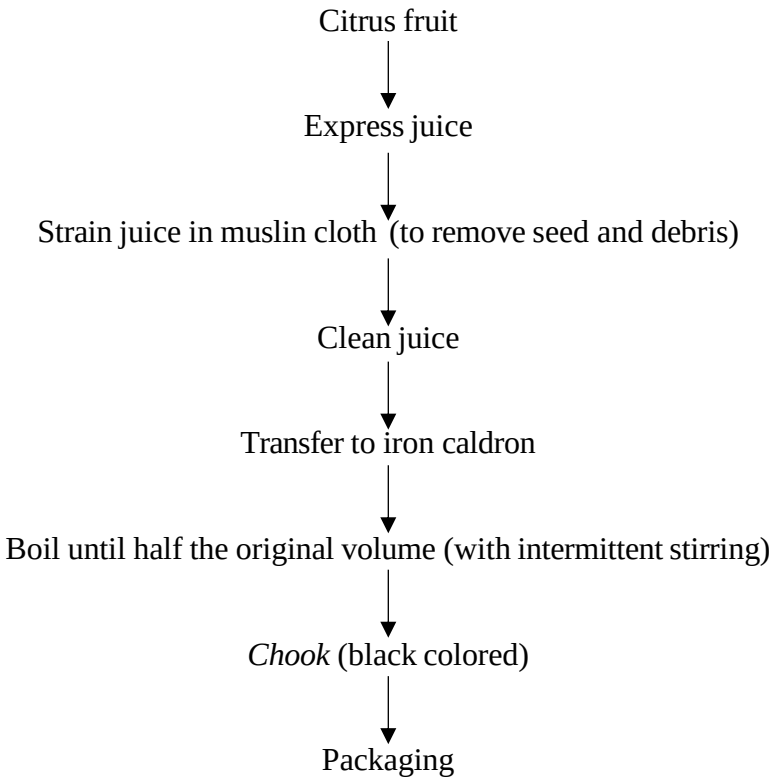

Fig 2.8 Traditional method of Preparation of lemon juice concentrate

Source: Kharel *et al*. (2009)

Part III

Materials and methods

3.1 Materials

All chemicals used were reagent grade unless specified otherwise and distilled water was used throughout the work. All operations were performed at room temperature unless otherwise stated.

3.1.1 Geographical location and collection of raw materials

The chief ingredient of the research, lemon (*Citrus limon* madrasi baramasi *)* was collected from single farm of Budhiganga Gaupalika located at geographic coordinate 26°34'59.7"N 87°17'38.1"E Hathimudha in Morang district on the month of July 2021. Samples were refrigerated at 7.5±0.5°C for further work. Market sample of chuk amilo was bought from supermarket of Dharan.

3.1.2 Chemicals required, equipment and utensils required

Chemicals required, equipment and utensils required were obtained from CCT and that used during this work are listed on appendix B

3.2 Experimental plan

At first collected sample were weighted by using weighing balance and after that washed with potable water. Juice was squeezed using handheld lemon squeezer and strained using muslin cloth. Fresh lemon juice was analyzed. The fresh juice was concentrated to 50°BX using vacuum evaporator by varying temperature 50°C, 60°C, 70°C and open pan boiling and were named as sample A, sample B, sample C, sample D respectively. Time taken for concentrating was approx. 8.5 hrs. for sample A, 5.5 hrs. for sample B, 2.5 hrs. for sample C and 45 minutes for sample D. Sample E was Coded for market sample. Sample prepared were packed into glass bottle and stored at refrigerator. All the concentrated sample and market sample were diluted to 5°BX and analysis was carried out along with fresh sample. The experiment was done as shown in figure 3.1.

The general flow diagram of preparation of concentrates is shown in fig. 3.1

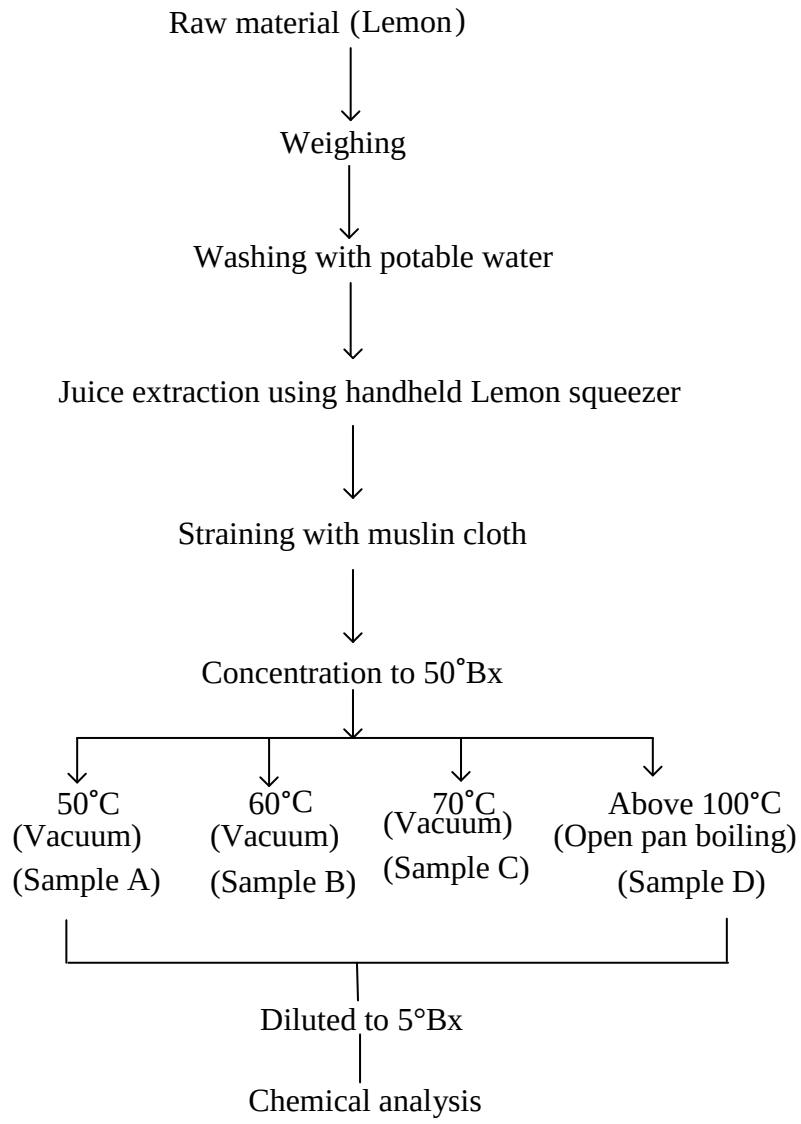

Fig. 3.1 Flow diagram for preparation of concentrate

3.2.1 Analytical methods

3.2.1.1 Determination of TSS

TSS of juice and product were determined by use of handheld Refractometer (optical Beer Brix– black RHB-32ATC).

3.2.1.2 Determination of juice Yield

The yield of juice was calculated by the ratio of amount of juice to amount of fruit.

3.2.1.3 Determination of pH

The pH of lemon was measured using a digital pH meter (HANNA HI 96017, Sensitivity ± 0.01) at 25°C.

3.2.1.4 Determination of physical parameters

Weight and diameter of lemon was measured using weighing balance and Vernier caliper respectively.

3.2.1.5 Determination of Browning index

5 mL of lemon juice sample was mixed with 5 mL ethyl alcohol (95 %) and centrifuged (4000 rpm, 10 min, at 4 °C). The supernatant was passed through a whatman No.42 filter and the absorbance of the supernatant was obtained at 420 nm in a Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO-291) (Meydav *et al.*, 1977).

3.1.1.6 Determination of total phenolic content

The total phenolic content of lemon juice extract was calculated using the Folin- Ciocalteu method, which was modified considerably (Abdullakasim *et al.*, 2007). For measurement of total phenolic content, 5 ml of lemon juice was mixed with 5 ml of 80% methanol and were centrifuged at 4000 rpm for 20 minutes at 4°C. For analysis, 100 µL of sample were mixed thoroughly with 100 µL of Folin- Ciocalteu reagent and 3000 µL of distilled water. After incubation for 10 min at room temperature, 100 µL of 20% Na_2CO_3 solution was added with intermediate mixing and was further incubated at room temperature for 2 hr. in dark a spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO-291) was used to detect the mixture absorbance at 765 nm. The total phenolic contents of the samples were reported in milligrams per L as Gallic acid equivalents (mgGAE/L), with Gallic acid as the standard.

3.1.1.7 Determination of antioxidant activity (DPPH free radical scavenging activity)

The antioxidant activity of the lemon juices was evaluated using the DPPH* free radical-scavenging method. DPPH* free radical-scavenging activity measurements were carried out

according to the procedure of (Klimczak *et al.,* 2007) with some modifications. 5 mL of lemon juices were mixed with 5 mL of methyl alcohol (80 %) and then centrifuged (4000 rpm, 10 min, at 4 °C). Briefly, 0.1 mL of supernatant was added to 2.46 mL of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH*; 0.025 gL^{-1} in 80 % methyl alcohol) and mixed by vortex. After incubating for 10 min in the dark, absorbance of the samples was measured at 515 nm using the spectrophotometer. Antioxidant activity was expressed as the percentage decline of the absorbance:

Antioxidant activity (%) = (A control – Asample)/A control*100

Where, $A_{control}$ was the absorbance of the control, and A_{sample} was the absorbance of the sample.

3.1.1.8 Determination total flavonoid content

Total flavonoid content is determined using a modified aluminium chloride assay method as described by (Barek *et al.,* 2015). 2ml of extract was mixed with 0.2ml of 5% $NaNO_3$ and stand for 1 min and 0.2 ml of 5% $AlCl_3$ and stand for 5 min after that 2 ml of 1N NaOH was added, volume was made up to 5ml using distilled water. After incubation for 15 min absorbance was measured at 510 nm against blank reagent in spectrophotometer. Standard curve was prepared and total flavonoid content was calculated from standard curve.

3.1.1.9 Total tannin content

The tannins are determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract is added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture is 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) are prepared in the same manner as described earlier. Absorbance for test and standard solutions are measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content is expressed in terms of mg of GAE /L of extract (Mythili *et al.,* 2014).

3.2.2 Sensory evaluation of lemon juice concentrate

Statistical analysis of the sensory scores was obtained from 14 semi-trained Panelist using 9- point hedonic rating scale (9=like extremely, 1= dislike extremely) for lemon juice concentrate. Sensory analysis was performed with the aid of different panelist evaluating

appearance, body, flavour, astringency and overall acceptance of lemon juice concentrate. For the sensory analysis of concentrate, the concentrate of lemon was diluted to 5° BX and analysis was carried out. From the sensory evaluation for appearance of the five different samples A, B, C, D and E of lemon juice concentrate. Here sample A, B, C, D are concentrated sample at 50°C, 60°C, 70°C and open pan boiling respectively. Sample E is the market sample.

3.3 Data analysis

Analysis of experimental data were done by using MS Excel (2013) for bar-diagram GenStat 12th edition, for one way ANOVA at 5% level of significance.

Part IV`

Results and discussion

This work was done in order to prepare concentrates with different processing temperatures and to analyze effect of temperature on bioactive composition. Juice concentrates from lemon juice were made in a rotary vacuum evaporator at 50°C, 60°C, and 70°C, as well as in open pan by open pan boiling method. Lemons were initially exposed to preliminary and physiochemical testing.

4.1 Preliminary analysis of lemon

Preliminary analysis of lemon and lime fruit as obtained in laboratory are tabulated in the table 4.1

Table 4.1 Preliminary analysis of lemon

Physical parameters	**Lemon**
Weight (g)	102.3±12.68
Diameter (cm)	5.38±0.46
Length (cm)	5.99±0.43
Juice Yield (%)	26.96±2.93

* Values presented are the means ± standard deviation.

Weight of lemon was found to be 102.3 gram on average which was slightly higher than the values found by Sadat *et al.* (2012) which is in range of 50-80 gram. Ghimire (2020) found the average weight of lemon as 104 gram this result is supported by this value. The juice yield of lemon is found to be 26.96% which is lesser than the result found by Al Juhaimi and Ghafoor (2013) which is 42.83% the juice yield depends on varieties and geographical location.

4.2 Physiochemical parameters of raw juice

The physiochemical parameters of raw juice is given in table 4.2

Table 4.2 physiochemical parameters of raw juice

Sample/Parameters	Lemon
Acidity % (as citric acid)	5.02±0.20
pH	2.2±0
TSS (°Bx)	5.0
Ascorbic acid (mg/L)	455.0±3.862
Total polyphenol content (mg/L)	926.63±2.164
Total tannin (mg/L)	510.61±0.934
Total flavonoids (mg/L)	320.22±0.88
Antioxidant activity (DPPH scavenging activity)	81.01%
Browning Index (A_{420nm})	0.268

* Values presented are means ± standard deviation.

The acidity of lemon juice was found to be 5.02% which is in accordance of the values found by Penniston *et al.* (2008) which is 4.8 %. Total soluble solid, ascorbic acid of fresh lemon juice was in line with several research works carried out. The total phenolic content of fresh lemon juice was found to be 926.63 mg GAE/L which is in the range that is found by Xu *et al.* (2008) in total phenolics in juices from 15 citrus varieties (seven mandarins, four sweet oranges, one lemon, one grapefruit, and two pummeloes) of China were in the range of 751.82–1555.49 mg GAE/L. Total phenolic content of lemon juice was reported as 751.82 mg GAE/L. Hussain *et al.* (2011) also studied on total phenolics in juices from four different varieties of citrus lemon, indigenous to Pakistan and the total phenolic contents from juices of citrus lemon following the Folin-Ciocalteu assay were in the range of 690.62–998.29 mg/L. Total tannin present in fresh lemon juice was found to be 510.61±0.934 mg/L which less than the value found by Sindhu and Khatkar (2018) where the concentration of tannin in fresh lemon juice was 52.55 mg/100ml. Browning index of lemon juice was measured as optical density at 440nm and it was 0.268. Total flavonoids in fresh lemon juice was found to be 320.22 mg/L which is greater than the value found by Sicari *et al.* (2016).

DPPH free radical scavenging activity of lemon juice was found to be 81.01%. Antioxidant activity of lemon juice was found 81.20% by (Uçan *et al.*, 2016). Munwar *et al.* (2015) found 76.28% free radical scavenging activity of *Citrus medica*. Choi *et al.* (2000) found 69.31% Radical-scavenging activities of essential oils of orange.

4.3 Effect of processing conditions on lemon juice concentrates

4.3.1 Effect on Browning

The Browning value increased from 0.268 to 1.13. It was found that the browning index of the raw juice was 0.268, which increased to 0.327, 0.526, 0.764, and 1.13 at processing temperatures of 50 ° C, 60 ° C, 70 ° C and open pan boiling, respectively. Uçan *et al.* (2016) found browning index values of extraction, pulp removing and pasteurization samples were determined as absorbance 0.132, 0.127 and 0.142, respectively which is less than this result. Browning at difference processing conditions of lemon presented in Fig. 4.1

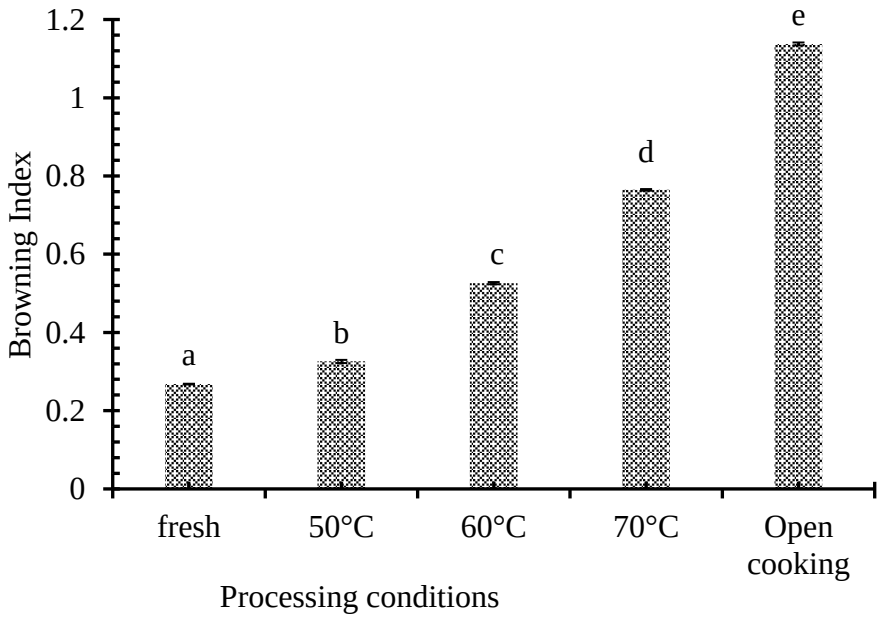

Fig. 4.1 Effect of different processing conditions on browning

[Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different (p>0.05) at 5% level of significance.]

Thermal treatment is used to preserve fruit derivatives in the production process. The negative effects of thermal treatments include a non-enzymatic browning, the loss of nutrients and the formation of unwanted products, such as 5-hydroxyMethylfurfurfurs (5-HMF). Browning's non-enzyme reactions mainly cause color changes, loss of sugar and vitamin C and 5-HMF formation, affecting the quality of fruit juices (Ibarz *et al.*, 1999). The reason for increasing browning is explained by Kus *et al.* (2005) where at the high evaporation temperatures, sucrose, glucose, fructose, and sometimes maltose, undergo changes. In highly acidic medium (pH < 3), transformation of fructose to hydroxymethyl furfural can occur. Hydroxymethyl furfural polymerizes or polycondenses with other compounds, forming dark-colored substances and in less acidic medium (pH > 3.5), glucose and fructose can form brown-colored substances by reacting with free amino acids present in the juices through non-enzymatic browning or Maillard-reaction.

To control browning and to preserve the quality of the juice, main factor involved should be identified for the design process and to optimize process conditions adequate kinetic models of the reactions are required (Damasceno *et al.*, 2008).

4.3.2 Effect on total phenolic content

The total phenolic content of concentrate significantly ($p<0.05$) decreases from raw juice. It decreases from raw 926.63 mg/L to 610.71 mg/L at 50^{o}C, 757.20 mg/L at 60^{o}C, 711.53 mg/L at 70^{o}C and 578.98 mg/L on open pan boiling respectively. All the concentrate has lower value of TPC than that of fresh juice. It may be due to heat processing which causes degradation of TPC. Also, the decrease in total phenolic contents after drying might be due to the binding of polyphenols with other compounds (proteins) or the alterations in the chemical structures of polyphenols (Qu *et al.*, 2010). The maximum polyphenol content was found in the concentrate processed at 60^{o}C and lowest TPC was found on open pan boiling. Similar kind of pattern was found by (Lopez *et al.*, 2017) in murta berries during vacuum drying at 50 ^{o}C, 60 ^{o}C and 70 ^{o}C.

At 50 ^{o}C much more reduction on total phenolic content was due to the longer drying time at lower temperature it was also stated by Madrau *et al.* (2009) in pomegranate arils during drying. They reported that the degradation of polyphenol content and loss of antioxidant activity was higher at 55°C than at 75°C drying temperature. The authors stated that a longer drying time caused a greater loss of phenolic compounds at a lower temperature. The effect

of different processing condition on total phenolic content of lemon juice concentrate is shown in Fig. 4.2

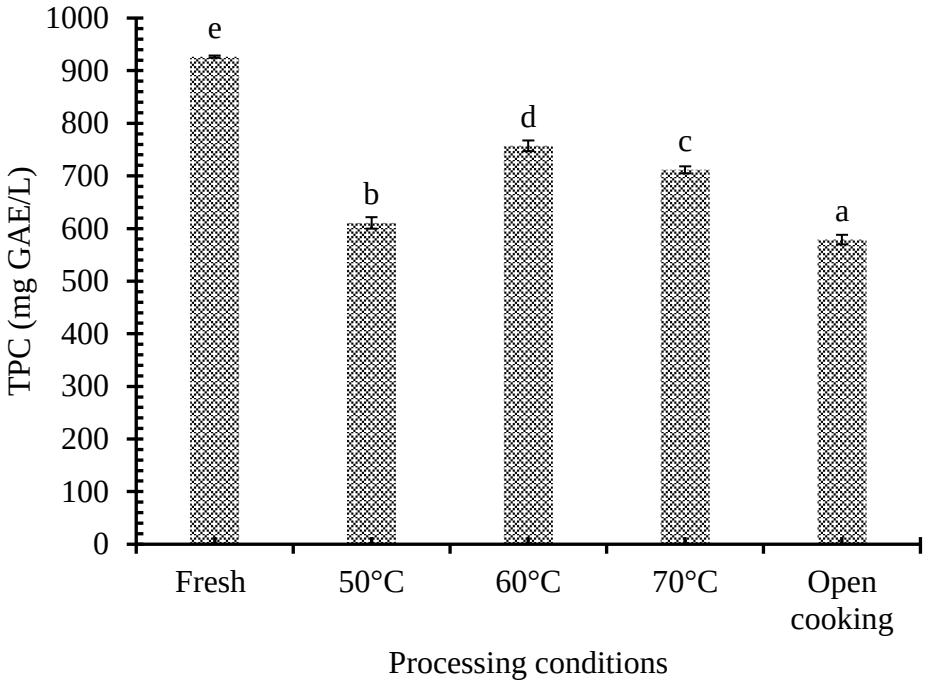

Fig. 4.2 Effect of processing conditions on total phenolic content

At 50^{o}C the average total phenolic content retention of lemon juice concentrate was found to be 65.92%, at 60^{o}C found to be 81.7331% and 76.80% at 70^{o}C and 62.49% on open pan boiling respectively. Elik *et al.* (2016) found phenolics content retained by 65.8% the end of concentration process by rotary vacuum concentration at 45^{o}C at 65 ^{o}Bx and 63.46% by open pan concentrating process of blueberry juice at 65 ^{o}Bx, here 66.07% found in 50^{o}C concentrated to 50 ^{o}Bx in vacuum evaporator it may be due to lesser TSS and more time for making concentrate.

Minimum TPC was found in open pan boiling it may be due to the presence of oxygen and high temperature processing which cause oxidation and degradation of total phenolic content.

4.3.3 Effect on flavonoid content

The total flavonoid content of lemon juice concentrate concentrated at 50°C was found to be 222.86 mg/L, 241.23 mg/L at 60°C, 232.93 mg/L at 70°C and 227.0142 mg/L on open pan boiling. Higher amount of total flavonoid was found in fresh juice i.e. 320.22 mg/L and

decreases in all the concentrate. Among the concentrate higher amount of flavonoid was found at 60°C and lower at open pan boiling. Similar kind of pattern was found by Lopez *et al.* (2017) in murta berries during vacuum drying at 50 ^{o}C, 60 ^{o}C and 70 ^{o}C. These findings can be explained by the longer times required by lower drying temperatures, thus the leaves had longer exposure to heat, which resulted in degradation of TPC and TFC, which were unstable. Similar findings were observed by (Vu *et al.*, 2017) during drying of banana (*Musa cavendish*) peels. The effect of different processing conditions on total flavonoid content of lemon juice concentrate is shown in Fig. 4.3

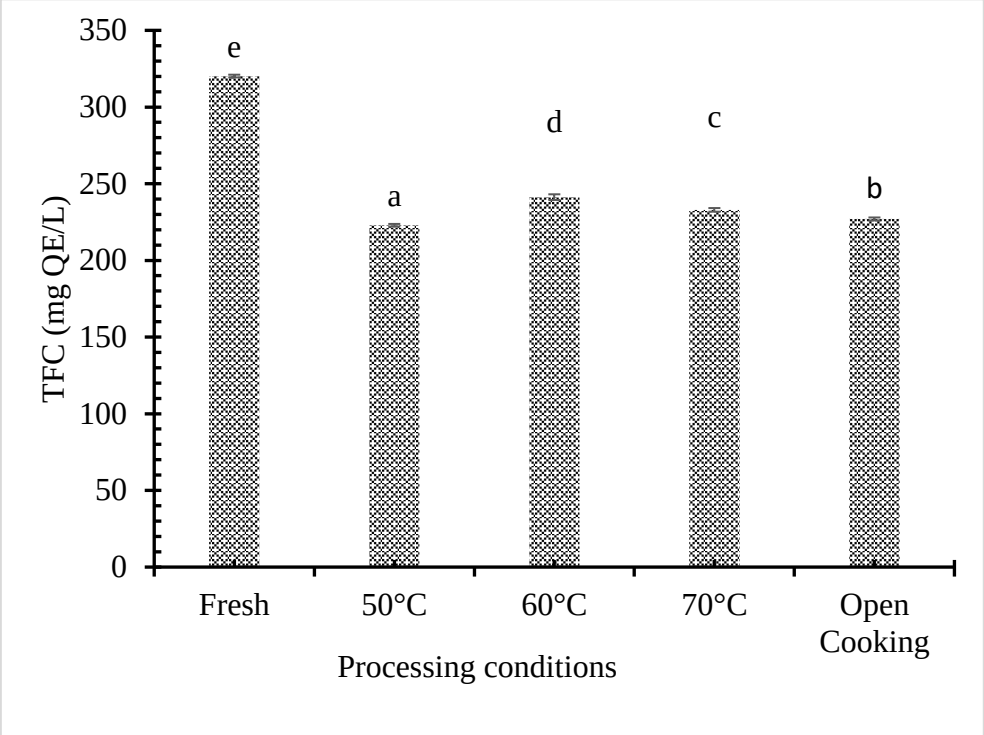

Fig. 4.3 Effect of processing conditions on total flavonoid content

At 50^{o}C the average total flavonoid content retention of lemon juice concentrate was found to be 69.59 %, at 60^{o}C found to be 75.33 % and 72.74 % at 70^{o}C and 70.8932 % on open pan boiling respectively. Mohd Zainol *et al.* (2009) found the loss of flavonoid 63 to 87 % in root, leaves and petiole of *Centella asiatica* dried at 45 ^{o}C in vacuum. According to Schieber *et al.* (2001), the loss of macromolecules like flavonoid during heat treatment might be due to the harsh drying conditions, in particular, the temperature and duration used. Similarly, Davey *et al.* (2000) reported that wet thermal processing can affect the phytochemicals by thermal breakdown that affect the integrity of the cell structure which

then resulted in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen.

4.3.4 Effect on tannin content

The tannin content of lemon juice concentrate decreases from raw juice 510.61 mg/L to 454.84 mg/L at 50°C, 485.96 mg/L at 60°C, 465.28 mg/L at 70°C and 406.60 mg/L on open pan boiling. The effect of different processing conditions on tannin content of lemon juice concentrate is shown in Fig. 4.4

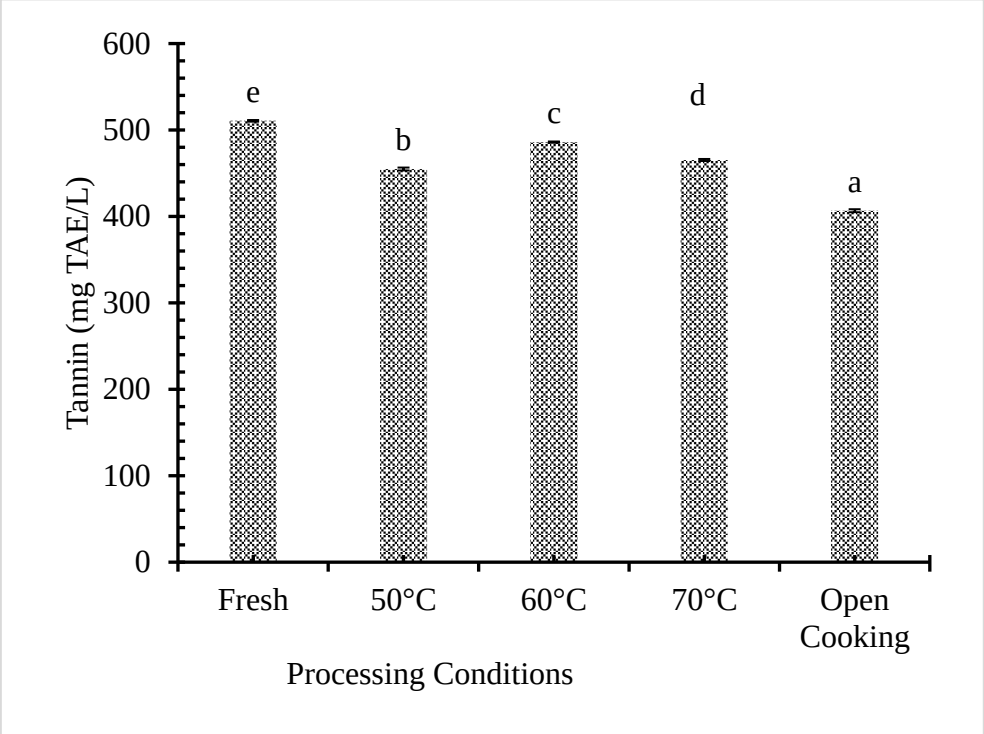

Fig. 4.4 Effect of processing conditions on tannin content

All the concentrated prepared has the lowest amount of tannin than that of fresh juice. The tannin content of juice concentrated has decreased at 50° Bx and gradually increased up to 60° Bx and lowest amount of tannin was found in open pan boiling. The heat possibly has the ability to enhance the recovery of phenolic compounds whereby higher temperatures of extraction is able to exhibit higher amount of phenolic compounds significantly in extracting both Indian medicinal plant and mashua respectively (Lim and Murtijaya, 2007; Silva *et al.*, 2007). This was further clarified by scientific literature conducted by (Al-Farsi and Lee, 2008) in which increment of extraction temperatures promote high extraction of active compounds especially those from phenolic groups whereby the increase of diffusion

coefficient and its solid to solvent solubility are also increased that lead to greater extraction of active compounds. Prolonged exposure or treatment with high temperatures could also lead to active compounds degradation (Wang *et al.*, 2007). Some compounds could not withstand high temperatures which will lead to deterioration of their active compounds. This matter could also explained the reduction of tannin content at open pan boiling, the total amount of tannin reduced gradually.

Retention of tannin on the concentrate prepared at 50°C was 89.063%, at 60°C 95.15%, at 70°C 91.10% and at open pan boiling 79.91%. Highest amount off tannin was retained on the concentrate prepared at 60°C and lowest at open pan boiling. During research conducted by (Kaul and Saini, 2000b) they found that tannin content was 27.13 mg/100 g in final concentrate which was prepared from juice of kagzi lime 28.53 mg/100 g at TSS 48.77° Bx. The retention of tannin on that research was 95.05% in that research which somehow support our research lesser retention may be due to the ° Bx of concentrate and temperature.

4.3.5 Effect on antioxidant activity (DPPH free radical scavenging activity)

To study the effect of temperature on then antioxidant activity of lemon juice, juice was concentrated to 50° Bx at the temperature of 50°C, 60°C, 70°C and open pan boiling. All the concentrated sample was diluted to 5° Bx and analysis was carried out. The antioxidant activity of lemon juice was found to be 81.01%. The antioxidant activity of lemon juice concentrate concentrated at 50°C was found to be 70.87%, 74.47% at 60°C, 70.45% at 70°C and 64.09% on open pan boiling. Higher amount of antioxidant activity was found in fresh juice and decreases in all the concentrate. Among the concentrate higher amount of flavonoid was found at 60°C and lower at open pan boiling. Among vacuum evaporated sample concentrate at 50 ^{o}C has lowest and 60^{o}C has greater amount of antioxidant activity. Phenolic compounds are important compounds due to their antioxidant properties, which facilitate the removal of free radicals and prevent the conversion of hydro peroxides to free radicals (Jimoh *et al.*, 2008). The effect of different processing conditions on antioxidant activity of lemon juice concentrate is shown in Fig. 4.5

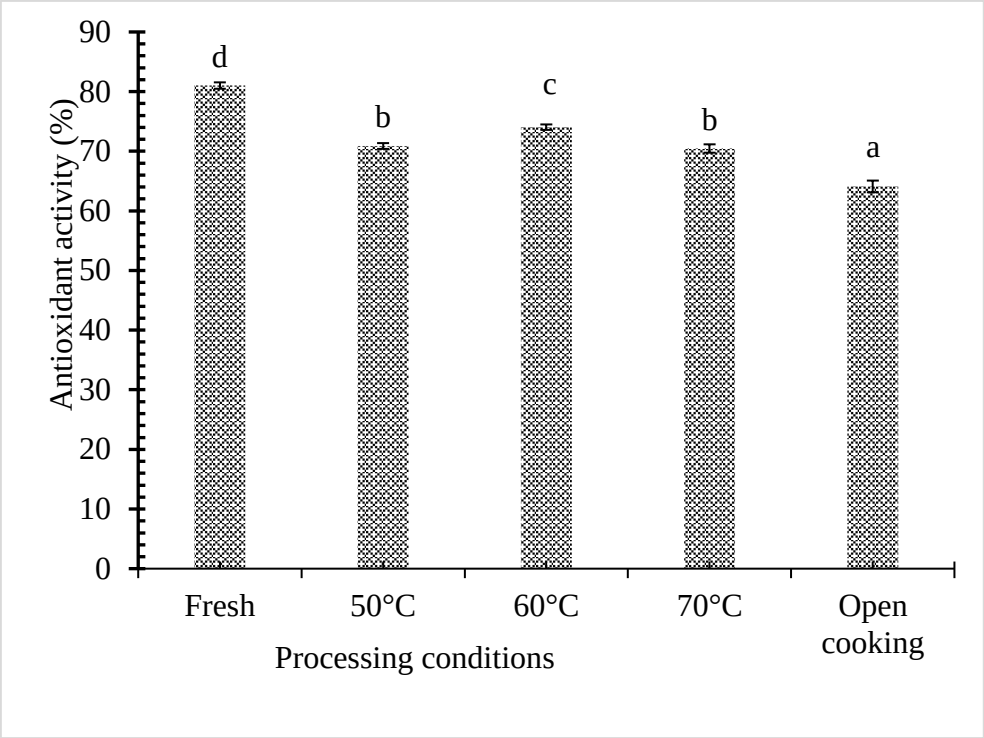

Fig. 4.5 Effect of processing conditions on Antioxidant activity

Several authors have observed a direct correlation between polyphenolic content and antioxidant activity (Rice-Evans *et al.*, 1997). In addition, Nicoli *et al.* (1999) indicated that the decrease in antioxidant activity due to drying was related to the degradation of biologically active compounds at high temperatures from chemical, enzymatic, or thermal decomposition. Evidently, the drying process would generally result in a depletion of naturally occurring antioxidants in raw materials from plants. Intense and/or prolonged thermal treatment may be responsible for a significant loss of natural antioxidants, as most of these compounds are relatively unstable (Gupta *et al.*, 2011).

4.4 Sensory evaluation of lemon juice concentrate

Statistical analysis of the sensory scores was obtained from 14 semi-trained Panelist using 9- point hedonic rating scale (9=like extremely, 1= dislike extremely) for lemon juice concentrate.

4.4.1 Appearance

The mean sensory score for appearance of sample B was found to be 7.8 and was highest of all other concentrated sample. The lowest score was 6.53 for sample D. Among all the samples sample A, D, E has no significant difference on appearance for Panelist. Sample B

is different from other sample and sample C has the appearance as sample A, B, D, and E respectively. The similar alphabets in the bar diagram i.e. Fig. 4.6 indicates that the samples are not significantly different ($p>0.05$) at 5% level of significance. Statistical analysis showed that there is significant difference ($p<0.05$) within the samples at 5% level of significance.

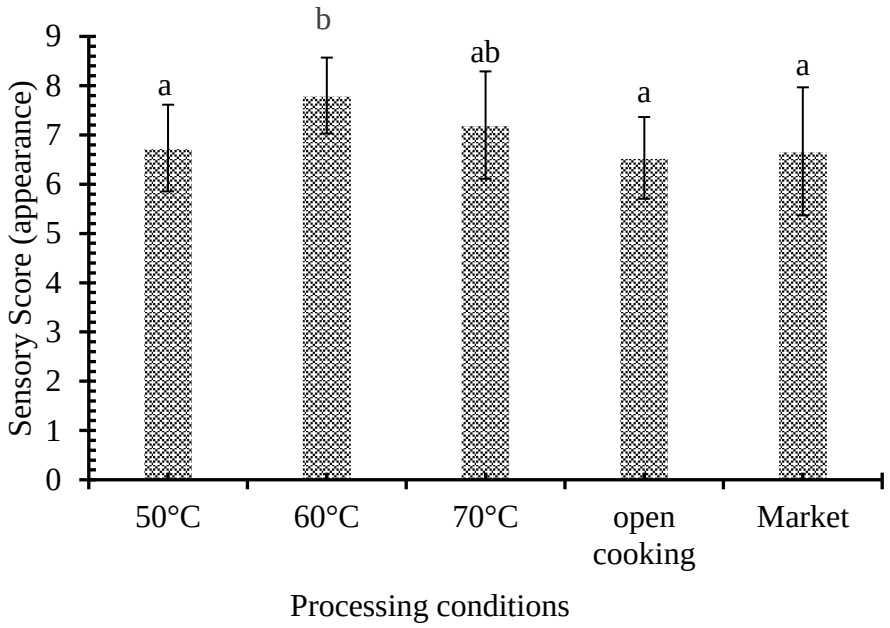

Fig. 4.6 Effect of processing conditions on appearance of lemon juice concentrate

*Bars sharing similar letters are not significantly different by LSD at $p>0.05$. Vertical error bars represent standard deviation of scores given by Panelist.

4.4.2 Body

The mean sensory score for body of sample A was found to be 7.07 and was highest of all the concentrated sample. The lowest score was 6.73 for sample D. The TSS of the sample was maintained at 5 °BX that might be the reason for all the sample with no difference on body. The similar alphabets in the bar diagram i.e. Fig. 4.7 indicates that the samples are not significantly different ($p>0.05$). Statistical analysis showed that there is no significant difference ($p>0.05$) within the samples at 5% level of significance.

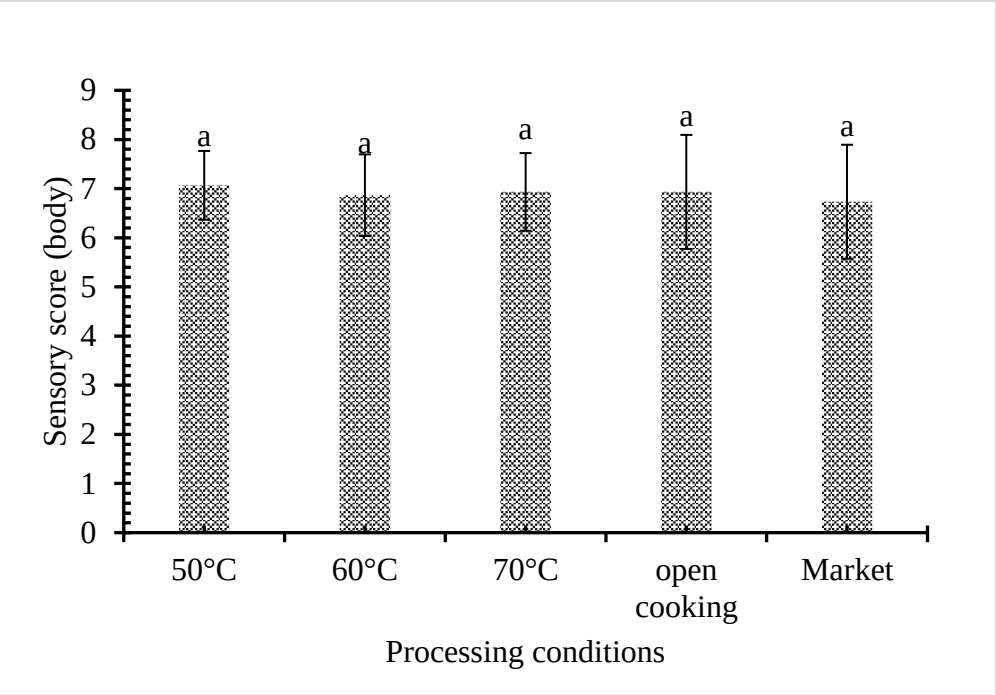

Fig. 4.7 Effect of processing conditions on body of lemon juice concentrate

*Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by Panelist.

4.4.3 Flavour

The mean sensory score for flavour of sample C was found to be 7.47 and was highest of all other concentrated sample. The lowest score was 6.33 for sample E sample. Among all the samples sample A, B and D has no significant difference and sample C and E are different from each other. The similar alphabets in the bar diagram i.e. Fig. 4.8 indicates that the samples are not significantly different (p>0.05) at 5% level of significance. Statistical analysis showed that there is significant difference (p<0.05) within the samples at 5% level of significance. LSD shows that sample A, B and D are same and sample C and E are different.

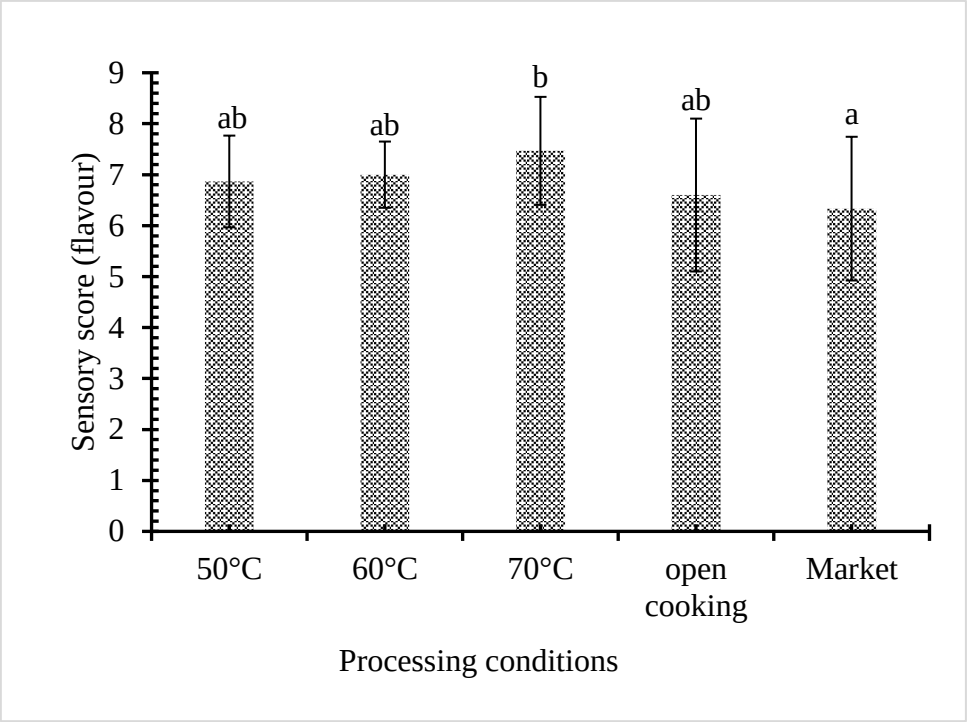

Fig. 4.8 Effect of processing conditions on flavour of lemon juice concentrate

*Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by Panelist.

4.4.4 Astringency

The mean sensory score for astringency of sample C was found to be 7.4 and was highest of all other concentrated sample. The lowest score was 6.2 for sample E sample. The similar alphabets in the bar diagram i.e. Fig. 4.9 indicates that the samples are not significantly different (p>0.05) at 5% level of significance. Statistical analysis showed that there is significant difference (p<0.05) within the samples at 5% level of significance. LSD shows that sample B, C and D are same E is different and sample A is same as B, C, D and E.

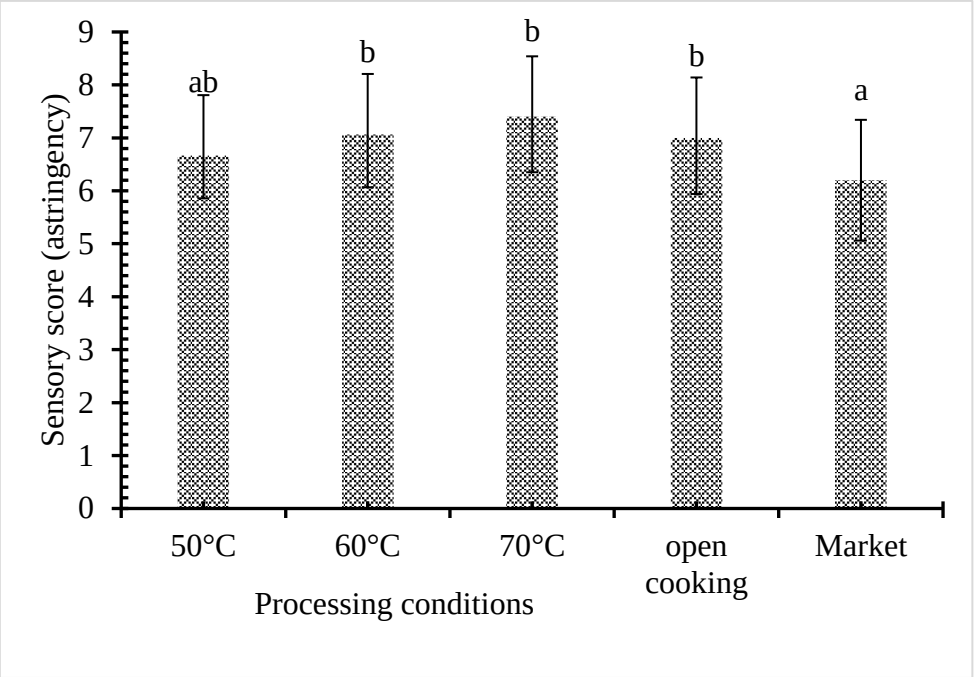

Fig. 4.9 Effect of processing conditions on astringency of lemon juice concentrate

*Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by Panelist.

4.4.5 Overall acceptance

The similar alphabets in the bar diagram i.e. Fig. 4.10 indicates that the samples are not significantly different (p>0.05) at 5% level of significance. Statistical analysis showed that there is significant difference (p<0.05) within the samples at 5% level of significance. LSD shows that sample A, B and D are same and sample C and E are different. The mean sensory score for appearance of sample C was found to be 7.47 and was highest of all other concentrated sample. The lowest score was 6.33 for sample E sample. Among all the samples sample A, B and D has no significant difference and sample C and E are different from each other.

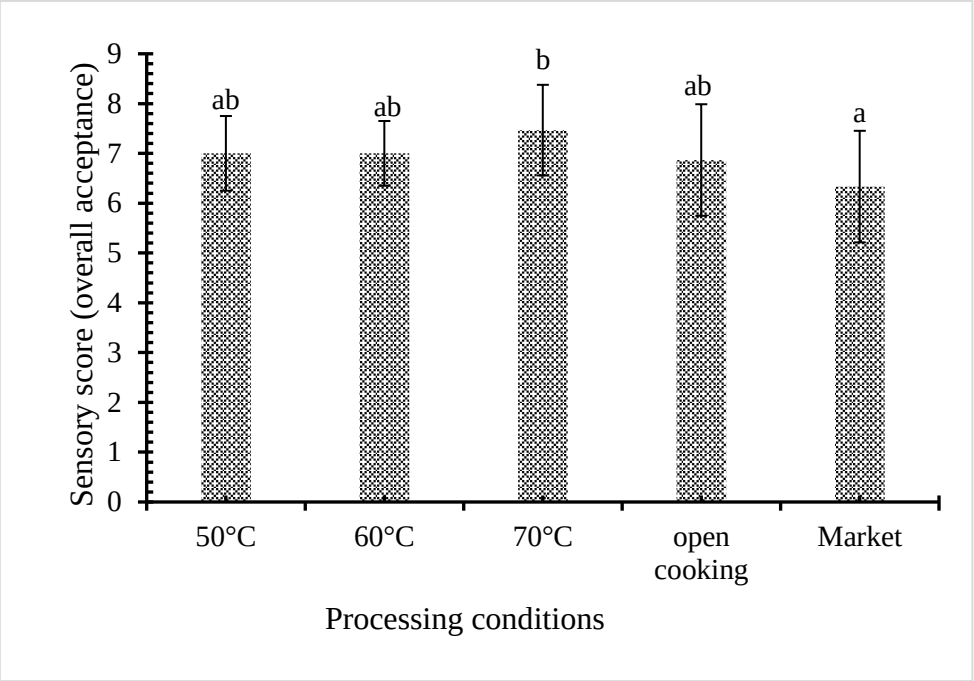

Fig. 4.0 Effect of processing conditions on overall acceptance of lemon juice concentrate

*Bars sharing similar letters are not significantly different by LSD at p>0.05. Vertical error bars represent standard deviation of scores given by Panelist.

Among all the sample samples sample C is found to be best sample which is vacuum concentrated sample at 70 °C.

4.5 Analysis on market sample

Market sample was analysed in lab as the concentrated sample by diluting to 5°Bx and certain results were found as shown in table 4.3

Table 4.3 physiochemical parameters of market sample

Parameters	**Market sample**
TSS	15°Bx
Acidity	10.85±0.27%
Total phenolic content	513.75± 0.522 mg/L GAE
Total tannin content	379.86±0.590 mg/L TAE
Total flavonoid content	219±0.5585 mg/L QE
Antioxidant activity	58.45±1.273%
Browning index (BI)	1.9533±0.009

* Values presented are means ± standard deviation.

From the analysis of market sample all the bioactive components and antioxidant activity was lesser that that present on vacuum evaporated sample ita may be due to the uncontrolled heating and time of treatment. From analysis it is also concluded that due to the loss in bioactive components market sample of concentrate has less nutrient than in vacuum evaporated sample. That's why sample prepared on vacuum evaporator is best for preservation of bioactive components.

4.6 Physiochemical parameters and bioactive components of all concentrated sample

Physicochemical parameters and bioactive compounds of vacuum evaporated sample and open pan boiled lemon juice concentrates (Chook amilo) in the laboratory are tabulated in Table 4.4.

Table 4.4 Physicochemical parameters and bioactive compounds of all concentrated sample

Parameters	Sample A 50°C	Sample B 60°C	Sample C 70°C	Sample D (Open pan boiling)
TSS (°Bx)	50	50	50	50
Acidity (%)	44.42±0.71	42.58±0.51	43.12±0.32	41.38±0.68
Total phenolic content (mg GAE/L)	610.71±10.8	757.20±10.16	711.53±6.6	578.98±9.06
Total tannin content (mg TAE/L)	454.84±1.68	485.96±0.72	465.28±1.1	406.60±1.72
Total flavonoid content (mg QE/L)	222.86±0.90	241.4297±1.86	232.93±1.1	227.01±0.96
Antioxidant activity (%)	70.87±0.48	74.47±0.45	70.45±0.70	64.09%
Browning index	0.32	0.526±0.002	0.76±0.003	1.13

From diluted sample of concentrate all the analysis was carried out and data was found as shown in table 4.4. The total phenolic content, tannin content, flavonoid content, antioxidant activity decreased from raw juice as temperature increased and at 50°C lowest amount of all the bioactive components were found this may be due to longer time taken for concentrating juice. It was also stated by Madrau *et al.* (2009) in pomegranate arils during drying. They reported that the degradation of polyphenol content and loss of antioxidant activity was higher at 55°C than at 75°C drying temperature. The authors stated that a longer drying time caused a greater loss of phenolic compounds at a lower temperature. Browning index of concentrates goes on increasing from raw juice as the temperature of evaporation increased it may be due to the formation of colored compounds due to millard browning.

Part V

Conclusions and recommendations

5.1 Conclusions

In this study Lemon juice concentrate was prepared at different processing temperature 50^{o}C, 60^{o}C, 70^{o}C (under vacuum at rotary vacuum evaporator) and open boiling method and variation in bioactive components of concentrate was analyzed in the lab. Following conclusions can be drawn from this work.

i. All the bioactive components decreased from raw juice as temperature increased. Concentrate prepared at 60°C was found to be best in retaining bioactive components.

ii. From the sensory analysis it was shown that temperature of evaporation has no effect on sensory quality of lemon juice concentrate.

iii. Market sample was found to be poor quality in terms of bioactive components and sensory quality.

5.2 Recommendations

Based on the present research work, the following recommendations could be made for further study:

i. Chuk amilo with high level of bioactive components and with good sensory profile can be prepared using vacuum evaporation.
ii. Other bioactive components such as anthocyanins, betalains, plant sterols, carotenoids and glucosinolates could be studied.

Part VI

Summary

Citrus fruits are also known to contain bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils which are believed to be responsible for a range of protective health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities (Ramful *et al.*, 2011).

In this study, Lemon (*Citrus limon* madrasi baramasi) was collected from Budhiganga Rural Municipality, Morang and their juice concentrate was prepared at different processing temperature under vacuum (50^oC, 60^oC and 70^oC) and open pan cooking method. The effect of temperature on bioactive components (total phenolic content, tannin content, flavonoid content, antioxidant activity and browning) of juice during preparation of concentrate was investigated.

The mean values of TSS, acidity, ascorbic acid, browning for lemon was found to be 5.0^oBx, 5.02%, 455 mg/L, and 0.268. Optimal temperature of 60^oC of was found to be most effective on preserving juice quality of lemon juice concentrate. For all bioactive components there was significant difference ($p<0.05$) in its properties due to difference in evaporating temperature. From sensory analysis sample C i.e. sample concentrated at $70\ ^oC$ under vacuum was found to be best sample. Analysis of market sample shows that all the bioactive components are degraded during processing and it has low amount of antioxidant capacity.

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Appendices

Appendix A

Scorecard: Hedonic rating scale

Panelist No. ……. Name:
……...…….

Please evaluate the lemon juice concentrates samples and indicate how much you like or dislike it for Appearance, Body, Flavour, Astringency and overall acceptability on 1-9 ranking scale.

1	2	3	4	5	6	7	8	9
dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely

Sample	Appearance	Body	Flavour	Astringency	Overall acceptance
A					
B					
C					
D					
E					

Comments if any:
……

Signature

Appendix B

Chemicals required

i. NaOH (HIMEDIA- GRM1183, Assay 97.00-103.50 %)

ii. Oxalic acid (Qualigens, Assay 99.5%)

iii. Indicators (Methyl blue, Phenolphthalein)

iv. Absolute Alcohol (Bengal Chemicals and pharmaceuticals)

v. Na_2CO_3 (Qualigens, Assay 99-101%)

vi. 2,4-dichlorophenol indophenol dye (HIMEDIA-RM350)

vii. Folin-Ciocalteuphenol reagent (FC reagent)

viii. $Alcl_3$

ix. Ascorbic acid (s.d fine-CHEM Limited, Assay 99.0%),

x. 2, 2-Diphenyl-1- picrylhydrazyl (DPPH), etc.

Equipment required

i. Water bath (Intake Serological Water Bath)

ii. Rotary vacuum evaporator (IKA® RV 10 Model-HB 10 D S96)

iii. Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO-291)

iv. Weighing Balance (AMPUT Electronic Balance Model No-457, Sensitivity ± 0.01g)

v. Refractometer (Portable Refractometer Optical Beer Brix– black RHB-32ATC)

vi. pH meter (HANNA HI 96017, Sensitivity ± 0.01)

vii. Heating arrangement, etc.

Glassware and utensils required

Beakers, conical flask, volumetric flasks, burette, pipettes**,** test tubes, stands**,** filter paper**,** filter aids**,** etc.

Appendix C

ANOVA result for analysis of bioactive components of lemon juice concentrate

Table B.7 One way ANOVA (no blocking) for total phenolic content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Processing Temperature	4	455649.14	113912.28	1608.00	<.001
Residual	25	1771.02	70.84		
Total	29	457420.16			

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

Table B.8 One way ANOVA (no blocking) for total tannin content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Processing Temperature	4	36198.030	9049.507	4366.67	<.001
Residual	25	51.810	2.072		
Total	29	36249.840			

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

Table B.9 One way ANOVA (no blocking) for browning index

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Processing Temperature	4	3.03975853	0.75993963	72884.24	<.001
Residual	25	0.00026067	0.00001043		
Total	29	3.04001920			

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

Table B.10 One way ANOVA (no blocking) for total flavonoid content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Processing Temperature	4	39323.853	9830.963	5528.74	<.001
Residual	25	44.454	1.778		
Total	29	39368.307			

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

Table A.4 One way ANOVA (no blocking) for Antioxidant activity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Processing Temperature	4	920.0543	230.0136	438.13	<.001
Residual	25	13.1248	0.5250		
Total	29	933.1791			

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

ANOVA result for sensory analysis of concentrate

Table A: Mean sensory scores for different attributes

Samples	Quality attributes				
	Appearance	Body	Flavor	Astringency	Overall Acceptance
A	6.73^{a}(0.88)	7.07^{a}(0.70)	6.87^{ab}(0.9)	6.67^{ab}(0.81)	7^{ab}(0.75)
B	7.8^{b}(0.77)	6.87^{a}(0.83)	7^{ab}(0.65)	7^{b}(1)	7^{ab}(0.65)
C	7.2^{ab}(1.09)	6.93^{a}(0.79)	7.47^{b}(1.06)	7.4^{b}(1.05)	7.47^{b}(0.91)
D	6.53^{a}(0.83)	6.93^{a}(1.16)	6.6^{ab}(1.50)	7^{b}(1.06)	6.87^{ab}(1.12)
E	6.67^{a}(1.3)	6.73^{a}(1.16)	6.33^{a}(1.41)	6.2^{a}(1.14)	6.33^{a}(1.12)

Figures in the parenthesis are the standard deviation.

Table B.1 Two way ANOVA (no blocking) for appearance

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Sample	4	16.1867	4.0467	5.27	0.001
Panelist	14	21.7867	1.5562	2.03	0.032
Residual	56	43.0133	0.7681		
Total	74	80.9867			

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

Table B.2 Two way ANOVA (no blocking) for body

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Sample	4	0.8800	0.2200	0.48	0.754
Panelist	14	37.5467	2.6819	5.79	<.001
Residual	56	25.9200	0.4629		
Total	74	64.3467			

Since, F pr. > 0.05, there is no significant difference between the sample at 5% level of significance so LSD testing is not necessary.

Table B.3 Two way ANOVA (no blocking) for flavor

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Sample	4	10.987	2.747	2.52	0.051
Panelist	14	37.387	2.670	2.45	0.009
Residual	56	61.013	1.090		
Total	74	109.387			

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

Table B.4 Two way ANOVA (no blocking) for astringency

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Sample	4	12.0533	3.0133	5.71	<.001
Panelist	14	43.7867	3.1276	5.93	<.001
Residual	56	29.5467	0.5276		
Total	74	85.3867			

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

Table B.5 Two way ANOVA (no blocking) for overall acceptance

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Sample	4	9.8667	2.4667	3.82	0.008
Panelist	14	28.6667	2.0476	3.17	0.001
Residual	56	36.1333	0.6452		
Total	74	74.6667			

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

Appendix D

Standard curve of gallic acid is shown in figure D.1

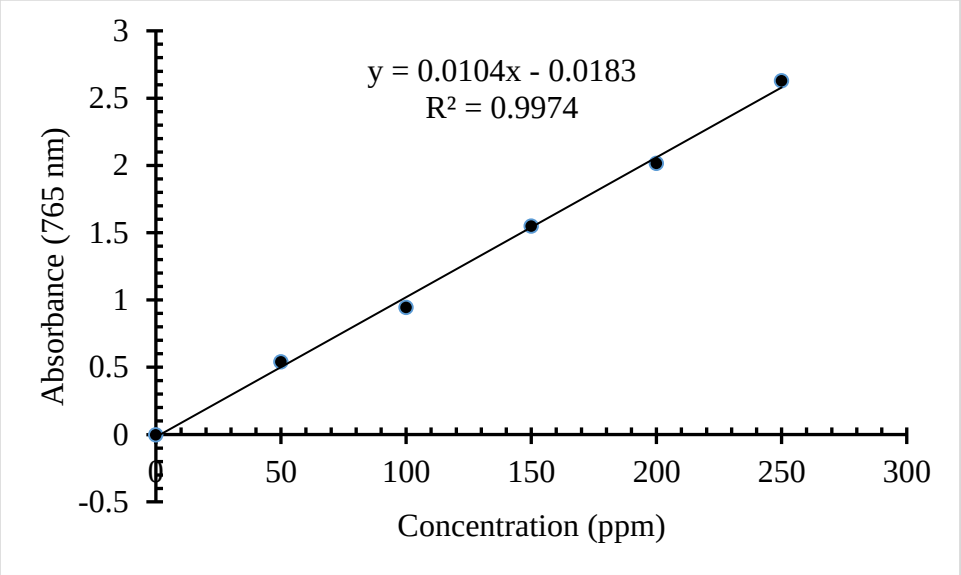

Fig: Standard curve of gallic acid

Standard curve of tannic acid is shown in figure D.2

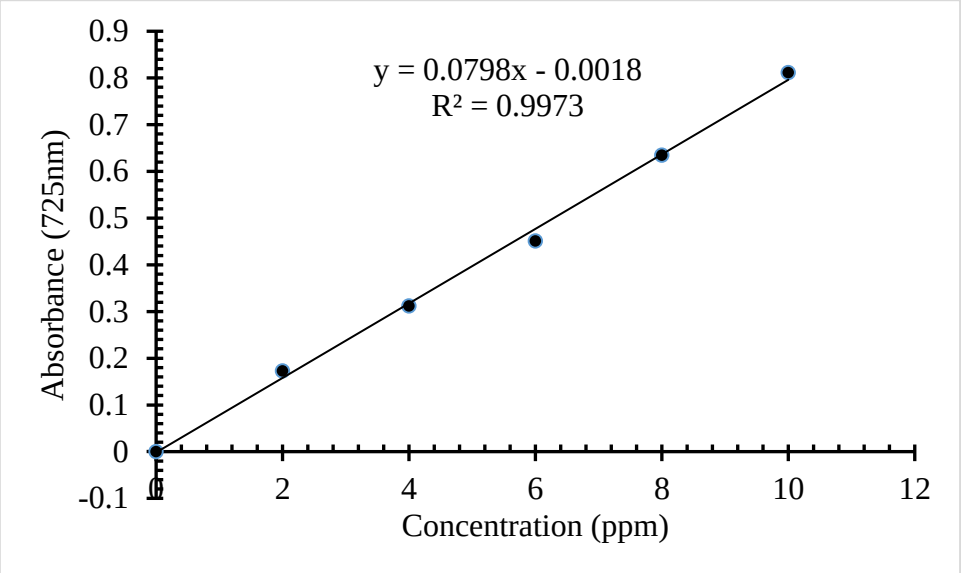

Fig: Standard curve of tannic acid

Standard curve of quercetin is shown in figure D.3

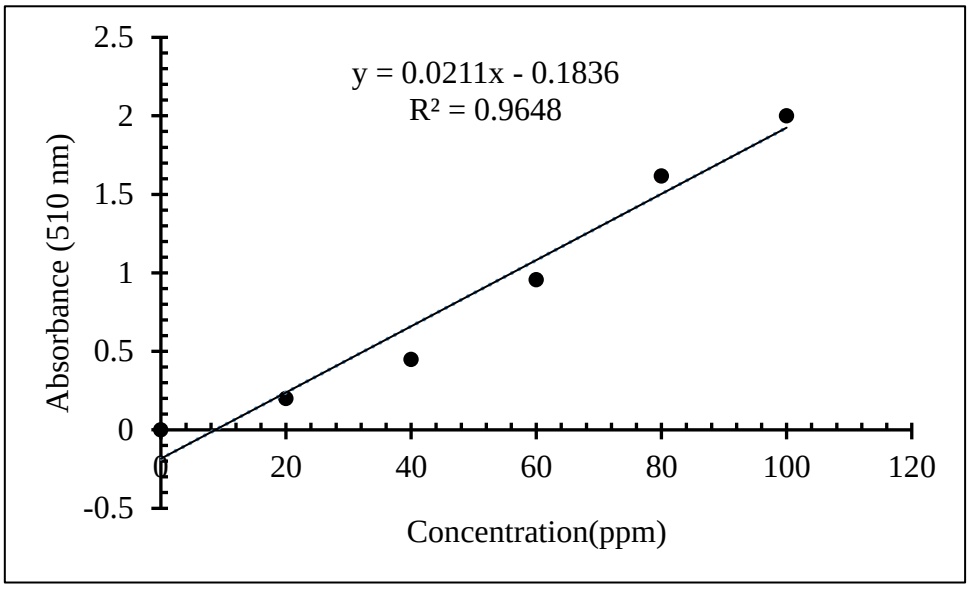

Fig: Standard curve of quercetin

Color plates

Plate 1 Field visit for sample collection

Plate 2 Lemon sample for analysis

Plate 3 Juice squeezing for making

Plate 4 making concentrate at 70°C

Plate 5 making lemon juice concentrate

Plate 6 concentrates ready for analysis

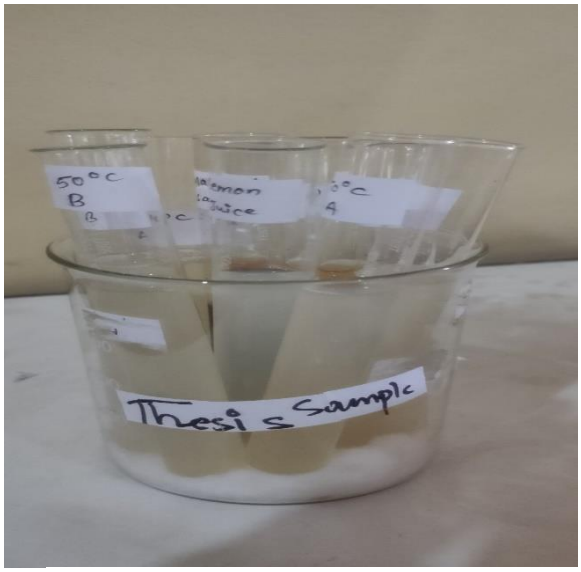

Plate 7 diluted concentrated sample

Plate 8 spectrophotometric analysis of sample

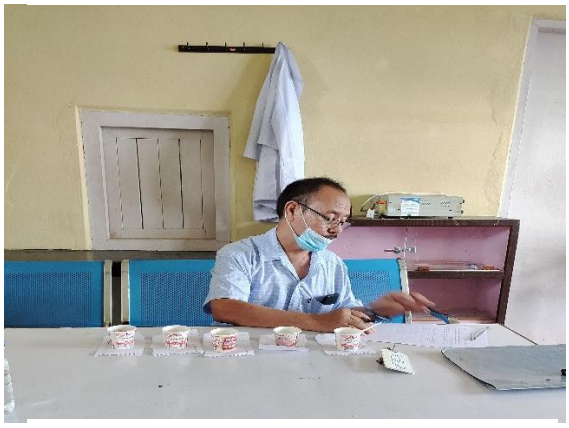

Plate 9 sensory analysis of sample

Plate 10 sensory analysis of sample