COMPARATIVE STUDY OF BLACK PEPPER (*Piper nigrum*) AND CARDAMOM (*Amoum sublatum roxb*) ESSENTIAL OIL AS NATURAL FOOD PRESERVATIVE FOR PLUM READY TO SERVE (RTS) DRINKS

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Comparative Study of Black Pepper (*Piper nigrum)* and Cardamom (*Amoum sublatum roxb)* Essential Oil as Natural Food Preservative for Plum Ready to Serve (RTS) Drinks

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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Approval Letter

This *dissertation* entitled of *Comparative Study of Black Pepper* (*Piper nigrium) and Cardamom (Amoum subulatum roxb) Essential Oil as Natural Food Preservative for Plum ready to serve (RTS) drinks* presented by Ganga Sangroula has been accepted as the partial fulfillment of the requirement for the B.tech. degree in Food Technology.

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(Ganga Sangroula)

Abstract

Ready-to-serve (RTS) drinks and fruit juices are crucial due to their high calorie, fiber, and vitamin content. However, their limited shelf life and rapid nutrient loss raise food safety concerns. The aim of this study was to extract essential oil from black pepper and cardamom and to compare the effects of extracted essential oils as a natural food preservative for plum RTS drinks. The essential oil was extracted by hydro-distillation using clevanger type apparatus and stored at 5±1ºC.The plum juice was extracted by screw press juice extractor. Plum RTS was maintained at 0.3% acidity and 15ºBx TSS and was pasteurized for 90ºC for 10 min and stored at refrigeration temperature. The plum RTS was divided into four equal batches of 250 ml with no additives (A), 100µL ethanol (B), 90µL ethanol and 10µL essential oil of black pepper (C) and 90µL ethanol and 10µL essential oil of cardamom (D).

The samples were stored at 5±1ºC and analysis were carried out at fixed interval of 7 days for 28 days. At the end of 28 days, pH, % titrable acidity, % ascorbic acid, TSS, % total sugar, % reducing sugar, %RSA total microbial count and yeast-mold count of sample A was 3.09, 0.49%, 2.85%, 17, 14.8%, 5.18%, 8.97%,7732 and 3324 respectively and that of sample B was 3.18, 0.45%, 4.38%, 16.4, 14.2%, 4.44%,10.44%, 6632 and 2106 respectively and sample C was 3.39, 0.38%, 6.1%, 16.1, 13.19%, 4.2%, 13.37%, 1964 and 1664 respectively and sample D was 3.44, 0.36%, 6.6%, 15.8, 13.09%, 3.88%,14.94%, 1284 and 994 respectively. Significant changes were observed during the storage period. The essential oil of cardamom show best preservative effect on overall observation in plum RTS.

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List of Abbreviation

Abbreviation	Full form
AOAC	Association of Analytical Communities
ANOVA	Analysis of Variance
DFTQC	Department of Food Technology and Quality Control
DPPH	2,2-Diphenylpicrylhydrazyl
EO	Essential oil
FAOSTAT	Food and agriculture organization statistics
GRAS	Generally recognized as safe
ISO	International Organization for Standardization
HD	Hydro-distillation
MOALD	Ministry of agriculture and livestock development
LSD	Least Significant Difference
PEF	Pulsed electric field
RTS	Ready to serve
SD	Standard deviation
SPSS	Statistical packaging for social science
TPC	Total plate count
TSS	Total soluble solids

Part I

Introduction

1.1 General introduction

Ready- to-serve drinks are a class of drinks that aren't diluted before being served. Typically, it is made from juice, pulp, or both, and then sweeteners (sugar), acidulants (citric acid), coloring agents, and flavoring agents are added as desired. Ready-to-drink beverages are carbonated (Thapaliya, 2004).

One of the most significant stone fruit harvests in the world is the plum. Several well-known stone fruits, such as apricot, peach and cherry. There are more than 2000 different types of plums, although only a small number of them are significant commercially (Birwal *et al.*, 2017). Plums (*Prunus cerasifera)* are a significant source of substances that affect human health and prevent the development of numerous diseases (Stacewicz-Sapuntzakis *et al.*, 2001) . The bioactive substances found abundance in plums include phenolic acids, anthocyanins, carotenoids, minerals, and pectin. Plums are an important part of our diet, both in terms of their dietary and nutritional benefits. These fruits are being used in more and more nutritional research studies on both humans and animals to determine how eating plums affects the body's ability to operate (Nakatani *et al.*, 2000). All around the world, they are mainly eaten raw. The common methods for processing plums include drying of fresh plums, canning, and beverage preparation. Although plums used to be primarily dehydrated today, sun drying was once highly widespread (Bhutani and Joshi, 1995).

Ready-to-serve drinks and fruit juices are a great source of calories, dietary fiber, protein, and vitamins are regarded as being an essential component of any diet plan (Debnath *et al.*, 2021). Fresh fruit juices are therefore in high demand on the market. Fruit juices, however, have a short shelf life and quickly lose their freshness and other nutrients. Because of this, both the food and fruit sectors and consumers are deeply concerned about food safety. A variety of physical and chemical techniques to maintain the quality of the fruit juice for the sole purpose of protection against microbial infection and other nutrient damages (King and Bolin, 1989). Due to the development of bitterness, non-enzymatic browning, enzymatic activity, change in color, aroma, and taste occurring in processed fruit juices that prevents microbial infection and other damages, the storage of fruit juice is

limited, and some preservatives have also been used. The chemical preservatives such benzoic acid, Potassium metabisulfite (KMS), formic acid, etc are involved to preserve the fruit juices and ready-to-serve drinks (Ranganna, 1986).

The low pH of plum products prevents a wide range of bacteria from growing. In plum products, only yeasts, molds, and lactic acid bacteria may proliferate. In processed plum products, yeast growth may result in the creation of bad tastes, turbidity, alcohol, and gas. Storage of the raw juice in a refrigerator can prevent microbial deterioration for a few days, but pasteurization or the application of legal preservatives can safeguard it indefinitely. Such juice is typically turbid, dark in color, and has a tendency to settle out when stored (Ashurst, 1994).

Black pepper as *Piper nigrum* is the king of spices, belonging to the piperceae family. Black pepper is a tropical plant that is indigenous to south asia. It is the most significant, well liked and commonly utilized spice in the entire planet (P. Ravindran *et al.*, 2012). It is significant medicinally and wide range of culinary purposes, including flavouring and preserving processed foods. Essential oil obtained from black pepper is widely applied in modern medicine as an aromatic stimulant in the treatment of cholera, weakness following fevers, vertigo, coma etc. (Rinzler, 1990).

Cardamom as *Amoum sublatum roxb* is a medicinal plant belonging to family Zingiberaceae. The plant is perennial herb with underground rhizomes and grown typically at elevations between 765 to 1675 m above the sea level. The larger or Neplease cardamom sometimes known as gaint cardamom (*Amoum sublutum roxb.)* are indigenous to eastern Himalayas (RA01 *et al.*, 1993). It is widely applied for flavouring as a aromatic compound and preservative of food and foods products. The seed and essential oils is used for ayurvedic medicine to treat head, mouth and rectum conditions as well as indigestion, itch, enlarged spleen, stomach ache and other conditions (Jafri *et al.*, 2001).

Herbs and spices have been used by humans as a means of reducing food degradation and food-borne illnesses since ancient times. The antibacterial and anti-browning properties of herbs and spices had already been studied by the turn of the 20th century, and it was recognized that their esential oils might delay microbiological spoilage and prevent enzymatic browning in food products. Utilizing natural antibacterial and anti-browning substances, particularly those derived from plants, for the preservation of food goods is

gaining popularity (Dorantes *et al.*, 2000). On the other hand, several species and herbs provide attractive flavors while masking undesirable flavors (Charles, 2012).

1.2 Statement of problem

The majority of the economy in Nepal is based on agriculture. Various crops are grown all around the nation. Horticulture crops, among other agricultural products, are crucial to the nation's economic development (Bhurtel *et al.*, 1996). Under typical ambient temperature and humidity levels, fresh fruit and vegetables have a short shelf life. Due to their very perishable nature, they quickly lose their freshness, are vulnerable to bacterial and mold attack, and as a result degrade and lose their usefulness as food for humans (Lal *et al.*, 1960).

Plum RTS is the most unstable fruit juice in terms of chemical and microbes. The low pH of plum products prevents a wide range of bacteria from growing. In plum products, only yeasts, molds, and lactic acid bacteria may proliferate. Fruit juices have been kept using a variety of chemical preservatives, which may be allergic and cause a number of negative health impacts on human beings. Different preservation techniques are used to destroy spoilage bacteria and inactivate degradation enzymes in fruits and their products (Eissa *et al.*, 2003). Hence, attempts have been made to enhance the shelf life of plum ready-to-serve drinks by essential oils derived from black pepper and cardamom which possesses remarkable antioxidant and antimicrobial properties.

1.3 Objective

The objective of research is divided into two parts:

1.3.1 General objective

To compare the effects of essential oils of black pepper and cardamom as a natural food preservative for plum ready to serve drinks.

1.3.2 Specific objectives

1. To carry out extraction of essential oil from black pepper and cardamom.
2. To study the effect of essential oil in microliter (μL) on plum RTS during storage.
3. To compare the preservative effect of black pepper and cardamom on plum RTS.

1.4 Significance of study

The use of chemical preservatives such as sodium benzoate, benzoic acid, Potassium metabisulfite (KMS), sodium sorbate and potassium sorbate etc. in the juice posses adverse health effects on human. This is an attempt to replace to such artificial preservatives using spices essential oil as a natural preservatives. Spices have been used as preservative in many food products. In the current context of world, people are conscious of using natural preservative for preservation of juice. Black pepper and cardamom are rich sources of antioxidant and antimicrobial properties. Despite of there high health benefits majority, people are totally unknown about there importance. Hence, this work might also provide enthusiastic market for plum and spices which would also help the financial status of people involved in its cultivation, production and marketing, ultimately uplifting their living standards (Nair, 2011).

1.5 Limitations of the study

1. Storage stability analysis was carried out only for 28 days.
2. Preservative properties of oleoresins of spices were not carried out.

Part II

Literature review

2.1 Background

The need for nutritious foods and the growing fitness consciousness of consumers are likely driving the worldwide juice industry. Juice producers today put the needs of the consumer first and concentrate on introducing various juice kinds, tastes, and mix juices along with cutting-edge packaging and specific nutrition and health claims. The market for juice is expected to increase rapidly between 2016-20 at a compound annual growth rate of 3% (Ceclu and Nistor, 2020).

2.2 Plum

Plum and prunes are among the fruits which belong to Rosaceae family, cherries, apples, peaches, pears and various berry includes in these family. The family Rosaceae includes the genus prunus, which comprises all true plums. There are many different types of plums, but the two most notable are European (*Prunus cerasifera*) and Japanese (*Prunus salicina Lindl*) plums (El-Sharkawy *et al.*, 2016).

In many regions, plums (*Prunus cerasifera*) can be harvested between cherry and apple crops and has the potential to be a fresh market and processing crop. Only about half of the plums are eaten fresh; the remainder are processed (Chang *et al.*, 1994). In addition to simple sugars, proteins, fats, vitamins, and minerals, plums are low in calories. There are several identified phytochemicals, including polyphenols, carotenoids, titerpenes, and unstable combinations. Consuming plums has been shown to have several health benefits, including higher levels of antioxidants and antiallergic characteristics (Igwe and Charlton, 2016). Due to its appealing color, flavor, and nutritional content, red plum juice is well-liked on a global scale. The nutritional value of red plums is mostly attributable to its phenolic components, including flavonoids and phenolic acid, which lower the risk of oxidative damage and prevent certain cancers (Kim *et al.*, 2003).

Kingdom:	Plantae
Sub kingdom:	Viridiplantae
Division :	Tracheophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Rosaceae
Genus :	*Prunus*
Species:	*P.cerasifera*

Source: Gull *et al* (2023)

2.2.1 Historical background of plum

Different species of plums have evolved and been domesticated independently throughout Europe, Asia, and North America. Due to the origins of *P.Cerasifera* and *Prunus Spinosa* in western and central Asia, astrological data suggests that *Prunus domestica* originated in Europe. The prunocerasus species, including *P. americana*, originated in North America, while *P. Salicina* was originated in China (Milosevic and Milosevic, 2018) . In accordance with the trade routes and historical occurrences that brought the Persian Empire into contact with the fourth century, it expanded toward the Near East and the Mediterranean. The initial entry into Europe would take place via Greece and Italy via Iran or Armenia. In the latter half of the 7^{th} century, the species also traveled from the Southern Mediterranean to Spain via the Arabs. Finally, in the 16^{th} and 17^{th} centuries, these fruit trees would spread from Europe to North America, Mexico, and South Africa (Salazar *et al.*, 2022).

2.2.2 Plum production

Review of the current state of global plum production, market, and commerce. With seven million tons and a revenue of USD 10 billion, China is the world's largest producer of plums and sole. It is followed in importance by Romania, Serbia, Chile, and the United States (Gull *et al.*, 2023).

2.2.2.1 Plum production in Nepal

The total area, production area, production and yield of plum in Fiscal year 2077/78 (2020/21) is given by Ministry of Agriculture and Livestock Development (MOALD) of all the seven province and it is found that the highest production is found in Bagmati province and there is no production in Madesh province which is given in Table 2.1.

Table 2.1 Plum production of Nepal in Fiscal year 2077/78 (2020/21)

Province	Total Area(Ha)	Production Area(Ha)	Production (Mt)	Yield (Ha/Mt)
Koshi Province	466	308	2035	6.61
Madesh Province	-	-	-	-
Bagmati Province	329	294	2586	8.79
Gandaki Province	102	89	627	7.03
Lumbini Province	747	538	2675	4.97
Karnali Province	334	244	1554	6.37
SudhurPaschim Province	155	112	807	7.23
Average production	2132	1585	10284	6.49

Source: MOALD (2021)

2.2.3 Nutritional value of plum

Like all fruits, the plum has a modest amount of starch but is a very good source of sugar. The quinic acid and malic acid found in plums are the acids that give fruits their distinctively acidic flavor. Plum is a good source of vitamin C as well. The phenolic compounds that give plum its distinct astringent properties include catechin, caffeic acid, chlorogenic acid, rutin, and phenolic acid. Plum is a rich source of antioxidants (Gunduz and Saracoglu, 2012) . The Nutritional composition of plum (*Prunus cerasifera*) per 100g is given in Table 2.2.

Table 2.2 Nutritional composition of plum fruit per 100g

Constituents	Amount
Energy	52 K.cal
Carbohydrate	11.1 g
Moisture	86.9 g
Protein	0.7 g
Fat	0.5 g
Fibre	0.4 g
Calcium	10 mg
Potassium	12 mg
Iron	0.6 mg
Magnesium	7 mg
Zinc	0.1 mg
Vitamin C	9.5 g
Thiamine (Vit.B_1)	0.028 mg
Riboflavin (Vit.B_2)	0.026 mg
Niacin	0.417 mg

Source: Gull *et al* (2023)

2.2.4 Products of plums

One of the most common plum products is prune, which often refers to dried plums produced from *Prunus cerasifera* processing variations. In addition to prunes, other goods made from plums include canned plums, plum sauce, plum juice, dried plum puree, and

plum powder. Prunes are known to include a wide range of biochemical substances that are good for human health because they are natural products. In the fields of food science and nutrition, research on the two plum species *Prunus cerasifera* and *Prunus salicina* is extensive (Gull *et al.,* 2023).

2.2.5 Health benefits of plums

Fruit juices are a crucial part of the average person's diet. They are recognized as top-notch suppliers of micronutrients like vitamins, minerals, and some phytochemicals that have positive effects on one's diet and general health (Shahbaz *et al.,* 2018). The benefits of plums consumption are discussed below:

1. Due to the isatin, sorbitol, and dietary fiber included in plums, one's digestive system is kept in check, which relieves constipation.
2. The vitamin C in plums scavengers free radicals and helps to protect against infectious diseases.
3. Its moderate beta-carotene content guards against lung and mouth cancer.
4. Carotenoids, such as cryptoxanthin, lutein and zeaxanthin, are present.
5. Plums include a number of minerals, including iron, potassium, and fluoride, which support healthy biological activities.
6. B-complex vitamins, which aid in the metabolism of carbohydrates, proteins, and lipids, are present in moderate amounts in plums.
7. According to studies, plums can prevent macular degeneration, shield against heart disease, and neuronal damage (Birwal *et al.,* 2017).

2.3 Fruit juices

Fruit juice is defined as the fermentable but unfermented product made from the edible part of fruit that is sound and ripe, fresh or preserved by chilling or freezing of one or more kinds mixed together, and has the same color, flavor, and taste as the juice of the fruit that it originates from, i.e., the juice made from fruit itself. Although the terms "direct juice" and "not from concentrate (NFC) juice") are frequently used to describe this product, they are not subject to legal restrictions (Mihalev *et al.,* 2018).

2.3.1 Types of fruit juices

According to their dispersion system composition, fruit juices can be divided into the following four main types

2.3.1.1 Clear/clarified (transparent) juice

This is an example of a water solution of so-called soluble solids, such as sugars, organic acids, salts, free amino acids, water-soluble vitamins, pigments, and others, with particle sizes less than 0.001 m. With the help of the cell sap found in plant cell vacuoles, it may be estimated. Freshly squeezed fruit juice is processed technologically to create clear juice (clarification) (Mihalev *et al.*, 2018).

2.3.1.2 Opalescent (translucent) juice

The transparent juice also contains colloidal particles with a 0.10-0.001 m distribution spectrum in addition to the soluble solids. This dispersed phase contains dissolved starch, proteins, protein polyphenol complexes, pectin, and hemicelluloses (Mihalev *et al.*, 2018).

2.3.1.3 Cloudy (turbid) juice

This juice has not undergone any clarifying processes; it is squeezed juice. It is possible to partially remove coarse particles (e.g., by centrifugation) to increase cloud stability because they are typically unstable and prone to rapid sedimentation. 95% of the particles in hazy apple juice are smaller than 2.5 mm in diameter, with 0.60 to 8 mm being the most frequent. The proteins, carbohydrates, lipids, and polyphenols that make up these rather stable tiny cloud particles are also present. Although they appear to be more than just cell waste, fine cloud particles most likely originate from cell membranes and walls. The stability of the cloud appears to be influenced by natively adsorbed pectin, which appears to be associated with cell membrane/wall fragments and colloidally dissolved macromolecules (Mihalev *et al.*, 2018).

2.3.1.4 Pulp-enriched juice

The majority of the coarse cloud particles in this mixture are fragments of fruit flesh, such as juice sacs from the endocarp of citrus fruits, and have diameters of over 100 m. These coarse cloud particles are also frequently referred to as pulp particles. By combining cloudy juice with fruit purée, pulp-enriched juice can be created. This type of dispersion

mechanism might also be applied to smoothies, which are blended drinks made from juices and mashed fruits or purées. Fruit and vegetable purees contain the greatest number of coarse cloud particles (Mihalev *et al.*, 2018).

2.3.1.5 Ready to serve

Ready-to-serve drinks refers to a category of beverages that have not been diluted before consumption. Typically, it is made from juice, pulp, or both, and is then colored and flavored with optional ingredients. Sweeteners like sugar and acidifiers like citric acid are also mixed in. Carbonation is present in ready-to-drink beverages. Depending on the product, different levels of carbonation are used. RTS are required to meet certain criteria, like having fruit components that are at least 10%, 10% TSS, and 0.3% acidity etc. as given in Table 2.3.

Table 2.3 Specifications for ready-to- serve drinks (RTS)

Parameters	Value
Fruit content	Not less than 10%
Total soluble solid (TSS)	Not less than 10%
Acidity	0.2-0.3%
Sulphur dioxide	Not more than 70ppm

Source : DFTQC (2018)

2.4. Juice processing

Fruit juice manufacturing and preservation are significantly hampered by their perishable nature. The major problem up until the development of preservation procedures was the rapid fermentation of juice after squeezing (Correa *et al.*, 2010). According to studies, the quantity of nutraceuticals in fruit juices depends on how they are grown, prepared, and stored. Thus, it is necessary to not only record the conventional techniques for extracting and storing juices, but also to investigate how cutting-edge procedures might aid to lessen the difficulties that the juice sector faces. Extensions in shelf life have been achieved using conventional methods like canning, pasteurization, concentrating, freezing, evaporation,

and spray drying. High-pressure processing, however, has a significant impact on the microbial, physical, and chemical properties of fruit juice at the expense of any nutritional or health benefits (Bull *et al.*, 2004).

A high-quality, nutrient-dense, and microbiologically stable product is not always produced by thermal treatments. Recently, the focus has been on using cutting-edge techniques to increase the safety and shelf life of fruit juices while preserving their nutritional value. For the preservation, processing, and packaging of fruit juice, a variety of cutting-edge technologies have been used, such as high pressure processing, pulsed electric field (PEF) processing, ultrasound, ozone processing, light-based technologies, irradiation, and non-thermal plasma. Due to their effectiveness in extending shelf life, reducing enzymatic activity, and inactivating microorganisms while preserving the quality of the original, freshly pressed produce, these revolutionary approaches are quickly taking over the juice market (Mohamed and Eissa, 2012). Water quality for drinking must meet regulatory standards as reported for the creation of RTS Haldar *et al.* (2016).

2.5 Challenges associated with fruit juices production

Fruit juices are a more healthful option for consumers, but their quality and safety have long been a concern. As a result, they are subject to strict regulations that ensure they contain all the required information about their compositions and nutritional advantages (Rajauria and Tiwari, 2017)**.**

In addition to rigorous rules, there are also other elements that make it difficult to produce fruit juices and restrain the expansion of the worldwide juice business. One of the biggest problems is ensuring a steady supply of fruits because most fruits are seasonal, which has an impact on production**.** Additionally, there are challenges with manufacturing (homogenization, extraction, filtration, processing, preservation, packaging, and storage), ingredients (fruit components, sweeteners, flavors, colors, preservatives, nutraceutical ingredients, and miscellaneous additives), quality (color and flavor deterioration, appearance changes, packaging material, storage conditions, microbiological problems, shelf life, water quality, and bottling issues), and manufacturing (Rajauria and Tiwari, 2017).

2.5.1 Microbiological background in fruit juices

2.5.1.1 *Escherichia coli, Salmonella, and Listeria monocytogenes*

Fruit juices can become more safer by the process of pasteurization. Juice processors may choose *E. coli* O157:H7 or *Salmonella* as the target microorganism to determine the lethality of a pasteurization treatment due to the numerous outbreaks that have been linked to them in unpasteurized juices, or *L. monocytogenes* due to its widespread prevalence. The most heat-resistant pathogen that is anticipated to be present in the juice should be the target microorganism since other microorganisms are also destroyed when the most heat-resistant pathogen is inactivated (Agcam *et al.*, 2018; Burt and Reinders, 2003).

Some food borne pathogens can develop acid adaptation systems that induce cross-protection, and make them more resistant against other environmental stresses, thus increasing their ability to survive in juice. *E. coli* O157:H7, *L. monocytogenes*, *Salmonella spp*., and *C. parvum* can tolerate low pH values and survive in fruit juices and juice concentrates longer than cells that are unable to adapt. The acid adaptation of *Salmonella spp., L. monocytogenes*, and, *E. coli* O157:H7 also increases the heat resistance of these bacteria in apple, orange, white grape juices, apple cider, juice blends, cantaloupe, and watermelon juice (Agcam *et al.*, 2018).

2.5.1.2 *Alicyclobacillus*

Currently, the *Alicyclobacillus* genus contains more than 20 known species, although only four of these species (*A. acidoterrestris, A. pomorum, A. herbarius, and A. acidiphillus*) have been implicated in the spoiling of fruit juice or other beverages. However, because of the prevalence of spoiling episodes and *A. acidoterrestris,* it is thought to be the most significant. Because *Alicyclobacillus* is capable of producing Guaiacol, 2,6-dibromophenol, and 2,6-dichlorophenol, Alicyclobacillus spoilage is distinguished by a phenolic off flavor (Agcam *et al.*, 2018).

2.5.1.3 Molds and yeasts

The fungus that can develop mycotoxins should not be allowed to thrive in fruit juices to protect the general public's health. Most molds' spores and vegetative cells are rendered inactive after being exposed to 60^0C for 5 min in order to prevent the growth of fungi and the production of mycotoxin in food. There are several notable exceptions, including the

ascospores of specific strains of the molds *Byssochlamys nivea*, *Byssochlamys fulva*, *Neosartorya fischeri*, *Talaromyces flavus*, and *Eupenicillium javanicum* in high-acid fruit pulps/juices (Agcam *et al.*, 2018).

In the juice industry, yeast and mold fermentation can be problematic, but the main issue with plum juice is patulin, a mycotoxin generated by numerous species of mold. According to Agcam *et al.* (2018) patulin is mutagenic, carcinogenic, and teratogenic. The total viable microbial count in fruit juices is shown to have a maximum permissible level of 1×10^4. Microbiological contamination can happen at any stage, from manufacture to consumption, although microbial growth during storage depends on the packaging's quality, the storage environment's temperature, and the presence of preservatives (Rahman *et al.*, 2011).

2.6 Preservation of juice

Juices that have just been extracted are very appealing to look at, have a pleasant flavor, and smell good, but if maintained for a while, they quickly lose their quality. Juice must be preserved as soon as it is extracted, without being left to stand for an extended period of time, in order to maintain its natural flavor and aroma. There are several preservation techniques used, and each has techniques have own advantages (Parajuli, 2010). The methods generally used are:

- Pasteurization
- Addition of chemicals or sugar
- Drying and freezing
- Freezing
- Filtration

2.6.1 Pasteurization

Louis Pasteur, a French scientist who developed the technique of heating liquids (such as wine and beer) at a low temperature for a brief period of time, is credited with the invention of the term "pasteurization." In order to destroy relatively heat-sensitive microorganisms like vegetative bacteria, yeasts, and molds that cause food deterioration or food poisoning, thermal pasteurization, a mild kind of heat treatment, is utilized (Agcam *et al.*, 2018).

Fruit juices are pasteurized at a temperature and for a time that would sterilize them without compromising their flavor. Juices are often pasteurized based on the type of juice and the volume of the container. Acid fruit juice needs to be pasteurized at a lower temperature and for a shorter period than less acidic fruit juice. The juice can be heated to the pasteurized temperature to destroy pectin enzymes, which alter the flavor and also cause particles in the juice to clump together. Since enzymes need air to function, they can also be destroyed at a moderate temperature by removing the air from the juice. The task must be done in hygienic settings and with all equipment kept immaculately clean in order to produce excellent outcomes. Fruit juice pasteurization can be done using a variety of techniques (Parajuli, 2010).

2.7 Preservatives

2.7.1 Definition of preservatives

Preservatives are compounds that are purposefully added to food to lengthen its shelf life and improve or alter its qualities, such as flavor, appearance or structure. Natural or synthetic ingredients with no nutritional value added to food during manufacturing and production (Silva and Lidon, 2016).

2.7.2 Natural preservatives

Natural preservatives are defined as those that have been used in food for generations and are generally recognized as safe (GRAS). Some examples of natural preservatives are sugar, salt, vinegar, spices, and herbs with a natural source (Kharel and Hashinaga, 2004). Traditional natural preservatives include the use of castor oil, salt, sugar, vinegar, alcohol, diatomaceous earth, rosemary extract, hops, and other naturally occurring ingredients. Food preservation techniques include freezing, pickling, smoking, salting, and freezing (Dalton, 2002).

2.8 Spices

2.8.1 Definition of Spice

The Geneva-based International Organization for standardization (ISO) defines spices and condiments as: Vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning and imparting aroma in foods.

Depending on the nation or area of the world, a spice may be defined differently. Depending on where they are cultivated, whether they are dry or wet, or when they first began to be used as spices, spices can often be classified based on these factors. From the perspective of their roles and constituents, these definitions aren't always accurate, though. The dried components of a plant, like roots, leaves, and seeds, that give food a specific flavor and pungent sensations are known as spice (Hirasa and Takemasa, 1998).

The Latin word species, which means particular sort, is where the word spice originated. The name refers to the cultivation of all plant parts, such as seeds (such as aniseed, caraway, and coriander), leaves (such as cilantro, kari, bay, and mint), berries (such as allspice, juniper, and black pepper), arils (such as mace), stems (such as chives), stalks (such as lemongrass), rhizomes (such as ginger, turmeric, and galangal), roots (such as lovage) (Raghavan, 2007).

2.8.2 Basic uses for spices

When used in food, spices have a variety of impacts. It added flavor, pungency, and color, they also have antioxidant, antibacterial, medicinal, and nutritional qualities (Stuckey, 1997). The basic uses of spice are shown in Table 2.4.

Table 2.4 Basic uses of spice

Basic uses	Spice
Flavoring	All spice, cinnamon, basil, dill, nutmeg, fennel, parsley, anise, marjoram, cumin, mint, cardamom, mace
Masking/Deodorizing	Garlic, clove, rosemary, onion, bay leaves, thyme, sage, coriander, caraway, oregano
Pungency	Pepper, red pepper, mustard, Japanese pepper, ginger
Colorant	Turmeric, paprika, saffron

Source : Stuckey (1997)

2.8.3 Essential (volatile) oils

Essential oils are fragrant, flammable liquids made from plant material by the steam distillation process that bear the name of the plant from which they were made. Essential oils are substances that are both fragrant and odorless that are combined to create products or combinations. These fragrant chemicals are chemically pure molecules that are volatile in typical circumstances (Rios, 2016). The direct and complex effects of spices as shown in Table 2.5.

Table 2.5 Direct and complex effects of spices

Direct Effect	Complex Effect
Flavor	Increased appetite
Taste (pungency, bitterness, sweetness)	Masking effect
Color (red, green, yellow)	Improvement of texture
Antifungal Effect	Preservation
Antibacterial Effect	Preservation
Antioxidant Effect	Preservation

Source: Hirasa and Takemasa (1998)

There are several techniques for isolating essential oils, including hydrodistillation, steam distillation, and organic solvent extraction. Steam and hydrodistillation (HD) are well-known traditional techniques for extracting essential oils from medicinal plants and herbs. These techniques are known to be energy-intensive and have a number of drawbacks, such as volatile chemical losses and prolonged extraction periods. Furthermore, natural ingredients, particularly monoterpenes, which are susceptible to structural changes under steam distillation conditions, might be partially or completely degraded by high temperatures. More volatile chemicals may be lost during the solvent removal process when using the traditional solvent extraction (Gavahian *et al.*, 2012).

2.8.4 Antimicrobial properties of spices

Microorganisms are involved in numerous aspects of the food economy. Some are successfully used to create dairy products, pickles, and other fermented items, although they typically result in foods becoming unappealing or spoiling. Antimicrobial activity, which includes bacteriostatic or fungistatic action (preventing microbial development and propagation), is a board term used to describe an inhibitory action against microbial growth. Many spices include antimicrobial and/or antifungal qualities. Spices have long been used and known to have antibacterial effects. For instance, in ancient Egypt, cinnamon, cumin, and thyme were employed in the mummification process, while in ancient India and China, spices were utilized to preserve food as well as for therapeutic purposes. Coriander and mint were employed in ancient Greece and Rome to keep meat fresher longer and milk fresher longer, respectively. In the medieval era, spices were used to treat infectious diseases including typhus and cholera. Early investigations on the antibacterial benefits of spices, such as mustard, clove, and cinnamon, date back to the 1880. Research on spice extracts and their essential oils has been done in this context since the early 20th century (Hirasa and Takemasa, 1998). The characterizing essential oil components in some popular spices Table 2.6.

Table 2.6 Characterizing essential oil components in some popular spices

Spices	Components of Essential Oils
Allspice seed	Eugenol; 1,8-cineol; humulene,α-phellandrene
Basil, sweet	Linalool; 1,8-cineol; methyl chavicol, eugenol
Cardamom	1,8-cineole; linalool; limonene;α-terpineol acetate
Dill leaf	Carvone, limonene, dihydrocarvone,α-phellandrene
Epazote	Ascaridol, limonene, para-cymene, myrcene,α-pinene
Fennel	Anethole, fenchone, limonene,α-phellandrene
Ginger	Zingiberene, curcumene, farnescene, linalool, borneol
Juniper	α-pinene,β-pinene, thujene, sabinene, borneol
Kari leaf	Sabinene,α-pinene,β-caryophyllene
Lemongrass	Citral, myrcene, geranyl acetate, linalool
Marjoram	Cis-sabinene,α-terpinene, terpinene 4-ol, linalool
Nutmeg	Sabinene,α-pinene, limonene, 1,8-cineol
Oregano	Terpinene 4-ol,α-terpinene,cis-sabinene
Black pepper	Sabinene,α-pinene,β-pinene, limonene, 1,8-cineol
Rosemary	1,8-cineol, borneol, camphor, bornyl acetate
Star anise	Anethole,α-pinene,β-phellandrene, limonene
Turmeric	Turmerone, dihydrotumerone, sabinene, 1,8-cineol
Zeodary	Germacrone-4, furanodienone, curzerenone, camphor

Source: Raghavan (2007)

2.8.5 Antibacterial and antifungal properties of spices

The aromatic and biological qualities of aromatic and therapeutic plants are due to their major ingredients, mono- and sesquiterpenes, which also include carbohydrates, alcohols, ethers, aldehydes, and ketones. These qualities have led to the addition of spices and herbs to food from ancient times, both as flavoring and as preservation. Essential oils have been extracted over the years from various plant components and have comparable uses. A wide range of functions are covered by essential oils. Numerous essential oils have pharmacological effects that show they are anti-inflammatory, antioxidant, and anticancerogenic. Others work as biocides to kill a variety of creatures, including plants, insects, fungi, viruses, bacteria, and protozoa (Kalemba and Kunicka, 2003).

Numerous research have looked into how well spices can slow the growth of bacteria that cause disease and other microorganisms that produce toxins. The effectiveness of spices against tubercle bacillus, bacillary dysentery, and cholera emerged in the 1940. Ceylan and Fung (2004) examined the antimicrobial effects of different spices on eight microorganisms, including *Shigella dysenteriae* and *Salmonella typhi*, and discovered that garlic had a potent antimicrobial effect on all of them, while onion and clove were effective against all but *B. subtilis*. When *Salmonella* was introduced to oregano-containing pre-enrichment media, its ability to multiply was also hindered. Thyme and oregano were found to be effective against *Vibrio parahemolyticus* at concentrations of 0.5% when added to growth media in powder form (Hirasa and Takemasa, 1998).

2.9 Black pepper

Black pepper is the king of the spices. It is the most significant, well-liked, and commonly utilized spice in the entire planet. It is significant medicinally and has a wide range of culinary purposes, including flavoring and preserving processed foods (P. N. Ravindran and kallupurackal, 2001).

Belonging to the Piperaceae family is black pepper (*Piper nigrum*). It is grown for its dried fruit, which is used as a spice and flavoring. Green and white pepper are also produced from the same fruit. Black pepper is a tropical plant that is indigenous to South India, where it is widely cultivated. When dried, the peppercorn-shaped fruit is a little drupe with a diameter of 5 mm that is dark red in color and has one seed within. One of the most popular spices in European cooking is dried, ground pepper, which has been valued for both its flavor and medicinal properties since ancient times. Piperine is the molecule that gives black pepper its spiciness. In various regions of the world, table salt and ground black peppercorn, which is more commonly known simply as pepper, can be found on almost every dinner table. The most significant and popular spice consumed worldwide is black pepper, often known as "black gold" and the "king of spices." In contrast to many other spices, properly dried black pepper (moisture content 8–10%) can be kept in airtight containers for a long time without losing flavor or scent. The term "pepper" is derived from the Sanskrit word pippali through the Latin word "piper" and the Old English word pipor. Along with other related forms, the Latin term is also the basis for the German pfeffer, French poivre, Dutch peper, and others. Since at least the 1840, pepper has been used

figuratively to denote "spirit" or "energy" (Kunnumakkara *et al.*, 2009). The figure of pods with black pepper is shown in fig 2.1.

Fig. 2.1 Black pepper plant with its pods

The pepper plant inflorescence is a pendent spike (catkin) that grows on branches with plagiotropy opposing the leaf. Cultivar-specific spikes range from 3 to 15 cm in length and have 50 to 150 blooms. The tiny, spirally-organized flowers, which range in hue from white to pale yellow, are set on fleshy peduncles. The species self-pollinates spontaneously, and geitenogamy is used for pollination. The presence of water droplets aids in the dispersion of pollen. The fruit, which is a sessile, tiny, typically globular drupe with one seed but is frequently termed a berry, has a hard endocarp and a fleshy pericarp. Most of the time, the fruits are spherical, but some are obovate and some are oblong (Lin, 1994).

There are more over a hundred identified varieties, and some of them are still in demand. There are several well-liked varieties unique to the traditional pepper farming regions. The state of Kerala has the greatest diversity of cultivars. Between cultivars, piperine ranges from 2.0 to 7.4% and essential oil from 0.4 to 7.0% (Kumar *et al.*, 2021).

The quality of pepper is contributed by two components

- Piperine that contributes the pungency.
- Volatile oils that is responsible for the aroma and flavor.

Black pepper oleoresin is made by solvent extracting dried powdered pepper and contains both aromatic and pungent components. As a result, the chemical composition of pepper is made up of piperine and its essential (volatile) oil (Narayanan, 2000). The chemical composition of black pepper is shown in Table 2.7.

Table 2.7 Chemical composition of black pepper (%db)

Parameter	Value (%)
Moisture	8
Protein	10.0
Piperine	2 – 7.5
Fat	10.2
Total ash	3.43-5.09
Fiber	10.79-18.60
Carbohydrate	66.5

Source: Tainter and Grenis (2001)

Antipyretic, aromatic, carminative, rubefacient, and stimulant are all terms used to describe black pepper. Black pepper is used in modern medicine in India as an aromatic stimulant in the treatment of cholera, weakness following fevers, vertigo, coma, etc.; as a stomachic in the treatment of gas and indigestion; as a substitute in the treatment of arthritic conditions and paraplegia; and as an antimalarial (Rinzler, 1990). A traditional treatment for abdominal tumors uses pepper root in the form of ghee, powders, enemas, and balms. The leaves are applied topically by the Chinese to treat headaches and urinary calculi. Fruits that have been powdered are believed to reduce "superfluous flesh." It is claimed that an electuary made from the seed can treat hard tumors, and that a salve made from the seed can treat eye indurations and interior tumors. The grain is claimed to ease stomach indurations when combined with warm wine and egg. Making a poultice with pepper, salt, and vinegar could be beneficial. Similar to mustard plasters, pepper is also applied topically for the treatment of rheumatism, colic, headaches, and parturition.

Abortion is allegedly caused by eating wild bamboo shoots with a lot of pepper. Safrole, a known carcinogen, is present in pepper, but in far lower concentrations than in sassafras. Even more effective at lowering cholesterol than pepper phytosterols are several undiscovered pepper chemicals, some of which may do so via inhibiting HMG-CoA-reductase. In addition to accelerating peristalsis, black pepper also hastens intestinal transit (Kapoor *et al.*, 2008).

The stimulant piperine is used. Additionally, heliotropine, which has its own set of therapeutic uses, is produced using it. Combining it with astringents could render it inert, thus it shouldn't be done. A radioprotective effect of piperine and curcumin was also found. Micrococcus species *Lactobacillus plantarum*, and two fecal microorganisms (*E. coli and Streptococcus faecalis*) can all be inhibited by isolated piperine. With Leptospira, it is mutagenic; at high dosages, it has bactericidal effects. It blocks the dangerous, pervasive *Clostridium botulinum* bacteria. Additionally, pyrethrin is less poisonous to houseflies than piperine. More poisonous than 0.1% pyrethrin is a mixture of 0.05% piperine and 0.01% pyrethrins (Tainter and Grenis, 2001).

Ayurveda, Sidha, and Unani, three Indian medical traditions, all use black pepper as a therapeutic agent for a variety of illnesses. Many of these conventional usages have been supported by pharmacological investigations. According to some Vijayan and Thampuran (2000) pepper exhibits a number of useful qualities, such as:

- Analgesic and antipyretic properties
- Antioxidant effects
- Antimicrobial properties

Pepper's main component, piperine, has strong analgesic and antipyretic properties. discovered that piperine, at an oral dose of 50 mg/kg body weight, decreases inflammation in carrageenin-induced testing. The reduction in inflammation was supported by Nisha *et al.* (2009). Since piperine and its homologues are absorbed through the skin, they can affect blood vessels, nerves, and subcutaneous tissues. Additionally, pepper affects breastfeeding by boosting milk production. Pepper oil stimulates circulation by warming the skin and drawing blood to the surface. Pepper is used in Ayurveda to cure epileptic seizures and promote sleep. Both pentrazole-induced and electroshock-induced seizures were protected from by piperine. Additionally, piperine has a potentiating effect on the

hypnosis that hexobarbital causes in mice. It has been demonstrated that the pepper compound 1-(3-benzodioxol-5yl)-1-oxo-2-propenyl-piperidide, also known as antiepilepsirine, has potent antiepileptic effects. In Chinese hospitals, this is used to treat epilepsy (Ebenhoech and Spadaro, 1992).

Due to pepper's antibacterial characteristics, adding pepper to food improves its ability to keep and avoids food spoilage. According to research, *Vibrio cholerae, Staphylococcus albus, Clostridium diphthereae, Shigella dysenteriae, Streptomyces faecalis, Bacillus spp., Pseudomanas spp.*, etc. are all inhibited by pepper essential oil. At a dosage of 0.2% to 1%, pepper oil inhibited Aspergillus parasite development and aflatoxin formation Additionally, pepper leaf oil has antifungal properties. The bioavailability of medications such as ampicillin and synthetic medicines as well as the absorption of amino acids from food are both enhanced by pepper and piperine (Johri *et al.*, 1992).

2.10 Cardamom

In the family Zingiberaceae, a few kinds of plants produce dried seed capsules are cardamom. These capsules contain seeds with a distinct, pleasantly aromatic flavor. Two general categories can be drawn from them (Y. Rao *et al.*, 1993).

- Small cardamom-popularly known as chhota Elaichi (*Elettaria cardamoum)* or the true cardamom. It is also as ‘Queen of Spices’.
- Large cardamom- Bada Elaichi (*Aframomxum* and *amomum* species)

The larger or Nepalese cardamom, sometimes known as giant cardamom, is *Amomum subulatum roxb*. It is indigenous to the eastern Himalayas. The idea that the plant originated in Sikkim is supported by the existence of multiple wild cousins, including *A. delbatum, A. aromaticum, A. kinger, A. lingriformi, and A. corynostachium*, as well as the enormous variety within the cultivated species (Y. Rao *et al.*, 1993). The distribution and diversified uses of different species of cardamom in Table 2.8.

Table 2.8 Distribution and diversified uses of different species of cardamom

Species	Common name	Country	Use
A. aromaticum Roxb.	Bengal cardamom or Nepal cardamom	Eastern India, Pakistan	Rhizomes are used as condiment and flowering shoots are used in curries
A. compactum soland	Round cardamom	Malaysia Java	Fruits are used as condiment and spice
A. glabosum cour	Round Chinese cardamom	China	Seeds are used as cardamom
A. krervanw Pierre	-	Cambodia Indo-China	Fruits are used as condiment and to flavor curries, sausages and cordials
A. maximum Roxb.	Java cardamom	Malaysia	Condiment
A. xanthioides wall.	Wild bastard siamese cardamom	Burma India	Condiment

Source: Y. Rao *et al* (1993)

This species, which is one of eastern India's cash crops, is grown in swampy areas next to mountain streams in Nepal, Bengal, Sikkim, and Assam (in the eastern Himalayas). The plants are typically grown around jhoras (small springs), on the damp, shaded banks of mountain streams, and along the slopes of hills, typically at elevations between 765 and 1675 m above mean sea level. The plant is a perennial herb with underground rhizomes that produce spikes and green shoots. The plant reaches maturity in the third year of growth, reaching a height of 1.5 to 3.0 m. Long stalks that resemble sheaths that are wrapped around one another create leafy shoots. The leaves have an acuminate tip, are green or dark green, and are glabrous on both surfaces. With 40 to 50 flower buds arranged in an acropetal pattern, the inflorescence is a dense spike on a short peduncle. The fruit is a

multi-seeded trinocular capsule. The echinate capsule wall is reddish brown to dark pink in color (Y. Rao *et al.*, 1993). The pods and cardamom plant are shown in Table 2.2.

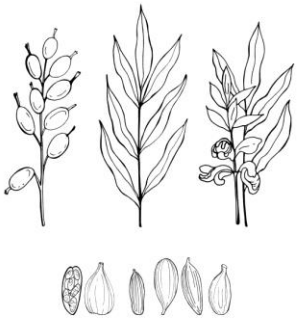

Fig. 2.2 Cardamom plant with its pods

Dried huge cardamom capsules range in size from oval to globose and are typically 25 mm long, greyish brown to dark red brown. The fruit's thick sweet pulp holds the fruit's 40–50 seeds together. The seeds are almost the same size as those of true cardamom, despite the fruits being easily distinguished from little cardamom by their bigger size and distinct forms (CFTRI., 1994). One of the main ingredients contributing to the distinctive odor is the volatile oil found in big cardamom seeds. When seeds are crushed and the essential oil is extracted using steam distillation, 2.5% of a dark brown mobile liquid with The variety Golsey Dwarf had the highest volatile oil content, measuring at 3.32%, while the variety White Ramna had the lowest at 1.95% (Gupta *et al.*, 1984). 1,8-cineole (65–80%) is the main component of large cardamom essential oil, and terpenyl acetate (traces to 5%) is a minor component. Lamonene, Sabeinene, Terpinenes, and Pinenes make up a sizable portion of the monoterpene hydrocarbon content, which ranges from 5 to 17%. About five to seven percent of the oil is made up of terpinols. The very strong aroma of this spice in comparison to that of authentic cardamom is likely caused by the high cineole and low terpenyl acetate content (Pruthi, 1993). The chemical composition of cardamom is shown in Fig 2.9.

Table 2.9 Chemical composition of cardamom (%db)

Parameter	Value (%)
Moisture	9.5-11
Protein	11.4-14.2
Fat	7.0-9.0
Fiber	8.5-13.8
Total Ash	6.5-9.0
Carbohydrate	42.1-49.5
Cineole	36.3

Source : Amma *et al* (2010)

The acrid seeds are used in Ayurvedic medicine to treat head, mouth, and rectum conditions as well as indigestion, itch, biliousness, enlarged spleen, stomach pain, and other conditions. The seeds have been given for stomach disorders, biliousness, dyspepsia, rectal disorders, and vomiting. They are also said to be alexeteric, astringent, stimulant, and stomachic. The seeds are used to treat neuralgia when consumed in big doses (30 grains) with quinine. For issues with the gums and teeth, use the seed decoction as mouthwash. The seeds are used as diuretics for kidney stones, together with melon seeds. Seeds aid in bile eviction, making them helpful for liver issues. Also utilized in gonorrhea are seeds. According to Unani, the seeds are stomachic, hypnotic, orexigenic, cardiotonic, and hepatotonic. The fruit's pericarp, or husk, is used to treat headaches and "heals stomatitis" The Unani medical system's use of giant cardamom (fruit of *A. subulatum*), also known as "Heel kalan" or "Bari Ilaichi," has been shown effective in treating digestive issues. The effectiveness of a crude methanolic extract and its various fractions, including EO, petroleum ether (60 to 80^0 C. ethyl acetate, and methanolic fractions, to prevent the stomach lesions brought on by aspirin, ethanol, and pylorus ligature was investigated in rats. Additionally, their impacts on pepsin content, stomach acid output, and wall mucus were noted. The extract and its fractions of *A. subulatum* considerably reduced the amount

of stomach lesions caused by ethanol, but not by pylorus ligation or aspirin (Jafri *et al.*, 2001).

2.11 Extraction of essential oils

The extraction of essential oils from plant material can be achieved by a number of methods. There are five methods of extraction:

- Expression
- Hydro or water – distillation
- Water and steam distillation
- Steam distillation
- Solvent extraction

There may be numerous modifications and improvements for each process, and the extraction may be carried out under reduced pressure (vacuum), ambient pressure, or excess pressure. The nature of the material, the stability of the chemical components, and the specifications of the desired result will all influence the choice of extraction technique (Lucchesi *et al.*, 2004; Tongnuanchan and Benjakul, 2014).

Citrus oil is only extracted from fruit peels using expression since heat easily damages the chemical components of the oil. A significant by product of the juice industry today is the manufacturing of citrus oil. The most cost-effective way to extract essential oils from herbs, spices, and aromatic plant material is through distillation. Distillation's key benefit is that it may be performed nearby the plant's production with a few pieces of relatively basic equipment. Large quantities of material can be processed quickly even in relatively remote locations (Elyemni *et al.*, 2019; Tongnuanchan and Benjakul, 2014).

The simplest of the three distillation techniques is water distillation. In a still pot, the plant material is combined immediately with water. To avoid the plant material sinking at the bottom and coming into touch with the hot base of the base and charring, a perforated grid may be put above the base of the still pot. The oils created using this approach typically have a darker color and harsher 'off-note' scents than oils made using other techniques, and as a result, they are typically of the lowest quality. Although it is likely the simplest and least expensive method of extracting essential oils, the effects of direct

heating and water contact have the most potential to change the amount of oil (Gavahian *et al.*, 2012; Tongnuanchan and Benjakul, 2014).

The fundamental still design for steam-and-water distillation is quite similar to that of water distillation. The plant material is tightly packed inside the still pot, which is perched above the boiling water on a grill or perforated plate. Due to the plant material not being suspended in water, the still pot's volume capacity is limited, although it would still be able to obtain a high packing density (Tongnuanchan and Benjakul, 2014).

Advantage of steam-and-water distillation over water distillation:

- Higher oil yield
- Oil component less susceptible to change due to wetness and thermal conductivity of the steel from the heat source
- The effect of refluxing is minimized
- Oil quality more reproducible.
- Faster process so more energy efficient (Mattoo, 1975; Sovova and Aleksovski, 2006).

Plant material is distilled using the steam produced outside the still in a standalone boiler by the technique of steam distillation. The plant material is supported on a perforated grid above the steam inlet, just like in the steam-and-water distillation method.

Advantages of steam distillation are as follow:

- The amount of steam and the quality of the steam can be controlled.
- Lower risk of thermal degradation as the temperature generally not above 100^0C.
- Most widely used process for the extraction of essential oils on a large scale.
- Throughout the flavor and fragrance supply industry, it is the standard method of extraction (Sovova and Aleksovski, 2006; Tongnuanchan and Benjakul, 2014)

Except for rose and orange blossom, flowers are typically solvent extracted rather than steam distilled. An isolate or essential oil fraction is favored over the entire oil in some applications (Tongnuanchan and Benjakul, 2014).

2.12 Storage stability of fruit juices

All food products are fundamentally unstable, and how well they maintain their quality depends on a variety of things, including how long they are stored at what temperature. This is acknowledged in all work on new products, in changes to or improvements to existing products, and in process modifications. Technologies for improving storage stability have advanced significantly in recent years. This can be seen visibly in the development of product holding systems for studying storage stability, starting with desiccators and progressing to programming storage cabinets and storage rooms that can be any location on earth's climate (Agcam *et al.*, 2018).

2.12.1 Change in color during storage

When maintained at 21°C and 37°C, products tend to darken whereas those kept at -17°C or -28°C appear slightly lighter. The millard reaction may be to blame for the color loss, however there may be further causes as well (Li *et al.*, 2018). Fruit juices' darker appearance is caused by browning. The millard reaction, a non-enzymatic chemical reaction between amino acids and reducing sugars, is what causes fruit juice to brown while it is being stored. The primary ingredient that causes food to turn brown while being stored is HMF (hydroxymethylfurfural), which is produced during the Maillard reaction as well as during caramelization (the pyrolysis of sugar) (Singh and Sharma, 2017).

2.12.2 Changes in TSS during storage

Fruit juice storage has been shown to experience a steady increase in TSS value over all storage conditions, which may be due to the ongoing hydrolysis of polysaccharides and acids (Bhardwaj and Pandey, 2011). The gradual lengthening of storage duration in response to an increase in TSS, which may be brought by more intense polysaccharide hydrolysis. Additionally, a small increase in TSS was seen during the production of RTS with mixed fruits (Deka and Sethi, 2001; Sharma *et al.*, 2011). However, this increase in TSS is related to storage temperature, and it has been seen that there is a direct correlation between the two. According to the Le Chatelier Principles of chemical reactions, this may be related to the slower rate of hydrolysis of sugars, polysaccharides, and organic acids at lower temperatures (Singh and Sharma, 2017).

2.12.3 Change in ascorbic acid (vitamin C) during storage

A crucial nutrient with antioxidant properties, vitamin C offers defense against free radicals (Esteve *et al.*, 2005). All consumables degrade ascorbic acid during storage, and this can happen both aerobically and anaerobically. However, aerobic degradation occurs at a rate that is 100–1000 times greater than anaerobic degradation (Krishnaveni *et al.*, 2001). Heat and light can damage vitamin C (Davey *et al.*, 2000) , Since first order kinetics governs vitamin C concentration, storage duration has an impact on vitamin C content (Burt and Reinders, 2003; Singh and Sharma, 2017). Ascorbic acid and other temperature-sensitive components in fruit juices degrade during regular room-temperature storage, and when they oxidize, they can produce an odd flavor (Oliveira *et al.*, 2012).

2.12.4 Change in acidity during storage

The organic acids that are primarily contained in fruits are included in the titratable acidity of fruits or fruit juice. These organic acids are beneficial for increasing the shelf life of fruit juice while being stored and have significant nutritional properties. These, however, are extremely sensitive to changes in temperature, storage conditions, and time. When organic acids are stored, they degrade, which may be caused by invertase enzymes turning them into sugar and salt (Singh and Sharma, 2017). Singh *et al.* (2005) argued that the amount of titrable acid in bael beverage drastically decreased after six months of storage. A similar pattern was also discovered in a juice mixture made of bottle guard and basil leaf juice. Acidity rise is caused by the creation of acid by sugars, the breakdown of polysaccharides and oxidation of reducing sugars, or by breakdown of pectic components (Majumdar *et al.*, 2011).

2.12.5 Changes in pH during storage

Fruit juice's pH is a negative function of its acidity; as a result, as pH decreases during storage, fruit juice's acidity also increases (Rehman *et al.*, 2014). The acid hydrolysis of the poly-saccharides into mono- and di-saccharides, which are in charge of increasing sweetness and increasing sourness, may be the cause of the pH decrease with longer storage of kinnow juice (Dhaka *et al.*, 2016).

2.12.6 Change in sugar during storage of RTS

The fruit juice contains a variety of reducing and non-reducing sugars, both of which are susceptible to interconversion processes that occur during storage. The conversion of polysaccharides including pectin, cellulose, and starch into simple sugars may be the cause of the rise in total sugars reported by (Singh and Sharma, 2017). They had suggested that the progressive conversion of non-reducing sugar and acids into reducing sugars may be the cause of an increase in reducing sugar during storage .Citrus juice undergoes a large rise in reducing sugar while being stored, which may be caused by the acid hydrolysis of sucrose (a non-reducing sugar) into glucose and fructose (Ahmed *et al.*, 2008).

Part III

Materials and methods

3.1 Materials

Fresh, mature and ripen variety of plum (*Prunus Cerasifera)* were purchased from local market of Dharan. Black pepper (*Piper nigrum)* was purchased from local market of Damak, Jhapa district. Cardamom (*Amoum sublatum roxb.)* pods were collected from Madi, Sankhuwasabha district.

3.1.1 Chemicals, glasswares and equipments

All the chemicals, glassware and equipment were used for the work as available from the laboratory at central campus of technology, Dharan. Details of chemicals and equipment are presented in Appendix A.1 and A.2 respectively.

3.2 Methods

3.2.1 Preparation of plum juice

a. Sorting/Grading

Unripen, damaged were sorted out from undamaged and sound fruits.

b. Washing

The selected fruits were washed with clean tap water to remove dusts, adhered impurities, mud etc.

c. Peeling

Manual peeling is done to remove outer skin of plum.

d. Seed removal

Hand removal.

e. Juice extraction

The juice was extracted by using the screw press juicer. The juice was filtered using a clean muslin clothes.

3.2.2 Preparation of RTS and addition of essential oil

The preparation of ready to serve juice was prepared as per (Paudel, 2022) with slight modification. Briefly describe self-explanatory flow diagram of plum RTS preparation in Fig 3.1

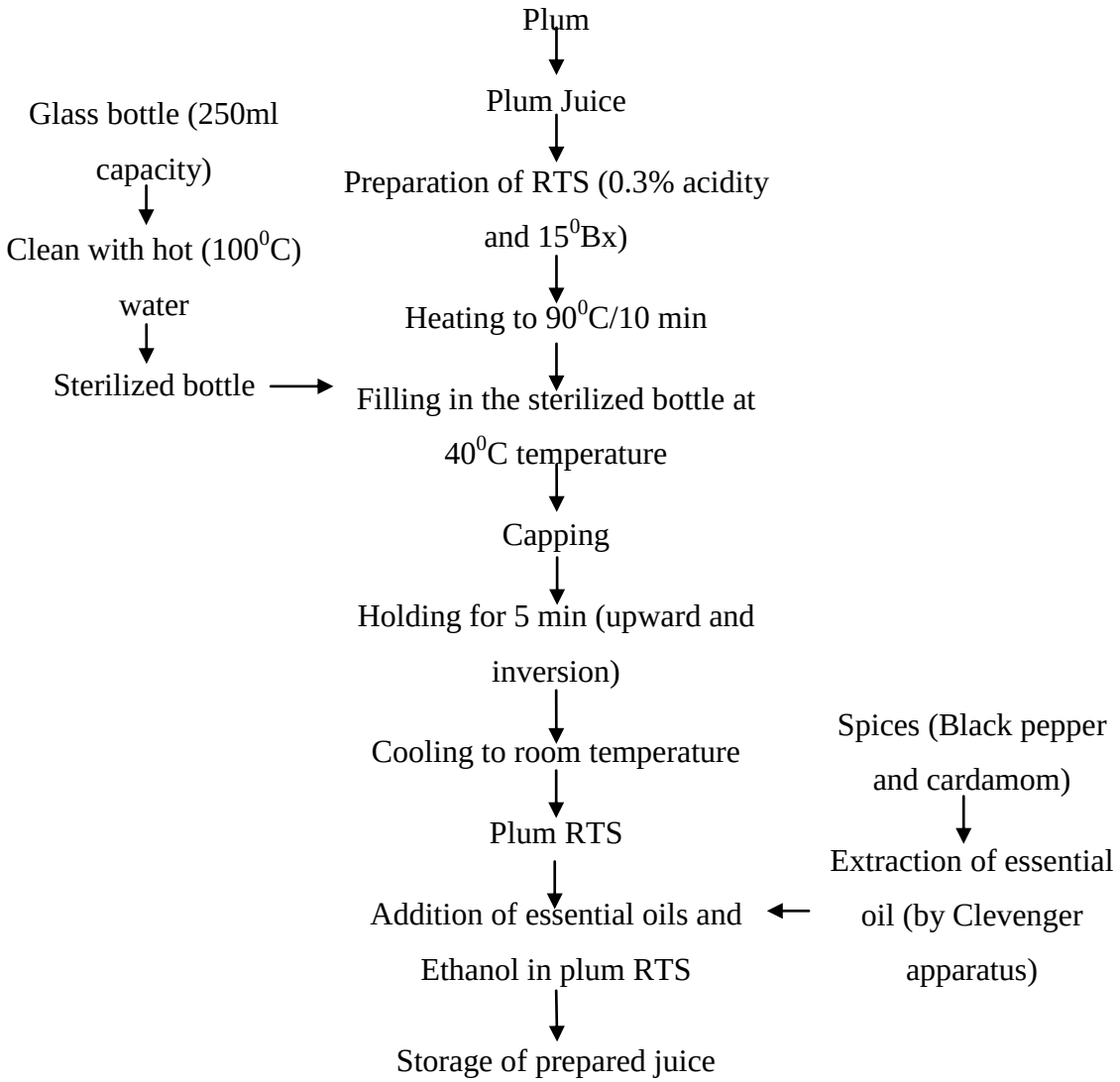

Fig. 3.1 Flow sheet for preparation of plum RTS

Source: Paudel (2022)

3.2.3 Sample formulation

As the RTS was prepared and divided into four equal batches of 250 ml each in bottles. The sample A was prepared without essential oil, sample B was prepared by adding 100 μL of ethanol. Sample C and D were prepared 90 μL of ethanol with 10 μL of essential oil

samples was predissolved in 90 μL of ethanol and then they were homogenously mixed with two batch of plum RTS. The predissolved of 10 μL of essential oil with 90 μL of ethanol was on the reference with slight modification to the research done by Kapoor *et al.* (2008). These samples were stored in refrigerator temperature for 28 days. The pH, titrable acidity, ascorbic acid, TSS, total sugar, reducing sugar, antioxidant activity and microbial growth were analyzed out at fixed interval for 28 days. B_p denoted as black pepper and C_d denoted as cardamom the sample formulation for storage of plum RTS is shown in Table 3.1.

Table 3.1 Plum RTS formulation

	A	B	C	D
Plum RTS (ml)	250	250	250	250
Ethanol (μL)	0	100	90	90
Essential oil B_p (μL)	0	0	10	0
Essential oil C_d (μL)	0	0	0	10

3.2.4 Analytical methods

3.2.4.1 Moisture content

The moisture determination of the plum was determined carried out by hot air oven at $(103\pm2)^0$C to the constant weight and moisture content of spice was determined by using Dean and stark apparatus (Ranganna, 1986).

3.2.4.2 Total soluble solids

Total soluble solid was determined with hand refractometer (0-30 ºBx) and values were expressed as degree brix according to Ranganna (1986).

3.2.4.3 Total and reducing sugar

Total sugar and reducing sugar was determined by Lane and Enyon's method from the process given by Ranganna (1986).

3.2.4.4 Titrable acidity and pH

Acidity of plum was determined by titration with the standard sodium hydroxide (0.1N) solution and expressed as % malic acid (Rangana,2008). pH was determined using pH meter.

3.2.4.5 Ascorbic acid

The ascorbic acid content of plum was determined by 2,6-Dichlorophenol-indophenol visual titration method (Ranganna, 1986).

3.2.4.6 Crude protein

The crude protein of sample was determined by measuring the total nitrogen content by Kjeldahl method from the process given by Ranganna (1986)

3.2.4.7 Crude fiber

Crude fiber of black pepper and cardamom was determined by the method described in Ranganna (1986).

3.2.4.8 Crude fat

Crude fat of black pepper and cardamom was determined by solvent extraction method the method described in Ranganna (1986).

3.2.4.9 Total ash

Total ash of black pepper and cardamom was determined by dry ashing as described in Ranganna (1986).

3.2.4.10 Total carbohydrate

Total carbohydrate of black pepper and cardamom was determined by difference method (AACC., 2016).

Carbohydrate (%) = 100- (%crude protein + %crude fat +% ash +%crude fiber + %moisture).

3.2.4.11 Hardness

Total hardness of water was determined by titration with EDTA using Eriochrome black T as an indicator and titrating till sky blue end point (Rai and KC, 2007).

3.2.4.12 Total dissolved solids (TDS)

TDS of water was determined as the dried residue left after evaporation of filtered water sample as given by Rai and KC (2007).

3.2.4.13 Alkalinity

Total alkalinity of water was determined by titration with HCl using methyl orange to pink end point (Rai and KC, 2007).

3.2.5 Yield of essential oil by hydro-distillation

The percentage yield of black pepper and Cardamom was computed by using weight difference by using the formula given below:

$$\% \text{ yield of essential oil} = \frac{\text{weight (ml) of essential oil after hydrodistillation}}{\text{Weight of sample before hydrodistillation}} \times 100\%$$

3.2.6 Microbial analysis

The total microbial count and yeast mold counts, total plate count were examination of juice samples using the plate count agar and potato-dextrose agar , respectively, were adopted (Aneja *et al.*, 2010). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37^0C/48h) (AOAC, 2005).

3.2.6.1 Total microbial count

The total microbial count was performed using plate count agar. In a petri plate, 10 mL of agar and 1 mL of sample were combined completely (Aneja *et al.*, 2010). Then it was incubated at 37°C.

3.2.6.2 Yeast and mold count

The experimental techniques for counting yeast and mold are identical. The sole variation was the use of a potato-dextrose agar medium instead of nutritional agar (Aneja *et al.*, 2010).

3.2.7 Free radical scavenging activity

DPPH free radical scavenging activities (antioxidant activities) of extracts was determined by the method described by vignoli *et al*. (2011). Using 80% methanol, several extract dilutions were created. Then, 2ml of 0.1 mM DPPH solution was combined with 1ml of the extract. After 30 minutes of incubation in the dark, the absorbance was measured at 517 nm. Finally, the following equation was used to calculate the percentage of scavenging activity.

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

Where Ac is the absorbance of control and As is the absorbance of the test sample.

3.2.8 Statistical analysis

The experiment was conducted in triplicates and all measurements were made in triplicate. A two-way ANOVA was used to examine the data with a 5% level of significance agricultural Trust, Roth Amsted Experimental, and GenStat 5 Release 12.1 software package were used to compare the treatment means. Independent t-test was performed by using IBM SPSS 20 (IMB Corporation, Marlborough, MA, USA). Microsoft corporation LTSC MSO (version 2207) developed by Microsoft Corporation (2021) was used for data documentation, calculation of mean and SD, graph plot of data calculated from the lab.

Part IV

Result and discussion

The present study was carried out to compare the preservative effect of essential oil extracted from black pepper and cardamom as a natural preservative on the plum (RTS). The RTS was divided into 250 ml batches and sample were prepared as sample A without any additives (essential oil), sample B with 100 µL ethanol, sample C with 90 µL ethanol and 10 µL essential oil extracted from black pepper and sample D with 90 µL ethanol and 10 µL essential oil extracted from cardamom. The samples were then kept in the refrigerator at 5 ± 1ºC for 28 days. The changes in pH, % titrable acidity, % ascorbic acid, TSS,% total sugar, % reducing sugar, % RSA and microbial analysis were examined in the interval for 7 days.

4.1 Chemical analysis of plum fruit

Chemical composition of plum (*Prunus cerasifera*) was determined and the chemical composition of fresh plum juice is presented in Table 4.1

Table 4.1 Chemical composition of fresh plum juice per 100ml

Components	Plum juice
Moisture (%)	84.6 ± 0.04
TSS ($^{\circ}$Bx)	9.5 ± 0.01
Total acidity (% as malic acid)	1.05 ± 0.02
pH	2.98 ± 0.02
Vitamin C (mg/100g)	12.4 ± 0.03
Reducing sugar (%dextrose)	4.04 ± 0.05
Total sugar (%)	8.8 ± 0.07
Juice yield (% total fresh weight)	53.4 ± 0.12
Antioxidant activity (% RSA)	34.67 ± 0.02
Crude fiber (%)	1.2 ± 0.12
Crude Protein (%)	0.5 ± 0.21
Crude fat	0.8 ± 0.16

*****Values were the means ± standard deviations of the three determinations.

The chemical composition of plum juice were analyzed and result revealed that moisture content was 84.6%, TSS 9.5^{0}BX, acidity 1.05 %, pH 2.98, vitamin C 12.4 (mg/100g) reducing sugar (%) 4.04, 53.4% juice yield ,16.67 % RSA, respectively result were similar with Gull *et al.* (2023).

4.2 Analysis of drinking water

The Chemical composition of water shows pH 6.9, hardness 54 ppm, TDS 171 (mg/L) , alkalinity 87 ppm respectively was given by Haldar *et al.* (2016) and shown in Table 4.2.

Table 4.2 Composition of drinking water

Parameter	Value
pH	6.9 ± 0.02
Hardness (ppm)	54 ± 0.24
TDS (mg/L)	171 ± 0.17
Alkalinity (ppm)	87 ± 0.19
Coliform	Nil

*****Values were the means ± standard deviations of the three determinations

4.3 Chemical composition of plum RTS

The chemical composition of fresh plum RTS was maintained at TSS 15°Bx, 0.3% acidity as presented in Table 4.3.

Table 4.3 Chemical composition of plum RTS

Parameter	Values
TSS (^{0}Bx)	15 ± 0.01
Acidity (% malic acid)	0.3 ± 0.02
Ascorbic acid (mg/100g)	8.76 ± 0.17
pH	3.57 ± 0.01
Total sugar (%)	12.49 ± 0.15
Reducing sugar (%)	3.2 ± 0.16
Antioxidant activity (%RSA)	9.42 ± 0.19

*****Values were the means ± standard deviations of the three determinations

4.4 Chemical composition of black pepper and cardamom

The proximate composition of black pepper and cardamom were determined, and the result were presented in Table 4.4.

Table 4.4 Proximate composition of black pepper and cardamom

Parameters	Black pepper	Cardamom
Moisture (g/100 g)	9.94 ± 0.10^a	10.60 ± 0.10^b
Crude protein (% db)	9.30 ± 0.08^a	12.40 ± 0.03^b
Crude fat (% db)	8.00 ± 0.05^a	9.00 ± 0.13^b
Total ash (% db)	4.20 ± 0.06^a	6.80 ± 0.05^b
Crude fiber (% db)	12.40 ± 0.07^a	12.6 ± 0.05^b
Carbohydrate (% db)	66.16 ± 0.17^a	59.4 ± 0.21^b
Antioxidant activity (% RSA)	43.1± 0.70 ^{a}	72.48 ±0.24^b
Yield (% essential oil)	2.25 ± 0.03^a	3.30 ± 0.08^b

*****Values were the means ± standard deviations of the three determinations. Means with similar superscripts are not significantly different at α =0.05 between rows. Significance testing results are presented in Appendix B.

The moisture content of the black pepper was found to be 9.94 which is in the normal range as described by Kunnumakkara *et al.* (2009). The moisture content of cardamom was found within the range of 9.5-11% as reported by Amma *et al.* (2010). The moisture content was found significant different ($p<0.05$) among black pepper and cardamom. The protein content of black pepper was within the range as described by Tainter and Grenis (2001). Protein content of black pepper and cardamom is highly influenced by the environment conditions, available nitrogen, and the variety genotype. Protein content of cardamom was within the range as described by Y. Rao *et al.* (1993). The crude protein was found significant different ($p<0.05$) among black pepper and cardamom.

The crude fat, total ash of black pepper was found significantly different ($p<0.05$) with cardamom. The crude fat of the black pepper and cardamom was found 8.0% and 9.0% respectively. The crude fat in black pepper was found to be slightly lower as reported by Vijayan and Thampuran (2000).The crude fat was found in cardamom within the range as described by Y. S. Rao *et al.* (1993). The total ash content in black pepper and cardamom is found to be 4.2% and 6.8%. The total ash in black pepper and cardamom was within the range as reported by Tongnuanchan and Benjakul (2014). The crude fiber content of cardamom was found significantly different ($p<0.05$) to black pepper. Tainter and Grenis (2001) stated the amount of crude fiber in the black pepper varies between 10.79 to 18.60% which was in our range. The crude fiber content in cardamom was within the range as described by Jafri *et al.* (2001).

Black pepper had significant different ($p<0.05$) in carbohydrate, % antioxidant activity and yield (% Essential oil) as compared to cardamom. The carbohydrate content of black pepper and cardamom was found to be 66.1% and 59.4% respectively where the black pepper had the highest and cardamom had least carbohydrate content. The carbohydrate content of black pepper was within the range as reported by Nisha *et al.* (2009) . The carbohydrate content in cardamom was within range as describe by Jafri *et al.* (2001) and slightly higher as reported by Amma *et al.* (2010). The antioxidant activity of cardamom was found higher as compared to black pepper as reported by Rinzler (1990). The higher in antioxidant of cardamom was due to chemical composition. Cardamom contains higher

concentration of antioxidants, such as polyphenols as reported by P. N. Ravindran and kallupurackal (2001). The yield of essential of black pepper and cardamom was found to be 2.25 and 3.3% respectively. The yield of essential oil of black pepper and cardamom was within the range as revealed by Gupta *et al.* (1984).

4.5 Comparison of different parameters during storage

The RTS was divided into 250 ml equal batches and sample were prepared as sample A, sample B, sample C and and sample D. The samples were then kept in the refrigerator at 5±1ºC for 28 days. The changes in pH, titrable acidity, ascorbic acid, TSS, total sugar, reducing sugar, antioxidant activity and microbial analysis were examined in the interval for 7 days.

4.5.1 Comparison of pH during storage of plum RTS

The change in pH was studied in four different samples. On the first day, pH was found to be 3.57 in plum RTS. The result of pH in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^{0}C was carried out and statistically analyzed as shown in Appendix C.2.

In First day of analysis, Statistical analysis showed that all the samples A, B, C and D were no significant different ($p>0.05$) with each other. On 7^{th} days of analysis, sample A was significant different ($p<0.05$) with samples B, C and D whereas samples B, C, D shows no significant difference ($p>0.05$) with each other. In 14^{th}, 21^{st} and 28 day, samples A and B were significant different ($p<0.05$) with each other but sample C and D were no significant different ($p>0.05$) with each other as revealed by Dhaka *et al.* (2016).During storage pH values gradually decreases, this might be due to the production of organic acids by the action of ascorbic acid on sugar and protein content of RTS drinks. In blank sample (A), the value of pH decreases of juice from 3.57 to 3.09 in the 28^{th} days of storage whereas there was least decrease in pH of sample having essential oil of cardamom (D) and black pepper(C) as similar result reported by Rehman *et al.* (2014).

4.5.1.1 Trend of pH change during storage

The Trend of pH change with respect to time (days) during storage was shown in Fig 4.1.

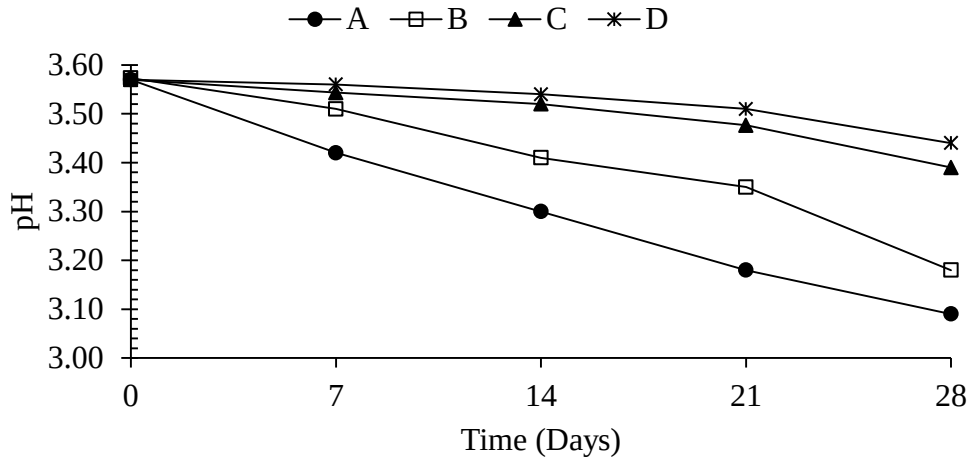

Fig 4.1 Trend of pH change with respect to time (days) during storage

The pH of RTS was found to be decreasing during 28 days of storage at $(5\pm1)^0$C. It was observed that pH decreases from 3.57 to 3.09 in sample A, 3.57 to 3.18 in sample B, 3.57 to 3.39 in sample C and 3.57 to 3.44 in sample D similar trends had been reported by Dhaka *et al.* (2016).

Above trend line shows that pH was continuously decreasing while the acidity was increasing. The blank sample RTS goes on decreasing compare to other sample and less decreasing in the sample having cardamom essential oil .The decrease in pH and increase in acidity was as suggested by Singh and Sharma (2017). From the trend line it was found that, cardamom essential shows less decrease in pH compare to black pepper.

4.5.2 Comparison of % titrable acidity during storage of plum RTS

The acidity of plum RTS was maintained at 0.3% and the change in titrable acidity of samples were studied in 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day stored at a refrigeration temperature 5 ± 1^0C. The statistical analysis of change in titrable acidity data was presented in Appendix C.4

In 0^{th} and 7^{th} days, statistical analysis shows that there was no significant difference ($p>0.05$) between sample A, B, C and D. In 14^{th} days, statistically analysis showed that sample A, B, C was no significant different ($p>0.05$) with each other whereas sample D was significant different ($p<0.05$) with samples A and B but no significant different ($p>0.05$) with sample C.

In 21st days, statistical analysis showed that samples B, C and D were no significant different ($p>0.05$) with each other whereas sample A show significant different ($p<0.05$) with samples C and D but no significant different ($p>0.05$) with B. In 28th days, statistical analysis showed that sample A and B were no significant different ($p>0.05$) with each other but significant different ($p<0.05$) with samples C and D whereas sample C and D shows no significant different ($p>0.05$) with each other as explained by Kapoor *et al.* (2008).

During storage of plum RTS, the value of acidity gradually increases due to the formation of organic acid from ascorbic acid and malic acid inherently present in plum juice and also due to the higher amount of phenolic content in beverage (Naik *et al.*, 2009). In blank sample (A), the value of acidity increases from 0.3% and reached to 0.48% in the 28th days of storage whereas there was less increase in acidity in sample having essential oil of black pepper (C) and cardamom (D) (Majumdar *et al.*, 2011).

4.5.2.1 Trend of titrable acidity change during storage

The trend of change in titrable acidity with respect to time during storage is shown in Fig 4.2**.**

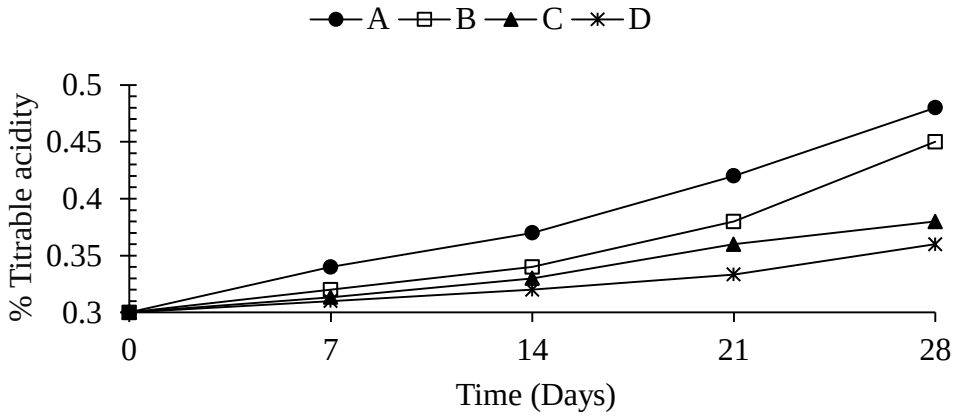

Fig 4.2 Trend of titrable acidity change with respect to time (days) during storage

The titrable acidity content of RTS samples were found to be increase during 28 days of storage at 5 ± 1^0C. It was observed that titrable acidity increases from 0.3 to 0.48% in sample A, 0.3 to 0.45 % in sample B, 0.3 to 0.38 % in sample C and 0.3 to 0.36 % in sample D similar trends have been reported by Singh and Sharma (2017).

The trend line shows that acidity was continuously increasing as the pH goes on decreasing. The blank sample RTS sharply increases compare to other sample while the sample having essential oil of black pepper and cardamom was slightly increasing. The decrease in pH and increase in acidity content shows the similar result as reported by Singh and Sharma (2017).

4.5.3 Comparison of ascorbic acid during storage of plum RTS

The change in ascorbic acid was studied in four different samples. Ascorbic acid of plum RTS was found to be 8.76%. Evaluation of ascorbic acid was an index of the nutrient quality of fruits, it is much more sensitive to various mode of degradation in food processing and storage as reported by Davey *et al.* (2000). The result of ascorbic acid in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^{0}C was carried out and statistically analyzed as shown in Appendix C.6.

In 0^{th} day, there was no significant different ($p>0.05$) with samples A, B, C and D. In 7^{th}, 14^{th} and 21^{st} days, statistical analysis showed samples A and B were significant different ($p<0.05$) with each other whereas samples C and D shows no significant different ($p>0.05$) with each other. In 28^{th} day, all the samples A, B, C and D were significant different ($P<0.05$) with each other (Davey *et al.*, 2000; Kapoor *et al.*, 2008).

During storage ascorbic acid content decreases due to oxidation of ascorbic acid to dehydroascorbic acid by the enzyme ascorbinase (Biswas *et al.*, 2016). In blank sample (A) ascorbic acid decreases juice from 8.76 and reached to 2.85 in 28^{th} days of storage. There was less decrease in ascorbic acid sample having essential oil of black pepper and cardamom. The possible reason may be that the cardamom (D) essential and black pepper (C) essential oil delayed the oxidation of ascorbic acid to dehydroascorbic acid by the enzyme ascorbinase to some extend (Krishnaveni *et al.*, 2001).Incorporation of air into juice during extraction has also been recognized as the cause of ascorbic acid loss (Farnworth et al., 2001).

4.5.3.1 Trend of ascorbic acid change during storage

The trend of change in ascorbic acid with respect to time during storage was shown in Fig 4.3.

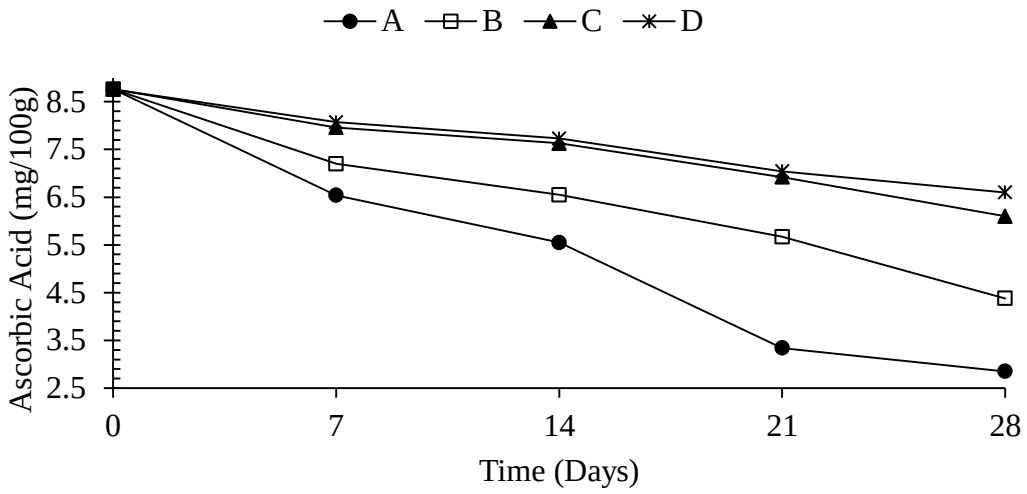

Fig 4.3 Trend of ascorbic acid change with respect to time (days) during storage

Vitamin C content of RTS samples were found to be decrease during 28 days of storage at 5 ± 1^0C. It was observed that ascorbic acid decreases from 8.76 to 2.85 mg/100g in sample A, 8.76 to 4.38 mg/100g in sample B, 8.76 to 6.1 mg/100g in sample C and 8.76 to 6.6 mg/100g in sample D similar trends have been reported by Thakur *et al.* (2018).

Vitamin C is an important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve *et al.*, 2005). It is also considered as an indicator of the nutritional quality of juices. Storage temperature, type of processing and packaging materials affect the rate of ascorbic acid degradation during storage (Bull *et al.*, 2004). From above trend line, it was observed highly decrease of ascorbic acid content in blank sample and less decrease in the plum RTS having black pepper and cardamom essential oil (Davey *et al.*, 2000).

4.5.4 Comparison of TSS during storage of plum RTS

The TSS of plum RTS was maintained at 15^0Bx and the change in TSS of samples were studied in 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day stored at a refrigeration temperature $(5\pm1)^0$C. The statistical analysis of change in TSS data was presented in Appendix C.8.

In first day, statistical analysis showed that was no significant difference ($p>0.05$). between sample A, B, C and D. In 7^{th} days, statistical analysis showed that samples A, B and D were significant difference ($p<0.05$) with each other whereas samples B and C were no significant different ($p>0.05$) with each other. In 14^{th} days, statistical analysis showed

that samples A and B was significant different ($p<0.05$) with each other whereas samples C and D shows no significant different ($p>0.05$) with each other as reported by (Deka and Sethi, 2001).

In 21st and 28th days, statistical analysis showed that there was significant different ($p<0.05$) between samples A, B, C and D. There was increase in TSS during storage of plum RTS is due to hydrolysis or inversion of non-reducing to reducing sugar. In blank sample, it was found that TSS increases from15^0 Bx and reached to 17^0 Bx during 28th days of storage whereas there was less increase in TSS of sample having an essential oil of black pepper (C) and cardamom sample (C) (Singh and Sharma, 2017).

4.5.4.1 Trend of total soluble solids (TSS) change during storage

The trend of total soluble solids (TSS) change with respect to time (days) during storage was shown in Fig 4.4.

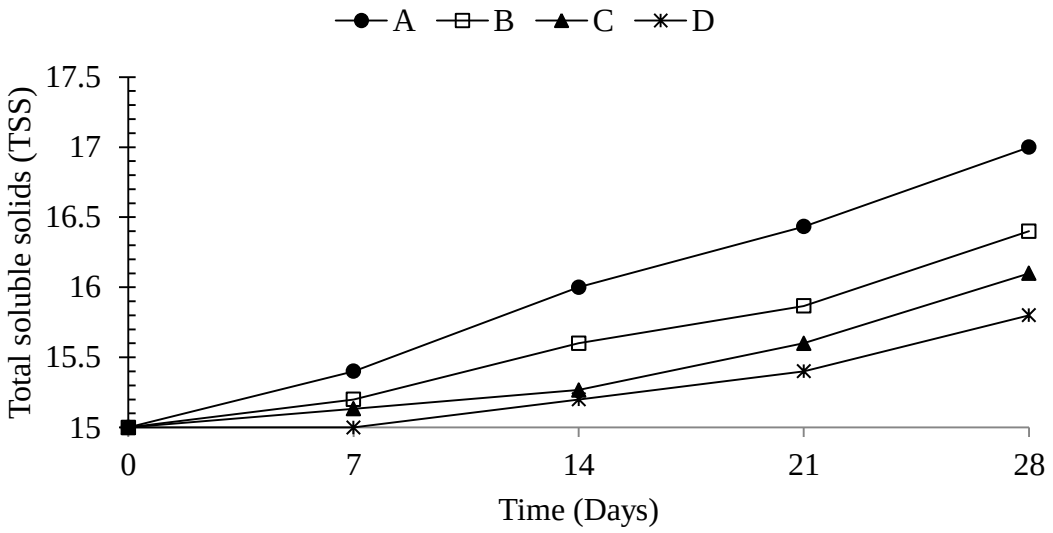

Fig 4.4. Trend of TSS change with respect to time (days) during storage

The total soluble solids (TSS) content of RTS samples were found to be increase during 28 days of storage at 5±1^{0}C. It was observed that TSS increases from 15 to 17 ^{0}Bx in sample A, 15 to 16.4^0 Bx in sample B, 15 to 16.1 ^{0}Bx in sample C and 15 to 15.8 ^{0}Bx in sample D similar trends have been reported by Bull *et al.* (2004). From the above trend line, it was observed that the RTS sample having essential oil of both black pepper and

cardamom data shows least increase in TSS whereas blank sample shows sharply increase in TSS during storage similar trend reported by Deka and Sethi (2001).

For the preservation of good juice quality, retention or rise in TSS level of juice during storage is preferred as per Bhardwaj and Pandey (2011) The above trend line depicts the pattern of TSS increasing over time, and identical outcomes were seen when mixed fruit RTS was similar result as reported by Bhurtel *et al*. (1996). The TSS value had been shown to gradually increase during the storage of plum RTS. According to the Le Chatelier principles of chemical reaction, this may be related to the slower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperatures (Sharma *et al.*, 2011).

4.5.5 Comparison of % total sugar during storage of plum RTS

The change in total sugar was studied in four different samples. Total sugar of plum RTS was found to be 12.49%. The result of total sugar in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^{0}C was carried out and statistically analyzed as shown in Appendix C.10.

Statistical analysis showed highly significant results among different treatments and storage intervals. In first days, statistical analysis showed there was no significant different ($p>0.05$) between samples A, B, C and D. In 7^{th} days, statistical analysis showed that there was no significant different ($p>0.05$) between samples B, C, D whereas sample A shows significant different ($p<0.05$) with samples C and D but no significant different ($p>0.05$) with sample B.

In 14^{th}, 21^{st} and 28^{th} day, statistical analysis showed that samples A and B were significant different ($p<0.05$) with each other but samples C and D shows no significant different ($p>0.05$) with each other. There was continuous increase in total sugar total sugar upto 28 days of storage. The increase in total sugar might be due to hydrolysis of polysaccharides like pectin, cellulose and starch into simple sugar. In blank sample (A), % of total sugar was sharply increases from 12.49% and reached to 14.8% during the 28 days of storage whereas there was least rise in total sugar having essential oil samples of black pepper (C) and cardamom (D) (Ahmed *et al.*, 2008).

4.5.5.1 Trend of % of total sugar change during storage

The Trend of total sugar change with respect to time (days) during storage was shown in Fig 4.5.

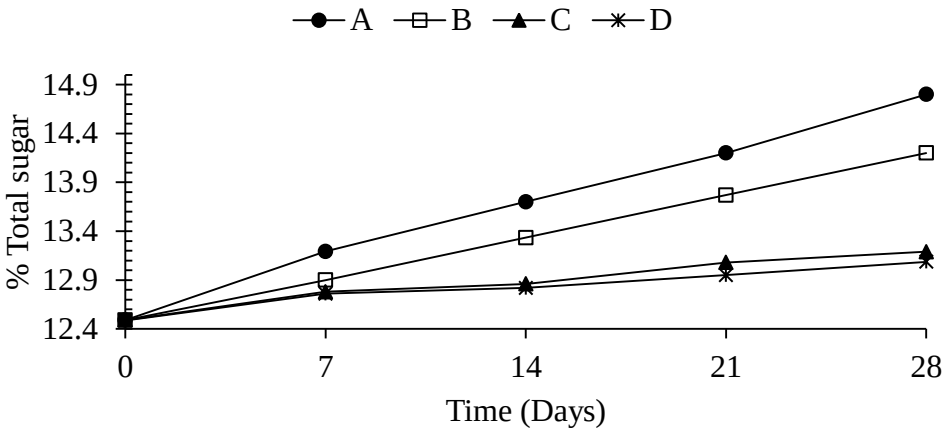

Fig 4.5 Trend of % total sugar change with respect to time (days) during storage

The % of total sugar content of RTS samples were found to be increase during 28 days of storage at 5 ± 1^0C. It was observed that % of total sugar increases from 12.49 to 14.8 % in sample A, 12.49 to 4.2 % in sample B, 12.49 to 13.19 % in sample C and 12.4 to 13.09 % in sample D similar trends have been reported by Singh and Sharma (2017).

The hydrolysis of polysaccharides causes the gradually rising levels of lowering sugars. Additionally, it could be brought on by dehydration with moisture loss and a reduction in fruit juice acidity due to physiological changes during storage as shown by Hussein *et al.* (2003). According to research, this progressive rise in the overall sugar percentage may be caused by fruits becoming more dehydrated, which led to a concentration of juice (Badshah and Safi, 1994). From the above trend line, it was reported that the blank sample show gradual increase in % of total sugar whereas the RTS samples having essential of black pepper and cardamom show least rise in % of total sugar.

4.5.6 Comparison of % reducing sugar during storage of plum RTS

The change in reducing sugar was studied in four different samples. Reducing sugar of plum RTS was found to be 3.22%. The result of reducing sugar in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^0C was carried out and statistically analyzed as shown in Appendix C.12.

Statistically analysis showed highly significant results among different treatments and storage intervals. In first days, statistical analysis showed there was no significant different ($p>0.05$) between sample A, B, C and D. In 7^{th} days, statistical analysis showed that samples A and B were significant different ($p<0.05$) with each other whereas samples C and D shows no significant different ($p>0.05$) with each other.

Statistical analysis showed that there was similar result in 14^{th}, 21^{st} and 28^{th} day, as there was significant different ($p<0.05$) between samples A, B, C and D. the gradual increase in reducing sugar might be due to hydrolysis of polysaccharides .Moreover, it could be due to the dehydration as a result of moisture loss and gradual inversion of non-non reducing sugars and acids into reducing sugar (S. Singh *et al.*, 1994).In blank sample(A), % of reducing sugar increases during the storage of juice from 3.22% and reached to 5.18% during the 28 days of storage whereas there was slight increase in reducing sugar in the sample having essential oil of black pepper (C) and cardamom (D) (Singh and Sharma, 2017).

4.5.6.1 Trend of % of reducing sugar change during storage

The trend of reducing sugar change with respect to time (days) during storage was shown in Fig 4.6.

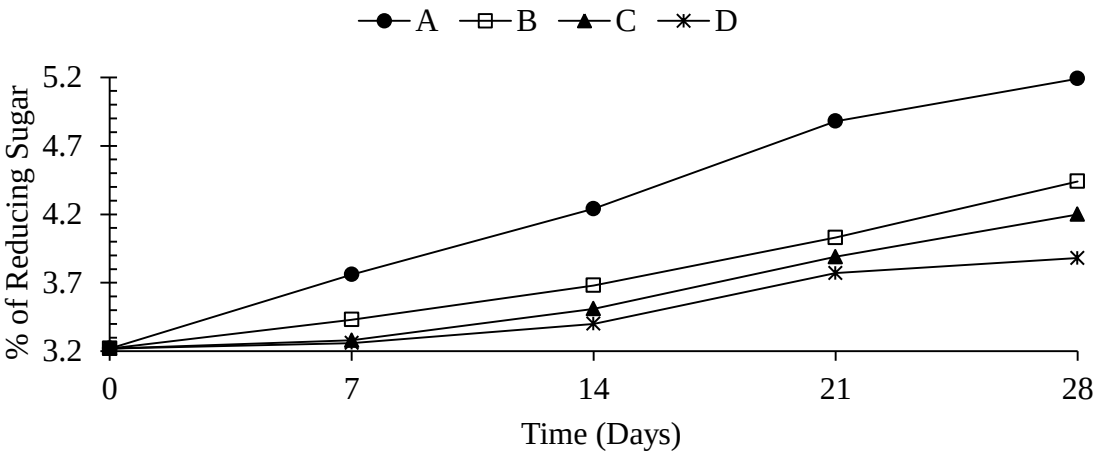

Fig 4.6 Trend of % reducing sugar change with respect to time (days) during storage

The % of reducing sugar content of RTS plum were found to be increase during 28 days of storage at 5 ± 1^0C between four different samples. It was observed that % of reducing sugar increases from 3.22 to 5.18 % in sample A, 3.22 to 4.44 % in sample B, 3.22 to 4.2

% in sample C and 3.22 to 3.88 % in sample D similar trends have been reported by Singh and Sharma (2017).

The conversion of polysaccharides into water soluble sugar during glycolysis may be the rise in sugar content. Similar alterations were also noted in the above trend line as followed by Mattoo (1975) demonstrating complete hydrolysis of starch into soluble sugars including glucose, fructose, and sucrose. The gradual inversion of non-reducing sugars and acids into reducing sugars may explain an increase in reducing sugar with longer storage times (Singh and Sharma, 2017). Above trend lines shows that the blank sample show gradual increase in % of reducing sugar and samples having essential oils show least rise in % of reducing sugar. Hence, cardamom possess remarkable best preservative action compare to black pepper.

4.5.7 Comparison of antioxidant activity during storage of plum RTS

The change in antioxidant activity (% DPPH inhibition) was studied four different samples using DPPH radical scavenging activity at 517nm.The antioxidant activity was found to be 9.42%. The result of antioxidant activity in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^{0}C was carried out and statistically analyzed as shown in Appendix C.14.

Statistical analysis showed highly significant results among different treatments and storage intervals. In 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days similar result was shown by statistical analysis as samples A, B, C and D were significantly different ($p<0.05$) with each other. The control sample B shows shows the increase antioxidant activity from 9.42 and reached to 10.44% and control sample A decreases from 9.42 and reached to 8.97%. There was rise of antioxidant in the sample having essential oils of black pepper (C) and cardamom (D) essential oil might be due to the presence of different polyphenolic compounds, flavonoids, phytochemical, anti- inflammatory, and other chemical compounds in the essential oil was reported by Pandareesh *et al.* (2018).

4.5.7.1 Trend of % antioxidant activity during storage

The trend of % antioxidant activity with respect to time (days) during storage was shown Fig 4.7.

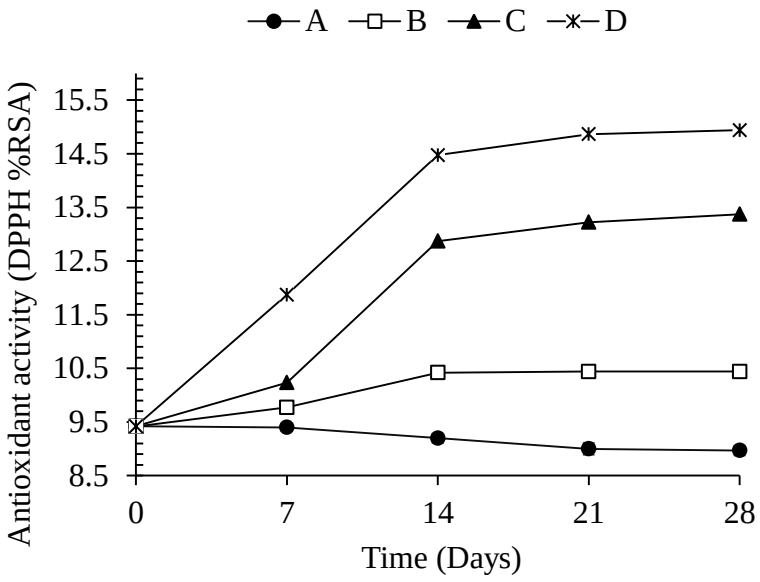

Fig 4.7 Trend of %RSA antioxidant activity respect to time (days) during storage

The % RSA antioxidant activity of RTS plum were found to be decrease in control (A) sample whereas increase trends in control (B) and samples having the black pepper (C) and cardamom sample (D) during 28 days of storage at 5 ± 1^0C. It was observed that % of RSA decreases from 9.42 to 8.97 % in sample A, increases from 9.42 to 10.44 % in sample B, increases from 9.42 to 13.37 % in sample C and increases 9.42 to 14.94 % in sample D similar trend line as reported by Nadeem *et al.* (2018).

Above trend line suggested that, there was slight decrease trend of antioxidant in the control sample (A) and in the samples B, C and D there was increasing of % RSA antioxidant activity. The decrease trend of % RSA in sample A was due to the exposure to oxygen, light exposure, temperature fluctuations, enzymatic activity, pH changes and other. The increase trend of % of antioxidant activity of control sample (B) is due to the content of polyphenols and other antioxidants present in ethanol. The sample having black pepper and cardamom essential oil shows increase pattern for 14 days and then steady increase after 14 days .The increase of antioxidant in samples C and D might be due to the presence of different polyphenolic compounds, flavonoids compounds as revealed by R. Singh *et al.* (2018). The sample having the essential oil of cardamom (D) wasfound to be high antioxidant value compare to black pepper essential oil (C) and other control sample A and B during the storage for 28 days.

4.5.8 Comparison of total plate count during storage of plum RTS

The RTS plum was prepared and pasteurized at 90^0 C for 10 minutes. The result of TPC in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days at a refrigeration temperature 5 ± 1^0C was carried out and statistically analyzed as shown in Appendix C.16.

Statistical analysis showed highly significant results among different treatments and storage intervals. In 0^{th} days, plum RTS was pasteurized at 90^0C for about 10 minutes. No microbial analysis was done. In 7^{th} days, statistically analysis showed that all the samples A, B, C and D were significant different ($p<0.05$) with each other. On moving from 7^{th} days to 14^{th}, 21^{st}, 28^{th} similar result was shown by statically analysis where samples A, B, C, D were significantly different ($p<0.05$) with each other as revealed by Agcam *et al.* (2018). The increase in microbial load during storage was due to the high moisture content in plum juice and improper storage conditions, temperature fluctuations etc. The number of microbial growth in blank sample was higher in comparison to the sample having essential oil of black pepper and cardamom (Agcam *et al.*, 2018).

4.5.8.1 Trend of total plate count during storage

The trend of total microbial count (cfu/ml) × 10^3 with respect to time (days) during storage was shown Fig 4.8

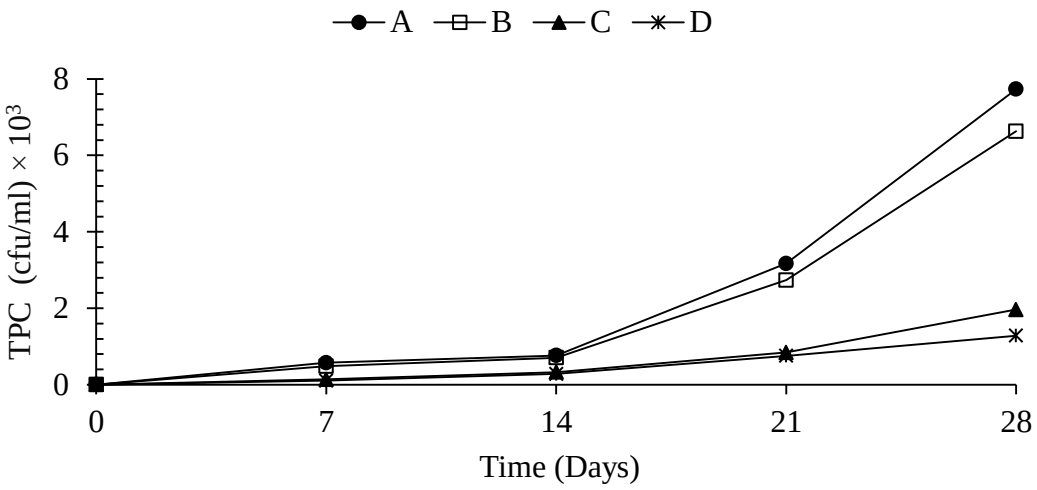

Fig 4.8 Trend of TPC (cfu/ml) with respect to time (days) during storage

The data indicate that there was rise of microbial load detected until 28 days with storage time. Microbial load had raised with longer storage times in all samples. The

addition of essential oils from different spices had positive effects on the microbial growth. It was observed that the both black pepper and cardamom essential oil had shown the most successful for preventing microbial growth. It was discovered that essential oils have high efficient monoterpene hydrocarbons, which ware important for suppressing microbial development, their activity as explained by Agcam *et al.* (2018).

Above trend line suggested that, microbial growth increased more at the end of the storage period, it was observed that the blank sample have comparatively higher increase of microbial load compare to sample having essential oil of black pepper and cardamom which may have been caused by storage temperature and change in pH of juice. The high moisture content in fruit juices, encourage the growth of yeast and bacteria (Rahman *et al.*, 2011). Furthermore, the synergistic effects of numerous phenolic compounds found in essential oils might be credited for the prevention of microbial growth. Hence, according to the above data, cardamom essential oil to black pepper essential oil, cardamom essential oil demonstrated the most preservative effect which was reported as:

$$D>C>B>A$$

4.5.9 Comparison of total yeast mold count during storage of plum RTS

The plum RTS was prepared and pasteurized at 90^0C for about 10 minutes. The result of TPC in 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days stored at a refrigeration temperature 5 ± 1^0C was carried out and statistically analyzed as shown in Appendix C.18.

Statistically analyzed showed highly significant results among different treatments and storage intervals. During the first days, the plum RTS was fully pasteurized at 90^0C for 10 minutes. In 7^{th} days, statistical analysis showed that all the samples A and D were significant different ($p<0.05$) with each other whereas samples B and C were no significant different ($p>0.05$) with each other. On moving from 7^{th} days , 14^{th}, 21^{st}, 28^{th} similar result was shown by statistically analysis where samples A, B, C, D were significantly different ($p<0.05$) to each other as reported by (Burt and Reinders, 2003). The continuous rise in the growth of yeast and mold count is due to different factors like exposure to air, improper sealing conditions, temperature fluctuations and naturally present yeast and mold in environment. The number of yeast and mold growth in blank sample was higher in comparison to the sample having essential oil of black pepper and cardamom (Rehman *et al.*, 2014).

4.5.9.1 Trend of total yeast mold count during storage

The trend of total yeast mold count (cfu/ml) × 10^3 with respect to time (days) during storage was shown in Fig 4.9.

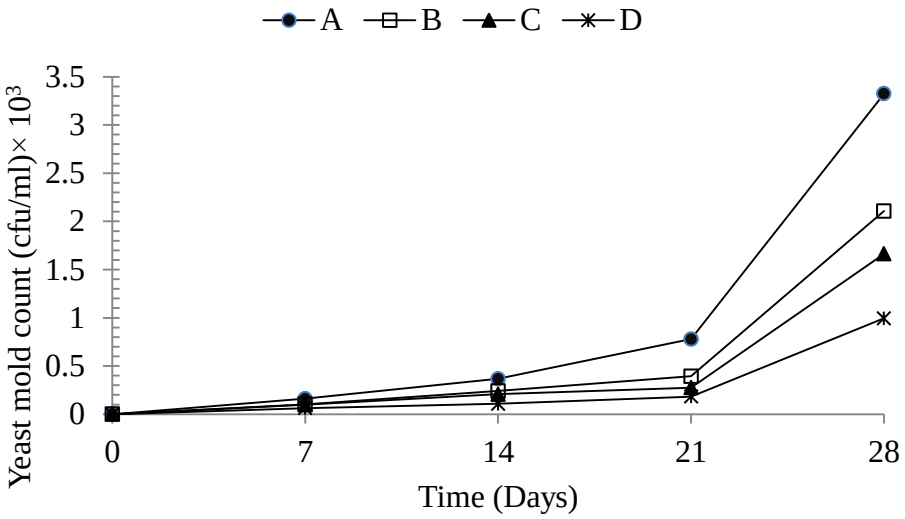

Fig 4.9 Trend of total YMC (cfu/ml) with respect to time (days) during storage

Longer storage periods result in higher yeast and mold count. This could be due to both external contamination and the development of yeast and mold that were already present in the juice. It's possible that the rise of bacteria that produce acid is also responsible for the increase in mold, yeast, and other microbes given by Burt and Reinders., (2003). Due to phenolic components, essential oils are known to have antibacterial properties as well as it increases flavour and quality of RTS (Karapinar and Aktug, 1987).

From above trend line, it was clear that microbes, yeast and mold were increased as the storage time increases. This may be due to the contamination of RTS during storage which leads to the growth of yeasts and mold occurs continuously. The reason for higher yeast and mold growth might be due to the increase in sugar content during the storage duration as their growth occurs in the presence of sugar. At the beginning, all the samples had almost similar count, but the later stages of storage, the yeast mold count goes on increasing. Cardamom essential oil had demonstrated the least amount of microbial deterioration growth. Cardamom essential oil may have key components such 1,8-cineol (43.7%), a-terpineol (9.5%), terpinene-4-ol (3.2%), spathulenol (2.7%), and (1.6%) that work together to prevent the growth of spoilage bacteria in plum RTS (Singh and Sharma,

2017). Additionally, several phenolic chemicals found in cardamom oil function to hinder the growth of microorganisms. Comparing cardamom essential oil to black pepper oil, cardamom essential oil demonstrated the most preservative effect.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the study carried out, the following conclusions were drawn:

1. It was observed that t-test of proximate composition of black pepper and cardamom show significant different ($p<0.05$) in all parameter.
2. % Titrable acidity, % Total sugar, % Reducing sugar, % RSA, Total microbial count, Total yeast and mold count were increased while pH and ascorbic acid content decreased during 28 days of storage.
3. Significant changes were observed in different parameters undertaken when compared to the control sample.
4. Plum RTS beverage containing the cardamom essential oil was found to be superior compare to black pepper. Total microbial count and yeast and mold count were inhibited to greater extent and antioxidant activity was found to be higher.

5.2 Recommendations

Based on the present research, the following recommendations are made for future work:

1. Long term study for shelf life of Plum RTS can be studied.
2. Preservative effects of oleoresins and essential oils of different spices for different fruit juices can be studied.
3. Enzymatic changes can be studied.
4. All the components of plum juice including bioactive component, anti-nutritional factors and so on further can be studied.

Part VI

Summary

The chemical composition of fresh plum, black pepper and cardamom were determined. The essential oils of the spices were extracted by hydro-distillation using cleavenger- type apparatus. The yield of essential oil was determined. Essential oils were stored at refrigeration temperature (5±1ºC) until further use in plum RTS. The juice was extracted using screw press and filtered through clean muslin cloth. Finally, plum RTS was maintained at 0.3% acidity and 15ºBx TSS and was pasteurized for 90ºC for 10 min and stored at refrigeration temperature. The plum RTS was divided into four equal batches of 250 ml with no additives (A), 100µL ethanol (B), 90µL ethanol and 10µL essential oil of black pepper (C) and 90µL ethanol and 10µL essential oil of cardamom (D) analysis were carried out at fixed interval of 7 days for 28 days.

The chemical analysis of plum juice for moisture, TSS, total acidity, pH, vitamin C, reducing sugar, total sugar , fat, juice yield, % RSA, crude fiber, protein was found to be 84.6%, 9.5 ºBx, 1.05 % as malic acid, 2.98, 12.4 mg/100g, 4.04%, 6.8%, 0.8%, 53.4 %, 34.67%, 1.2%, 0.5% respectively. The plum RTS samples at first day of analysis shows pH, % titrable acidity, % ascorbic acid, TSS, % total sugar, % reducing sugar, % RSA to be 3.57, 0.3%, 8.76 mg/100g, 15ºBx, 12.49, 3.22%, and 9.42% respectively and no microbial count and yeast-mold count was detected. At the end of 28 days, pH, % titrable acidity, % ascorbic acid, TSS, % total sugar, % reducing sugar, %RSA, total microbial count and yeast-mold count of sample A was 3.09, 0.49%, 2.85%, 17, 14.8%, 5.18%,8.97% 7732 and 3324 respectively and that of sample B was 3.18, 0.45%, 4.38%, 16.4, 14.2%, 4.44%, 10.44%, 6632 and 2106 respectively and sample C was 3.39, 0.38%, 6.1%, 16.1, 13.19%, 4.2%, 13.37% ,1964 and 1664 respectively and sample D was 3.44, 0.36%, 6.6%, 15.8, 13.09%, 3.88%, 14.94%, 1284 and 994 respectively.

These findings suggest that essential oil extracted from black pepper and cardamom shows preservative action with 90 µL ethanol and 10 µL essential oil in the plum RTS compared to the blank sample. From overall observation, cardamom essential shows the most preservative action on the sample.

References

AACC. (2016). "American Association of Community Colleges". Retrieved from http:www aacc. nche. edu/AboutCC/Documents/AACCFactSheetsR2.

Agcam, E., Akyıldız, A. and Dündar, B. (2018). Thermal pasteurization and microbial inactivation of fruit juices. *In:* "Fruit juices".). pp. 309-339. Turkey. Elsevier.

Ahmed, M., Ahmad, A., Chatha, Z. A. and Dilshad, S. M. R. (2008). Studies on preparation of ready to serve mandarin (*Citrus reticulata*) diet drink. *Pak. J. Agri. Sci.* **45** (4), 470-476.

Amma, K., Rani, M. P., Sasidharan, I. and Nisha, V. N. P. (2010). Chemical composition, flavonoid-phenolic contents and radical scavenging activity of four major varieties of cardamom. *Int. J. Biol. Med. Res.* **1** (3), 20-24.

Aneja, K., Sharma, C. and Joshi, R. (2010). Fungal infection of the ear: a common problem in the north eastern part of Haryana. *Int. j. ped. otorhinolaryngology*. **74** (6), 604-607. [doi:org/10.1016/j.ijporl.2010.03.001].

Ashurst, P. R. (1994). "Production and Packaging of Non-carbonated Fruit Juices and Fruit Beverages". Springer Science & Business Media. [ISBN 0834212897].

Badshah, N. and Safi, S. (1994). Role of calcium in prolonging the shelf life of apples. *Sarhad J. Agri. Pak.*

Bhardwaj, R. L. and Pandey, S. (2011). Juice blends a way of utilization of under-utilized fruits, vegetables, and spices: a review. *Crit. Rev. Food Sci. Nutri.* **51** (6), 563-570. [doi:org/10.1080/10408391003710654].

Bhurtel, P. R., Regmi, K. R. and Sapkota, R. P. (1996). Horticulture Research in NARC: Status and Prospectus. *1st national workshop on horticultural research at Lumle horticulture research centre*.

Bhutani, V. and Joshi, V. (1995). Plums: A Brief Introduction. *In:* "Handbook of fruit science and technology".). pp. 219-258. CRC Press. [ISBN 978-0-571-08989-5].

Birwal, P., Deshmukh, G., Saurabh, S. P. and Pragati, S. (2017). Plums: a brief introduction. *J. Food Nutr. Pop. Health*. **1** (1), 1-5.

Biswas, S., Das, R., Basu, M., Bandyopadhyay, R. and Pramanik, P. (2016). Synthesis of carbon nanoparticle embedded graphene for sensitive and selective determination of dopamine and ascorbic acid in biological fluids. **6** (103), 100723-100731.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L. and Stewart, C. M. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov. Food Sci. Emerg. Technol.* **5** (2), 135-149. [doi:org/10.1016/j.ifset.2003.11.005].

Burt, S. A. and Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against Escherichia coli O157: H7. *Innov. Food Sci. Emerg. Technol.* **36** (3), 162-167. [doi:org/10.1016/j.ifset.2003.11.005].

Ceclu, L. and Nistor, O. (2020). Red beetroot: Composition and health effects—A review. *J. Nutr. Med. Diet Care*. **6** (1), 1-9.

Ceylan, E. and Fung, D. Y. (2004). Antimicrobial activity of spices *J. Rap. Meth. Automation Microbiol.* **12** (1), 1-55. [doi:org10.1111/j.1745-4581.2004.tb00046.x].

CFTRI. (1994). "Studies on Post Harvest Technology, Product Development and Diversification of End Uses of Large Cardamoms". Mysore. p. 90.

Chang, T. S., Siddiq, M., Sinha, N. K. and Cash, J. N. (1994). Plum juice quality affected by enzyme treatment and fining. *J. Food Sci.* **59** (5), 1065-1069. [doi:10.1111/j.1365-2621.1994.tb08191.x].

Charles, D. J. (2012). "Antioxidant Properties of Spices, Herbs and other Sources". Springer Science & Business Media. USA. [1461443105].

Correa, M. I. C., Chaves, J. B. P., Jham, G. N., Ramos, A. M., Minim, V. P. R. and Yokota, S. R. C. (2010). Changes in guava (*Psidium guajava L. var. Paluma*) nectar volatile compounds concentration due to thermal processing and storage. *Food Sci. Technol.* **30** (4), 1061-1068. [doi:org/10.1590/S0101-20612010000400035].

Dalton, L. (2002). Food Preservatives [Newsletter]. Chemical and Engineering News. **80** (45), 40.[Accessed 13 December, 2012.]

Davey, M. W., Montagu, M. V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I., Strain, J. J., Favell, D. and Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* **80** (7), 825-860. [doi:org/10.1002/(SICI)1097-0010(20000515)80].

Debnath, B., Singh, W. S., Goswami, S. and Manna, K. (2021). Taxonomical, phytochemical, traditional explanation, nutritional values, and biological activities of certain edible medicinal plants *J. Nat. Remed.* **21** (3), 173-188.

Deka, B. and Sethi, V. (2001). Preparation of mixed fruit juice spiced RTS beverages. *Ind. Food Pack.* **55** (3), 58-61.

DFTQC.(2018)."Departmrnt of Food Technology and Quality Control". Agriculture Statistics (Ministry of Agriculture and Livestock Development)Nepal. pp. 212-214.Retrived from https://dftqc.gov.np/en/mandatory-standard/.

Dhaka, A., Sharma, M. and Singh, S. K. (2016). Use of additives to reduce browning, microbial load and quality loss of kinnow juice under ambient storage. *Ind. J. Sci. Technol.* **9**, 1-11. [doi:10.17485/ijst/2016/v9i14/80907].

Dorantes, L., Colmenero, R., Hernandez, H., Mota, L., Jaramillo, M. E., Fernandez, E. and Solano, C. (2000). "Inhibition of Growth of Some Foodborne Pathogenic Bacteria by Capsicum Annum extracts". Vol. 57.Int. J. Food Microbio. [ISBN 0168-1605].

Ebenhoech, A. and Spadaro, O. (1992). Antiepilepsirine: a new Chinese anticonvulsant herb drug. *J. Eco. Tax. Bot.*. **16**, 99-102.

Eissa, H. A., Nadir, A. M. and Ibrahim, K. E. (2003). Effect of anti-browning and anti-microbial constituents of some spices on the quality and safety of apple juice. *Amse Fra.* **64** (2), 43-57.

El-Sharkawy, I., Sherif, S., Qubbaj, T., Sullivan, A. J. and Jayasankar, S. (2016). Stimulated auxin levels enhance plum fruit ripening, but limit shelf-life characteristics. *Postharvest Biol. Technol.* **112**, 215-223. [doi:10.1016/j.postharvbio.2015.09.012].

Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., Chaouch, M. and Eloutassi, N. (2019). Extraction of essential oils of Rosmarinus officinalis L. by

two different methods: Hydrodistillation and microwave assisted hydrodistillation. *Scient. World J.* **2019**. [doi:org/10.1155/2019/3659432].

Esteve, M., Frigola, A., Rodrigo, C. and Rodrigo, D. (2005). Effect of storage period under variable conditions on the chemical and physical composition and colour of Spanish refrigerated orange juices. *Food Chem. Toxicol.* **43** (9), 1413-1422. [doi:org/10.1016/j.fct.2005.03.016].

Gavahian, M., Farahnaky, A., Javidnia, K. and Majzoobi, M. (2012). Comparison of Ohmic-assisted Hydrodistillation with Traditional Hydrodistillation for the Extraction of Essential Oils from *Thymus Vulgaris L*. **14**, 85-91. [doi:org/10.1016/j.ifset.2012.01.002].

Gull, A., Nayik, A. G., Wani, M. W. and Nanda, V. (2023). "Handbook of Plum Production". CRC Press. Boca Raton London. [ISBN: 978-1-032-06242-6].

Gunduz, K. and Saracoglu, O. (2012). Variation in total phenolic content and antioxidant activity of Prunus cerasifera Ehrh. selections from Mediterranean region of Turkey. *Scientia horti.* **134**, 88-92. [do:org/10.1016/j.scienta.2011.11.003].

Gupta, P. N., Naqvi, A., Misra, L. N., Sen, T. and Nigam, M. C. (1984). Gas chromatographic evaluation of the essential oils of different strains of Amomum subulatum growing wild in Sikkim. *Parfumerie und Kosmetik*. **65** (9), 528-529.

Haldar, D., Halder, S., Das, P. and Halder, G. (2016). Assessment of water quality of Damodar River in South Bengal region of India by Canadian Council of Ministers of Environment (CCME) Water Quality Index: a case study. *Desalinat. Water Treat.* **57** (8), 3489-3502. [doi:org/10.1080/19443994.2014.987168].

Hancock, J. F. (2008). "Temperate Fruit Crop Breeding: Germplasm to Genomics". Springer Science & Business Media. USA. [ISBN1402069073].

Hirasa, K. and Takemasa, M. (1998). "Spice Science and Technology". CRC Press. New york. [ISBN 0585367558].

Hussein, M., El-Mahdy, T. and Ibrahim, A. (2003). Effect of calcium chloride and gibberellic acid treatments on Anna and Dorsett Golden apples during storage. 2.-chemical characteristics of fruits. *Ast. J. Agri. Sci.* .

Igwe, E. O. and Charlton, K. E. (2016). A systematic review on the health effects of plums (*Prunus domestica and Prunus salicina*). *Phytothe. Res.* **30** (5), 701-731. [doi.org/10.1002/ptr.5581].

Jafri, M., Javed, K. and Singh, S. (2001). Evaluation of the gastric antiulcerogenic effect of large cardamom (fruits of Amomum subulatum Roxb). *J. Ethnopharmacol.* **75** (2-3), 89-94. [doi:org/10.1016/S0378-8741(00)00398-6].

Johri, R. K., Thusu, N., Khajuria, A. and Zutshi, U. (1992). Piperine-mediated changes in the permeability of rat intestinal epithelial cells: the status of γ-glutamyl transpeptidase activity, uptake of amino acids and lipid peroxidation. *Biochem. Pharmaco.* **43** (7), 1401-1407.

Kalemba, D. and Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current med. chem.* **10** (10), 813-829. [doi.org/10.2174/0929867033457719].

Kapoor, I., Singh, B. and Singh, G. (2008). Essential oil and oleoresins of Cinnamomum tamala (tejpat) as natural food preservatives for pineapple fruit juice. *J. food process. and prese.* **32** (5), 719-728. [doi.org:10.1111/j.1745-4549.2008.00200.x].

Karapinar, M. and Aktug, S. E. (1987). Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. *Int. J. Food Micro.* **4** (2), 161-166. doi:org/10.1016/0168-1605(87)90023-7].

Kharel, G. P. and Hashinaga, F. (2004). Principles of food preservation. *Shibundo Co. Ltd. Kagoshima Japan.*

Kim, D. k., Jeong, S. W. and Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chem.* **81** (3), 321-326. [doi:10.1016/S0308-8146(02)00423-5].

King, A. D. and Bolin, H. R. (1989). Physiological and microbiological storage stability of minimally processed fruits and vegetables. *Food Technol.* **43** (2).

Krishnaveni, A., Manimegalai, G. and Saravanakumar, R. (2001). Storage stability of jack fruit (Artocarpus heterophyllus) RTS beverage. *J. Food Sci. Technol.* **38** (6), 601-602.

Kumar, B. M., Sasikumar, B. and Kunhamu, T. (2021). Agroecological aspects of black pepper (*Piper nigrum L.*) cultivation in Kerala: A Review. *J. Agri. Sci.* **43** (3), 648-664.

Kunnumakkara, A. B., Koca, C., Dey, S., Gehlot, P., Yodkeeree, S., Danda, D., Sung, B. and Aggarwal, B. B. (2009). Traditional uses of spices: an overview. *Mol. targ. ther. uses spices*. 1-24. [doi:org/10.1142/9789812837912_0001].

Lal, G., Siddappa, G. S. and Tandon, G. L. (1960). "Preservation of Fruits and Vegetables" (1 ed.). Indian Council of Agricultural Research. New Delhi [ISBN 978-0-571-08989-5].

Li, H., Zhang, J., Wang, Y., Li, J., Yang, Y. and Liu, X. (2018). The effects of storage conditions on lycopene content and color of tomato hot pot sauce. *Intern. J. Analytical Chem.* [doi:org/10.1155/2018/1273907].

Lin, R. I. (1994). Pharmacological properties and medicinal use of pepper (*Piper nigrum L.*). *Dev. food sci.*

Lucchesi, M. E., Chemat, F. and Smadja, J. (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *J. Chromat.* **1043** (2), 323-327. [doi:org/10.1016/j.chroma.2004.05.083].

Majumdar, T., Wadikar, D., Vasudish, C., Premavalli, K. and Bawa, A. (2011). Effect of storage on physico-chemical, microbiological and sensory quality of bottlegourd-basil leaves juice. *Ameri. J. Food Technolo.* **6** (3), 226-234.

Mattoo, A. k. (1975). Chemical changes during ripening and senescence. *Postharv. physio. hand. utiliz. trop. subtrop. fruits vege.*, 104-127.

Mihalev, K., Dinkova, R., Shikov, V. and Mollov, P. (2018). Classification of fruit juices. *In:* "Fruit Juices".). pp. 33-44. Elsevier.

Milosevic, T. and Milosevic, N. (2018). Plum (*Prunus spp*.) breeding. *Adv. Plant Breed. Strat. Fruits*. **3**, 165-215.

MOALD. (2021). "Statical Information on Neplease Agriculture". Agriculture Statistics (Ministry of Agriculture and Livestock Development), Nepal. pp. 111-115. Retrieved from https://moald.gov.np/publication-types/agriculture-statistics/.

Mohamed, M. E. and Eissa, A. H. A. (2012). Pulsed electric fields for food processing technology. *Stru. Fun. food engin.* **11**, 275-306. [doi: org/10.57772/48678].

Nadeem, M., Ubaid, N., Qureshi, T. M., Munir, M. and Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrasoni. sonochem.* **45**, 1-6.

Naik, Y. K., Khare, A., Choudhary, P., Goel, B. and Shrivastava, A. (2009). Studies on physico-chemical and sensory characteristics of whey based watermelon beverage. *Asian J. Res. Chem.* **2** (1), 57-59.

Nair, K. P. (2011). Agronomy and Economy of Black Pepper and Cardamom. *The" King" and" Queen" of Spices*.

Nakatani, N., Kayano, S., Kikuzaki, H., Sumino, K., Katagiri, K. and Mitani, T. (2000). Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (*Prunus domestica L.*). *J. Agric. Food Chem.* **48** (11), 5512-5516.

Narayanan, C. (2000). Chemistry of black pepper. *Black pepper (piper nigrum L.). Harwood Acad., Amsterdam, The Nether.*, 143-162.

Nisha, P., Singhal, R. S. and Pandit, A. B. (2009). The degradation kinetics of flavor in black pepper (*Piper nigrum L.*). *J. Food Engr.* **92** (1), 44-49. [doi:org/10.1016/j.jfoodeng.2008.10.018].

Oliveira, A. N., Ramos, A. M., Minim, V. P. R. and Chaves, J. B. P. (2012). Estabilidade sensorial de suco integral de manga: influencia da temperatura do tempo de armazenamento. *Food Sci. Technol.* **32**, 819-825. [doi:org/10.1590/S0101-20612012005000115].

Pandareesh, M. D., Chauhan, V. and Chauhan, A. (2018). Walnut supplementation in the diet reduces oxidative damage and improves antioxidant status in transgenic mouse

model of Alzheimer's disease. *J. Alzheimer's Dis.* **64** (4), 1295-1305. [doi: 10.3233/JAD-180361}.

Parajuli, B. (2010). Preparation of watermelon (*Citrullus lanatus*) and amla (*Phyllantus emblica*) juice blended RTS and its quality evaluation. B.Tech. Dissertation. Tribhuvan Univ., Nepal.

Paudel, P. (2022). Prepration of beetroot-ginger ready to serve (RTS) juice and study of storage stability at different storage condition. B.Tech. Disseration. Tribhuvan univ., Nepal.

Pruthi, J. S. (1993). Major spices of India. *Crop mgmt. pht. technol.*

Rai, B.K. and KC, J.B. (2007). "Basic Food Analysis Handbook" (First ed.). Maya K.C. Nepal. [978-99949-2-796-7].

RA01, Y., Kumar, A., CHATTERJEE, S., Naidu, R. and George, C. (1993). Large cardamom (*Amomum subulatum Roxb*.). *J. spices Aromatic crops*. **2**, 1-15.

Raghavan, S. (2007). "Handbook of Spices, Seasonings and Flavorings" (2 ed.).

Rahman, T., Hasan, S. and Noor, R. (2011). An assessment of microbiological quality of some commercially packed and fresh fruit juice available in Dhaka city: A comparative study. *Stamford J. Microbiol.* **1** (1), 13-18.

Rajauria, G. and Tiwari, B. K. (2017). "Fruit Juices: Extraction, Composition, Quality and Analysis". Academic press. [0128024917].

Ranganna, S. (1986). "Handbook of Analysis and Quality Control for Fruit and Vegetable Products". Tata McGraw-Hill Education. New Delhi. [ISBN 0074518518].

Rao, Y., Kumar, A., Chatterjee, S., Naidu, R. and George, C. K. (1993). Large cardamom *(Amomum subulatum Roxb*.)-a review. *J. spices Aromatic crops*. (2), 1-15.

Rao, Y. S., Kumar, A., Chatterjee, S. and Naidu, R. (1993b). Large Cardamom *(Amomum subulatum Roxb*) Germplasm Collection. *J. spices Aromatic Crops*. (2), 77-80.

Ravindran, P., Divakaran, M. and Pillai, G. (2012). Handbook of herbs and spice. **1**, 86-115.

Ravindran, P. N. and kallupurackal, J. A. (2001). "Handbook of Herbs and Spices". Vol. 1. Woodhead Publishing Ltd. Washington DC.

Rehman, M. A., Khan, M. R., Sharif, M. K., Ahmad, S. and Shah, F. U. H. (2014). Study on the storage stability of fruit juice concentrates. *Pak. J. Food Sci.* **24** (1), 101-107.

Rinzler, C. A. (1990). "The complete book of herbs, spices, and condiments: from garden to kitchen to medicine chest".New york. [0816020086].

Rios, J. L. (2016). Essential oils: What they are and how the terms are used and defined. *In:* "Essential Oils in food Preservation, Flavor and Safety".). pp. 3-10. Elsevier.

Salazar, J. A., Ruiz, D., Zapata, P., Martínez-García, P. J. and P., M.-G. (2022). Whole Transcriptome Analyses of Apricots and Japanese Plum Fruits after 1-MCP (Ethylene-Inhibitor) and Ethrel (Ethylene-Precursor) Treatments Reveal New Insights into the Physiology of the Ripening Process. *Int. J. Mol. Sci.* **23** (19), 1105.

Shahbaz, H. M., Kim, J. U., Kim, S.-H. and Park, J. (2018). The Inactivation of pathogens in fruit juice: *Escherichia coli* O157: H7, *Salmonella Typhimurium, and Listeria monocytogenes*. *In:* "Fruit Juices".). pp. 341-361. Elsevier.

Sharma, N., Tanwer, B. S. and Vijayvergia, R. (2011). Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. *Int. J. Pharm.Tech. Res.* **3** (1), 110-113.

Silva, M. M. and Lidon, F. (2016). Food preservatives–An overview on applications and side effects. *Emirates J. Food Agric.* **28** (6), 366-373. [doi:org/10.9755/ejfa.2016-04-351].

Singh, Godara, R. K., Saini, R. S. and Sharma, J. R. (2005). Standardization of processing technology for Baell blended Bael (Aegele marmelos) ready-to-serve beverages. *Haryana J. Horticult. Sci.* **34** (3/4), 263.

Singh and Sharma, M. (2017). Review on biochemical changes associated with storage of fruit juice. *Int. J. Curr. Microbiol. Appl. Sci*. **6** (8), 236-245. [doi:org/10.20546/ijcmas.2016.501.032].

Singh, R., Kaushik, R. and Jaglan, V. (2018). Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review. *Pharma. Innov. J.* **7**, 568-573.

Singh, S., Ladkani, B. G., Kumar, A. and Mathur, B. N. (1994). Development of whey based beverages. *Ind. j. dai. sci.* **47**, 586-586.

Sovova, H. and Aleksovski, S. A. (2006). Mathematical model for hydrodistillation of essential oils. *Flav. frag. j.* **21** (6), 881-889. [doi:org/10.1002/ffj.1729].

Stacewicz-Sapuntzakis, M., Bowen, P. E., Hussain, E. A., Damayanti-Wood, B. and Farnsworth, N. R. (2001). Chemical composition and potential health effects of prunes: a functional food. *Crit. rev. food sci. nutr.* **41** (4), 251-286.

Stuckey, M. (1997). "The Complete Spice Book". St. Martin Press. New York. [ISBN 0-8247-0144-5].

Tainter, D. R. and Grenis, A. T. (2001). "Spices and Seasonings: Food Technology Handbook" (2 ed.). John Wiley & Sons. [0471355755].

Thakur, N., Thakur, N., Kumar, P. and Thakur, A. (2018). Formulation and optimization of vitamin C rich ready-to-serve juice based beverage from wild aonla fruits and its quality evaluation during storage. *J. Pharmaco. Phytochem.* **7** (1), 1796-1802.

Thapaliya, P. (2004). Formulation of dry mix for bael fruit (Ready-to-serve)RTS and quality evaluation. B.Tech. Dissertation. Tribhuvan univ., Nepal.

Tongnuanchan, P. and Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *J. Food sci.* **79** (7), R1231-R1249. [doi:org/10.1111/1750-3841.12492].

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

Vijayan, K. K. and Thampuran, R. A. (2000). "Pharmacology, Toxicology and Clinical Application of Black Pepper" (1st Edition ed.).[ISBN 9780429219719].

Appendices

Appendix A

A.1 Chemicals used

Chemicals specifications	Manufacturer
Acetic acid	Quailgens, india
Acetone	Fisher Scientific
Calcium acetate, 98%	Fisher Scientific
Calcium chloride, 99.8%	Fisher Scientific
Chloroform,99.5%	Fisher Scientific
Citric acid ,99.8%	HiMedia
Ethanol 99.9%	Changshu Hongsheng fine chem. Co.Ltd.
Hydrochloric acid , 36%	Fisher Scientific
Hydrogen peroxide, 100%	Fisher Scientific
L-Tyrosine	Quailgens, india
Oxalic acid, 99.5%	Fisher Scientific
Petroleum ether	Fisher Scientific
Phenolophthalein indicator	British drug houses Ltd
Phosphoric acid,85%	HiMedia
Potassium ferricynide, 98%	Fisher Scientific
Undenatured ethanol, 95%	Lab Alley
Starch indicator, 99%	Fisher scientific

A.2 Apparatus required

Apparatus	Manufacturer /specification
Centrifuge	Model: RM-12C,india
Electronic Balance	Model: HZT-A500, INDIA
Grinder	Baltra
Incubator	Model:DH5000BII, Fainthful
Magnetic stirrer	ITTLAB
Measuring cylinder	Merk
Micropipette	Micro-lot RBO
Muffle furnace	Thermotech
pH meter	OHAUS
Refirgerator	Tosiba, japan
Water bath	LABLINE

Appendix B

Table B.1 Independent sample t-test for composition of black pepper and cardamom.

		Levene's Test for Equality of Variances		t-test for Equality of Means					
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
									Lower	Upper
Moisture	Equal variances assumed	.000	1.000	-7.638	4	.002	-.66000	.08641	-.89991	-.42009
	Equal variances not assumed			-7.638	4.000	.002	-.66000	.08641	-.89991	-.42009
Protein	Equal variances assumed	.941	.387	-61.591	4	.000	-3.10000	.05033	-3.23974	-2.96026
	Equal variances not assumed			-61.591	2.725	.000	-3.10000	.05033	-3.26974	-2.93026
Fat	Equal variances assumed	3.724	.126	-12.157	4	.000	-1.00000	.08226	-1.22839	-.77161

	Equal variances not assumed			-12.157	2.624	.002	-1.00000	.08226	-1.28434	-.71566
Ash	Equal variances assumed	.571	.492	-51.657	4	.000	-2.60000	.05033	-2.73974	-2.46026
	Equal variances not assumed			-51.657	3.741	.000	-2.60000	.05033	-2.74365	-2.45635
CHOs	Equal variances assumed	.000	1.000	128.400	4	.000	7.56000	.05888	7.39653	7.72347
	Equal variances not assumed			128.400	4.000	.000	7.56000	.05888	7.39653	7.72347
Fiber	Equal variances assumed	.400	.561	-3.873	4	.018	-.20000	.05164	-.34338	-.05662
	Equal variances not assumed			-3.873	3.670	.021	-.20000	.05164	-.34861	-.05139
Antioxidant (RSA)	Equal variances assumed	5.131	.086	-73.341	4	.000	-31.38000	.42786	-32.56794	-30.19206
	Equal variances not assumed			-73.341	2.476	.000	-31.38000	.42786	-32.91998	-29.84002

Yield(essential oil)	Equal variances assumed	4.500	.101	-18.464	4	.000	-1.00000	.05416	-1.15037	-.84963
	Equal variances not assumed			-18.464	2.616	.001	-1.00000	.05416	-1.18759	-.81241

Appendix C

Table C.1 Least significant differences of means (5%level) for pH

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.03420	0.03824	0.04906

Table C.2 Two way ANOVA(no blocking) value of pH on different days.

Samples	pH				
	Day0	Day7	Day14	Day21	Day28
A	3.57^{a}	3.42^{def}	3.30^{g}	3.18^{h}	3.09^{i}
B	3.57^{a}	3.51^{abc}	3.41^{def}	3.35^{fg}	3.18^{h}
C	3.57^{a}	3.54^{ab}	3.52^{ab}	3.47^{bcd}	3.39^{ef}
D	3.57^{a}	3.56^{a}	3.54^{ab}	3.51^{abc}	3.44^{cde}

The values are the mean of pH on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.3 Least significant differences of means (5%level) for % titrable AcidityS

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.02194	0.02453	0.04906

Table C.4 Two way ANOVA (no blocking) value of % titrable acidity on different days

Samples	%Titrable acidity				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	0.3^{a}	0.34^{abcd}	0.37^{cd}	0.42^{ef}	0.48^{g}
B	0.3^{a}	0.32^{ab}	0.34^{cd}	0.38^{de}	$0.45f^{g}$
C	0.3^{a}	0.31^{ab}	0.33^{abc}	0.36^{bcd}	0.38^{de}
D	0.3^{a}	0.31^{a}	0.32^{ab}	0.33^{abcd}	0.36^{bcd}

The values are the mean of %titrable acidity on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.5 Least significant differences of means (5%level) for % Ascorbic Acid (vit-c)

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.05586	0.06246	0.12491

Table C.6 Two way ANOVA (no blocking) value of % ascorbic acid on different days

Samples	Ascorbic acid (mg/100g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	8.76^{a}	6.54^{f}	5.55^{h}	3.34^{j}	2.85^{k}
B	8.76^{a}	7.2^{d}	6.55^{f}	5.67^{h}	4.38^{i}
C	8.76^{a}	7.96^{b}	7.63^{c}	6.92^{e}	6.1^{g}
D	8.76^{a}	8.0^{b}	7.73^{c}	7.04^{e}	6.6^{f}

The values are the mean of ascorbic acid on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.7 Least significant differences of means (5%level) for Total soluble solids (TSS)

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f	38	38	38
S.D.	0.0802	0.0897	0.1794

Table C.8 Two way ANOVA (no blocking) value of total soluble solids (TSS) on different days.

Samples	Total soluble solids (TSS)				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	15^{a}	15.4^{c}	16.0^{f}	16.4^{h}	17.0^{i}
B	15^{a}	15.2^{b}	15.6^{d}	15.9^{ef}	16.4^{h}
C	15^{a}	15.1^{ab}	15.3^{bc}	15.6^{d}	16.1^{g}
D	15^{a}	15.0^{a}	15.2^{b}	15.4^{c}	15.8^{e}

The values are the mean of Total soluble solids(TSS) on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.9 Least significant differences of means (5%level) for % of total sugar

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.0789	0.0882	0.1765

Table C.10 Two way ANOVA (no blocking) value of % of total sugar on different days.

Samples	% Total Sugar				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	12.49^{a}	13.19^{cd}	13.7^{e}	14.2^{f}	14.8^{g}
B	12.49^{a}	12.9^{bc}	13.33^{d}	13.77^{e}	14.2^{f}
C	12.49^{a}	12.78^{ab}	12.86^{bc}	13.08^{bcd}	13.19^{cd}
D	12.49^{a}	12.76^{ab}	12.82^{ab}	12.95^{bc}	13.09^{bcd}

The values are the mean % of total sugar on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.11 Least significant differences of means (5%level) for % of reducing sugar

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f	38	38	38
S.D.	0.03195	0.03572	0.07144

Table C.12 Two way ANOVA (no blocking) value of % of reducing sugar on different days

Samples	% Reducing Sugar				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	3.22^{a}	3.76^{e}	4.24^{h}	4.88^{j}	5.18^{k}
B	3.22^{a}	3.43^{b}	3.68^{d}	4.03^{g}	4.44^{i}
C	3.22^{a}	3.28^{a}	3.51^{c}	3.89^{f}	4.2^{h}
D	3.22^{a}	13.26^{a}	3.40^{b}	3.77^{e}	3.88^{f}

The values are the mean % of reducing sugar on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.13 Least significant differences of means (5%level) for % RSA

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f	38	38	38
S.D.	0.0978	0.1094	0.2188

Table C.14 Two way ANOVA (no blocking) value of % of antioxidant activity on different days

Samples	% antioxidant activity				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	9.42^{d}	9.4^{cd}	9.2^{bc}	9.0^{ab}	8.97^{a}
B	9.42^{d}	9.77^{e}	10.42^{f}	10.44^{f}	10.44^{f}
C	9.42^{d}	10.23^{f}	12.87^{h}	13.22^{i}	13.37^{i}
D	9.42^{d}	11.87^{g}	14.47^{j}	14.87^{k}	14.94^{k}

The values are the mean % RSA on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Table C.15 Least significant differences of means (5%level) for total microbial count (cfu/ml).

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.00624	0.00689	0.01395

Table C.16 Two way ANOVA (no blocking) value total microbial count (cfu/ml) $\times 10^3$ on different days.

Samples	Total Microbial count (Cfu/ml) $\times 10^3$				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	0^{a}	0.573^{g}	0.762^{i}	3.710^{n}	7.732^{f}
B	0^{a}	0.483^{f}	0.703^{h}	2.734^{m}	6.632^{o}
C	0^{a}	0.140^{c}	0.326^{e}	0.844^{j}	1.964^{l}
D	0^{a}	0.110^{b}	0.280^{d}	0.756^{i}	1.284^{k}

The values are the mean of total microbial count on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio $\leq$ indicate significant difference at 5% level of significance.

Table C.17 Least significant differences of means (5%level) for % of total yeast mold count

Source of variation	Samples	Days	Samples Days
Rep	15	12	3
d.f.	38	38	38
S.D.	0.00619	0.00692	0.01385

Table C.18 Two way ANOVA (no blocking) value of yeast mold count (cfu/ml) $\times 10^3$ on different days

Samples	Total yeast mold count (Cfu/ml) $\times 10^3$				
	Day 0	Day 7	Day 14	Day 21	Day 28
A	0^{a}	0.160^{d}	0.366^{i}	0.778^{k}	3.324^{o}
B	0^{a}	0.102^{c}	0.240^{g}	0.393^{j}	2.106^{n}
C	0^{a}	0.098^{c}	0.208^{f}	0.276^{h}	1.664^{m}
D	0^{a}	0.062^{b}	0.108^{c}	0.183^{e}	0.994^{l}

The values are the mean of total yeast mold count on different days. The values having same superscript in column did not vary significantly at 5% level of significance. F-ratio ≤ indicate significant difference at 5% level of significance.

Appendix D

Photographs / color plate

Plate 1 Extraction of essential oil

Plate 2 Samples for Analysis

Plate 3 Total sugar Determination

Plate 4 pH Determination

Plate 5 Microbial Analysis

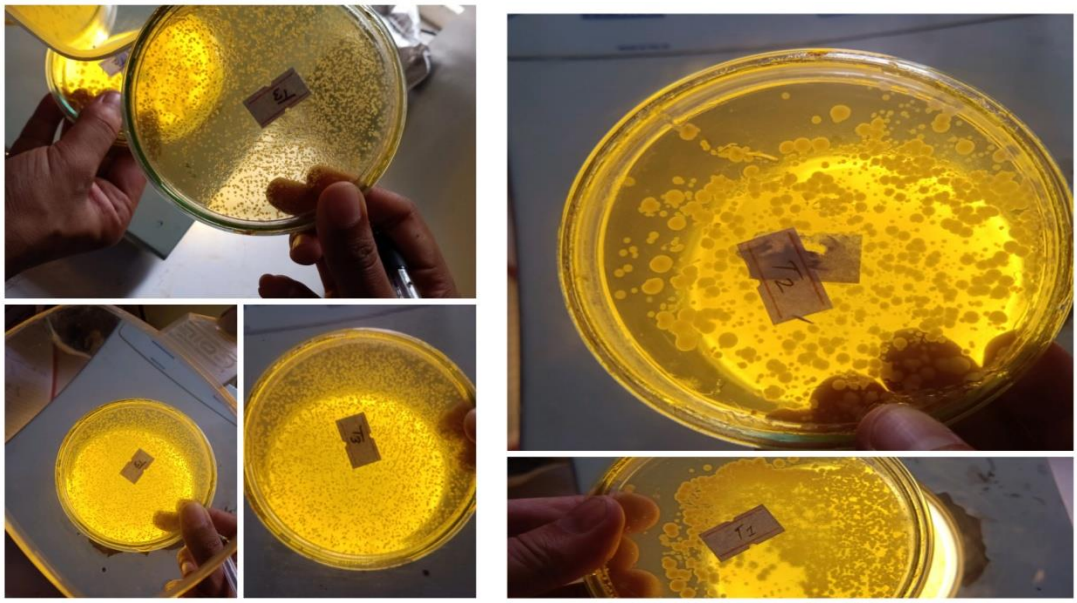

Plate 6 Some microbial (TPC and YMC) plates