COMPARATIVE STUDY ON ESSENTIAL OILS OF TEJPAT *(CINNAMOMUM TAMALA*), BLACK PEPPER (*PIPER NIGRUM*) AND CARDAMOM (*AMOMUM SUBULATUM ROXB.*) AS NATURAL FOOD PRESERVATIVES FOR APPLE JUICE.

by

Nibedita Chaudhary

Food Technology Instruction Committee

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University

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A dissertation submitted to the Food Technology Instruction Committee in Tribhuvan University in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Nibedita Chaudhary

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Tribhuvan University

Dharan, Nepal

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Tribhuvan University

Institute of Science and Technology

Food Technology Instruction Committee

Central Campus of Technology, Dharan

Approval Letter

This dissertation entitled *Comparative study on essential oils of Tejpat (Cinnamomum tamala), Black pepper (Piper nigrum) and Cardamom (Amomum subulatum Roxb.) as natural food preservative for apple juice* presented by *Nibedita Chaudhary* has been accepted as the partial fulfillment of the requirement for the B. Tech. in Food Technology.

Dissertation Committee

1. **Chairperson** _____
   (Assoc. Prof. Geeta Bhattarai*)*
2. **External Examiner** _____
   (Mr Achyut Mishra)
3. **Supervisor** _____
   (*Prof.* Dr Surendra B. Katawal*)*

Date: Sep, 2013

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Nibedita Chaudhary

Abstract

The present work was carried out to compare between spices (i.e. Tejpat, Black pepper and Cardamom) on the basis of their preservation properties. Preservation of apple juice was carried out using essential oil of above mentioned spices. The essential oils of spices were extracted by hydrodistillation using Clevenger-type apparatus. The chemical composition of fresh apples (*Malus domestica*) was determined. The value of moisture content, acidity, total sugar, reducing sugar, pH, protein, and vitamin C were found to be 85.79%, 0.77%, 10.49%, 6.66%, 4.1, 0.1% and 11.76mg % respectively.

Apple juice was extracted using electronic juicer and filtered through muslin cloth. Five equal batches of apple juice were prepared. Control sample was prepared without any additives i.e. essential oil. Control sample II was prepared adding 100 µL of ethanol. 10 µL of each essential oil was predissolved in 90 µL of ethanol and then homogeneously mixed with other three samples of juice. Those samples were then kept under refrigeration for 28 days and changes were examined every 7 days. During observation, change in pH, % total sugar, % reducing sugar, % titrable acidity, % ascorbic acid content and microbial count were evaluated. The notations S1, S2, S3, S4 and S5 stand for sample with no additives, sample with ethanol, black pepper oil, cardamom oil and tejpat oil respectively. In the control sample S1, there was extreme change in every parameters evaluated.

At the end of 28 day, % total sugar, % reducing sugar, % ascorbic acid, pH, % titrable acidity, total microbial count and yeast and mold count of Sample S1 were 12.9, 12.05, 3.05, 4.84, 0.373, 6832 and 5623 respectively and that of sample S2 were 12.2, 8.95, 4.48, 4.72, 0.53, 5690 and 4817 respectively. The % total sugar and % reducing sugar of Sample S3, S4 and S5 were 11.83, 11.68, 12.07, 8.03, 8.01 and 8.46 respectively. Ascorbic acid content of sample S3, S4 and S5 were 6.51, 7.37 and 5.38 respectively and that of pH and % titrable acidity were 4.53, 4.46, 4.64, 0.57, 0.62 and 0.61 respectively. The total microbial count and yeast & mold count of sample S3, S4 and S5 were 2033, 1210, 1737, 2240, 1780 and 2917 respectively. In reference to control sample, essential oils showed preservative properties. Among three of the spices used, cardamom oil showed the most preservative effect on overall observation. During 28 days of observation, there were gradual changes in the parameters of each sample.

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List of abbreviations

CCT Central Campus of Technology

MIC Minimum Inhibitory Concentration

HMG-CoA 3-hydroxy-3-methyl-glutaryl-CoA

GSH Gastricsulphydryls

CNS Central Nervous System

EO Essential Oil

GRAS Generally recognized as Safe

Part I

Introduction

1.1 General Introduction

Nepal is an agricultural country; most of the economy depends upon farming. Varieties of crops are grown throughout the country. Among the various agricultural commodities, horticulture crops play a significant role in the economic growth of the country (Bhurtyal *et al.*, 1996). The vegetables such as cauliflower, tomato, citrus family, apple, pear, mango, banana, in fruits are important horticulture commodities. Fruits together with vegetables provide vitamins and minerals essential for the proper maintenance of human health (Potter, 1986). Apple is one of such fruits that merit attention.

Fresh fruit juices are considered an integral part of any dietary system because they are a rich source of minerals, protein, vitamins, calories and dietary fiber (A. Singh and Goswami, 1996). Moreover, because freshly extracted fruit juices, which have always been considered a healthful food, may not always be safe owing to the heavy load of microbes (Choudhary, 1999; Kumari, 1995). So fresh fruit juices always enjoy good market demand. However, fruit juices have short shelf life, losing their freshness and other nutrients. That is why food safety is a fundamental concern with both consumers and the food and fruit industries. So for mere protection from microbial infection and other nutrient damages, many physical and chemical methods have been employed by King (1989) to keep the fruit juice in good condition. Some preservatives have also been used because the storage of fruit juice is limited due to development of bitterness, non-enzymatic browning, enzymatic activity, change in color, aroma, and taste taking place in processed fruit juices which prevents the microbial infection and other damages. Some chemical preservatives such as benzoic acid, SO_2, formic acid, etc, have been used for the preservation of fruit juices (Ranganna, 2008).

Fruits and their products are preserved by different methods to inactivate degradation enzymes and kill spoilage microorganisms. Fresh apple juice is the most unstable fruit juice from both the chemical and microbiological point of view. Consequently, certain types of apple juice that are available on the market largely reflect the preservation techniques that have been used for their production. Pure apple juice is a colorless and virtually odorless

liquid. Within seconds of its expression from the fruit, however, it undergoes a sequence of enzymatic changes to produce the color and the aroma which we are familiar with (El-Assi *et al.*, 1997; Spanos and Wrolstad, 1992)

The low pH of apple products restricts the growth of a wide variety of microorganisms. Only yeasts, molds and lactic acid bacteria are capable of prolific growth in apple products. Growth of yeast may lead to the production of off-flavors, turbidity, alcohol, and gas in processed apple products. The raw juice can be protected from microbiological degradation for a few days by storage in a refrigerator, or may be protected indefinitely by pasteurization or by the use of permitted preservatives. Such juice is nearly always turbid, brown in color and tends to sediment on storage (Lea, 1994).

While application of conventional chemical preservatives such as benzoic and sorbic acids and sulfite to fruit juices is an alternative, the practice is not common as indeed such preserved fruit juices and the chemicals are often imported. For developing countries, preservation should be inexpensive and simple but reliable (Leistner, 1994).

Since ancient times, people have used herbs and spices for preventing food deterioration and food borne diseases. At the end of the last century, antimicrobial and anti-browning activities of the herbs and spices had already been examined and their oils were known to retard microbial spoilage and inhibit enzymatic browning in food products. there are growing interests in using natural antimicrobial and anti-browning compounds, especially those extracted from plants, for the preservation of food products (Dorantes *et al.*, 2000). On the other hand, the species and herbs give a good flavor and musk the undesirable flavors.

Apple (*Malus domesticus*) is the leading edible member of family Rosaceae. It is the most popular fruit and its pleasant flavor, distinct aroma and exquisite taste make it one of the most chosen fruits all over the world. Its juice is a good source of carotene and ascorbic acid and is fairly rich in vitamins. To my knowledge, there is no report on the preservation of apple juice by essential oils.

1.2 Statement of the problem and justification

Nepal is an agricultural country; most of the economy depends upon farming. Varieties of crops are grown throughout the country. Among the various agricultural commodities,

horticulture crops play a significant role in the economic growth of the country (Bhurtyal *et al.*, 1996). Fresh fruit and vegetables have a short life under normal ambient conditions of temperature and humidity. Being a highly perishable nature, they soon lose their freshness and become subject to mould and bacterial attack, and consequently decay and become useless as articles of human food (Lal and Siddappa, 1960).

From chemical and microbiological point of view, fresh apple juice is the most unstable fruit juice. The low pH of apple products restricts the growth of a wide variety of microorganisms. Only yeasts, molds and lactic acid bacteria are capable of prolific growth in apple products. Fruit juices have been preserved using different chemical preservatives which may be allergic to certain people and may also result in various health effects. Fruits and their products are preserved by different methods to inactivate degradation enzymes and kill spoilage microorganisms. Most recent reviews have concentrated on technology for the improvement of fruit and their products quality without adding chemicals, without affecting their nutritional value and safety of products (Eissa *et al.*, 2003a; Eissa *et al.*, 2003b; Ejechi *et al.*, 1998). For this, use of essential oils from spices will be an approach towards such technology. Hence, attempts have been made to enhance the shelf life of apple juice by essential oils derived from Cinnamomum sp., Piper nigrum and Cardamom which possesses remarkable antioxidant and antimicrobial properties.

1.3 Objective

1.3.1 General objective

The objective of this study was to compare the effects of essential oils of black pepper, tejpat and cardamom as natural preservatives for apple fruit juice peservation.

1.3.2 Specific objectives

1. To carry out the extraction of essential oil from the spices.
2. To study the effect of essential oils on apple juice during storage.
3. To compare among three spices as the most effective natural preservative for fruit juice.

1.4 Significance of study

Use of chemical preservatives in preservation of fruit juice has always invited some kind of health effects on human. Regarding this, it is an attempt to create an alternative to such chemical preservatives by using spices, i.e., natural preservatives. From centuries, spices have been used as preservatives for various food products. In developing countries, preserved fruits as well as chemical preservatives are generally imported. Spices from within such country will be somehow economic for the preservation purpose. Even the farmers will be encouraged towards farming of spices which will eventually improve the economic status of the farmers as well as the country.

1.5 Limitations of study

1. Preservative properties of oleoresins of spices were not carried out.
2. The experiment was carried out only under refrigeration.

Part II

Literature Review

2.1 The apple plant

2.1.1 General description of the apple plant

The apple forms a tree that is small and deciduous, reaching 3 to 12 meters (9.8 to 39 ft) tall, with a broad, often densely twiggy crown. The leaves are alternately arranged simple ovals 5 to 12 cm long and 3–6 centimeters (1.2–2.4 in) broad on a 2 to 5 centimeters (0.79 to 2.0 in) petiole with an acute tip, serrated margin and a slightly downy underside. Blossoms are produced in spring simultaneously with the budding of the leaves. The flowers are white with a pink tinge that gradually fades, five petaled, and 2.5 to 3.5 centimeters (0.98 to 1.4 in) in diameter. The fruit matures in autumn, and is typically 5 to 9 centimeters (2.0 to 3.5 in) in diameter. The center of the fruit contains five carpels arranged in a five-point star, each carpel containing one to three seeds, called pips (Anonymous, 2008c). The apple tree is originated in the mineral-rich mountain ranges of Kazakhstan, and now being cultivated in many parts of the world. Apple fruit features oval or pear shape; and the outer skin has different colors depending upon the cultivar type. Internally, the juicy pulp has been off-white to cream in color and has to mix of mild sweet and tart taste. Its seeds are inedible because of their bitter taste. Hundreds of varieties of apples are either eaten as table fruits or as dessert fruit grown in the US and worldwide. Some of the apples are sought-after in cooking and baking too (Wikipedia, 2013).

Fig 2.1: An apple tree

2.1.2 Botanical classification

Kingdom:	Plantae
Sub-Kingdom:	Angiosperms
	Eudicots
	Rosids
Order :	Rosales
Family:	Rosaceae
Subfamily:	Maloideae or Spiraeoideae
Tribe:	Maleae
Genus:	*Malus*
Species:	*M.domestica*

Binomial name: Malus domestica

Nepali name: Shyau

Source: Borkh (1803)

Fig 2.2 : Blossoms, fruits, and leaves of the apple tree (*Malus domestica*)

2.2 General description of Apple fruit

The apple is the pomaceous fruit of the apple tree, species *Malus domestica* in the rose family (Rosaceae). It is one of the most widely cultivated tree fruits, and the most widely known of the many members of genus *Malus* that are used by humans. Apples grow on small, deciduous trees. The tree originated in Western Asia, where its wild ancestor, *Malus sieversii*, is still found today. Apples have been grown for thousands of years in Asia and Europe, and were brought to North America by European colonists. Apples have been present in the mythology and religions of many cultures, including Norse, Greek and Christian traditions. In 2010, the fruit's genome was decoded, leading to new understandings of disease control and selective breeding in apple production (Anonymous, 2008c) .

Fig.2.3: An apple fruit

(Source: Borkh,1803)

There are more than 7,500 known cultivars of apples, resulting in a range of desired characteristics. Different cultivars are bred for various tastes and uses, including in cooking, fresh eating and cider production. Domestic apples are generally propagated by grafting, although wild apples grow readily from seed. Trees are prone to a number of fungal, bacterial and pest problems, which can be controlled by a number of organic and non-organic means (Hendel, 2012).

About 69 million tonnes of apples were grown worldwide in 2010, and China produced almost half of this total. The United States is the second-leading producer, with more than 6% of world production. Turkey is third, followed by Italy, India and Poland (Bradley *et al.*, 2009;

Desmond and Andrew, 1994). Apples are often eaten raw, but can also be found in many foods (especially desserts) and drinks. Many beneficial health effects have been found from eating apples; however, the seeds are slightly poisonous and two forms of allergies are seen to various proteins found in the fruit (Wikipedia, 2013) .

2.3 Present scenario of apple in Nepal

Both high chilling and low chilling cultivars of apple are cultivated in Nepal. The principal high chilling cultivars are Red, Royal and Golden Delicious, Mc Intosh, Jonathan, Rome Beauty, Granny Smith, Richared, Golden Spur, etc. Among all these cultivars the Delicious group covers a major area as their fruit quality is excellent. The mid chilling cultivars are Katza, Red June, Cox Orange Pippin, Crispin and Summer Pippin. The low chilling cultivars are Anna, Vered, Tropical Beauty, Winter Banana etc (Devkota, 1999).

Production Areas: High and mid chilling cultivars are mostly grown in an altitude range of 1800 - 2800 m.a.s.l, where chilling is more than 1000 hours; low chilling types are cultivated at elevations as low as 1200 m.a.s.l. and where chilling is 600-1000 hours. As far as elevation is concerned, apple can be grown throughout mid and high mountain areas from Eastern to the far Western region; however, due to high humidity and heavy rainfall during the growing period the most suitable areas for quality apple production are confined to the mid and far Western region where dry to semi humid conditions exist .(Devkota, 1999)

2.4 Nutritional value of apple

Apples are a good source of dietary fiber and a good source of Vitamin A. Most of the Vitamin A, as well as the dietary fiber, are contained within the skin of fruit. The chemical composition of apple per 100g is given in Table 1.

Table 2.1 Chemical composition of Apple fruit per 100g (3.5oz) ORAC value- 5900

Constituents	Amount
Energy	50 Kcal (2.5%)
Carbohydrates	13.81 g(11%)
Protein	0.26 g(0.5%)
Total Fat	0.17 g(0.5%)
Cholesterol	0 mg(0%)
Dietary Fiber	2.40 g (6%)
Folates (Vit. B_9)	3 µg(1%)
Niacin (Vit. B_3)	0.091 mg(1%)
Pantothenic acid (Vit. B_5)	0.061 mg(1%)
Pyridoxine (Vit B_7)	0.041 mg(3%)
Riboflavin (Vit. B_2)	0.026 mg(2%)
Thiamin (Vit. B_1)	0.017 mg(1%)
Vitamin A	54 IU(2%)
Vitamin C	4.6 mg(8%)
Vitamin E	0.18 mg(1%)
Vitamin K	2.2 µg(2%)
Sodium	1 mg(0%)
Potassium	107 mg(2%)
Calcium	6 mg(0.6%)
Iron	0.12 mg(1%)
Magnesium	5 mg(1%)
Phosphorus	11 mg(2%)
Zinc	0.04 mg(0%)
Carotene-ß	27 µg
Crypto-xanthin-ß	11 µg
Lutein-zeaxanthin	29 µg

(Source: USDA National Nutrient data base)

2.5 Uses of apple

Apples are consumed fresh, canned, as juice, and dried. Apples or its juice can also be used in jellies and jam, usually in combination with other fruits or berries. They are milled to produce apple cider (non-alcoholic, sweet cider) and filtered for apple juice. The juice can be fermented to make cider (alcoholic, hard cider), ciderkin, and vinegar. Through distillation, various alcoholic beverages can be produced, such as applejack, Calvados, and apfelwein. Pectin and apple seed oil may also be produced. Apples are an important ingredient in many desserts, such as apple pie, apple crumble, apple crisp and apple cake. They are often eaten baked or stewed, and they can also be dried and eaten or reconstituted (soaked in water, alcohol or some other liquid) for later use. Puréed apples are generally known as apple sauce. Apples are also made into apple butter and apple jelly. They are also used (cooked) in meat dishes (Wikipedia, 2013).

2.5.1 Health benefits of apple consumption

The proverb "*An apple a day keeps the doctor away.*", addressing the health effects of the fruit, dates from 19th century Wales (Phillips, 1866). Research suggests that apples may reduce the risk of colon cancer, prostate cancer and lung cancer (Anonymous, 2008b). Compared to many other fruits and vegetables, apples contain relatively low amounts of vitamin C, but are a rich source of other antioxidant compounds (Boyer, 2004). Apple's antioxidant property prevents the damage to cells and tissues. The fiber content, while less than in most other fruits, helps regulate bowel movements and may thus reduce the risk of colon cancer. They may also help with heart disease, weight loss, and controlling cholesterol (Anonymous, 2008a). The fiber contained in apples reduces cholesterol by preventing reabsorption, and (like most fruits and vegetables) they are bulky for their caloric content (Sharma, 2005). However, apple seeds are mildly poisonous, containing a small amount of amygdalin, a cyanogenic glycoside. It is usually not enough to be dangerous to humans, but can deter birds (Juniper, 2006). There is evidence from laboratory experiments that apples possess phenolic compounds which may be cancer-protective and demonstrate antioxidant activity (Lee *et al.*, 2004). The predominant phenolic phytochemicals in apples are quercetin, epicatechin, and procyanidin B2 (Lee *et al.*, 2003).

Apple juice concentrate has been found to increase the production of the neurotransmitter acetylcholine in mice, providing a potential mechanism for the "prevention of the decline in cognitive performance that accompanies dietary and genetic deficiencies and aging." Other studies have shown an "alleviation of oxidative damage and cognitive decline" in mice after the administration of apple juice (Cha *et al.*, 2006). Researchers at the Chinese University of Hong Kong discovered that fruit flies who were fed an apple extract lived 10% longer than other flies who were fed a normal diet (Maher, 2011).

Apple juice is a fruit juice manufactured by the maceration and pressing of apples. The resulting expelled juice may be further treated by enzymatic and centrifugal clarification to remove the starch and pectin, which holds fine particulate in suspension, and then pasteurized for packaging in glass, metal or aseptic processing system containers, or further treated by dehydration processes to a concentrate. Apple Juice may also be sold in an untreated state (Wikipedia, 2013).

Due to the complex and costly equipment required to extract and clarify juice from apples in large volume, apple juice is normally commercially produced. In the United States, unfiltered fresh apple juice is produced by smaller operations in areas of high apple production, in the form of unclarified apple cider. Apple juice is one of the most common fruit juices in the world, with world production led by China, Poland, the United States, and Germany (Potter *et al.*, 2007).

2.5.2 Health effects of apple juice

Vitamin C is sometimes added by fortification, because content is variable, and much of that is lost in processing. Vitamin C also helps to prevent oxidation of the product. Other vitamin concentrations are low, but apple juice does contain various mineral nutrients, including boron, which may promote healthy bones. Apple juice has a significant concentration of natural phenols of low molecular weight (including chlorogenic acid, flavan-3-ols, and flavonols) and procyanidins that may protect from diseases associated with aging due to the antioxidant effects which help reduce the likelihood of developing cancer and Alzheimer's disease (Lawson, 2006) . Research suggests that apple juice increases acetylcholine in the brain, possibly resulting in improved memory. Despite having some health benefits, apple

juice is high in sugar. It has 28 g carbohydrates (24 g sugars) per 8 ounces. This results in 130 calories per 8 ounces (protein and fat are not significant), which is comparable to non-diet soft drinks such as Pepsi and 7 Up. Also like most fruit juice, apple juice contains a similar amount of sugar as the raw fruit, but lacks the fiber content (Ferree *et al.*, 1999).

While apple juice generally refers to the filtered, pasteurized product of apple pressing, an unfiltered and sometimes unpasteurized product commonly known as apple cider in the United States and parts of Canada may be packaged and sold as apple juice. In the U.S., there is an unclear distinction between filtered apple juice and natural apple cider. In other places such as New Zealand, Australia and the United Kingdom, apple cider is an alcoholic beverage. The alcoholic beverage referred to as cider in these areas is usually referred to as hard cider in Japan (Wikipedia, 2013).

2.6 Preservatives

2.6.1 Definition of preservatives

A preservative is a naturally occurring or synthetically produced substance that is added to products such as foods, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by undesirable chemical changes (Wikipedia, 2012).

2.6.2 Natural Preservatives

Natural substances which are employed in foods from centuries are generally recognized as safe (GRAS) are said to be natural preservatives. Sugar, salt, vinegar, spices and herbs of natural origin are some of the examples of natural preservatives (Kharel and Hashinaga, 2004). Naturally occurring substances such as rosemary extract, hops, salt, sugar, vinegar, alcohol, diatomaceous earth and castor oil are also used as traditional natural preservatives. Certain processes such as freezing, pickling, smoking and salting can also be used to preserve food (Dalton, 2002) .

2.7 Spices

2.7.1 Definition of Spice

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

Webster describes spices as any of various aromatic vegetable productions as pepper, cinnamon, nutmeg, mace, allspice, ginger, cloves, etc., used in cookery to season and to flavor sauces, pickles, etc.; a vegetable condiment or relish, usually in the form of a powder; also, as condiments collectively.

The definition of a spice differs according to the country or region in the world. Spices are sometimes defined according to where they are grown, whether they are dry or wet, or their historical background (i.e., when they started to be used as spices. However, these definitions are not always accurate from the viewpoint of their functions and constituents). The term "spice" can be defined as the dry parts of a plant, such as roots, leaves, and seeds, which impart to food a certain flavor and pungent stimuli (Hirasa and Takemasa, 1998) .

The word "spice" came from the Latin word "species," meaning specific kind. The name reflects the fact that all plant parts have been cultivated for their aromatic, fragrant, pungent, or any other desirable properties including the seed (e.g., aniseed, caraway, and coriander), leaf (cilantro, kari, bay, and mint), berry (allspice, juniper, and black pepper), bark (cinnamon), kernel(nutmeg), aril (mace), stem (chives), stalk (lemongrass), rhizome (ginger, turmeric,and galangal), root (lovage and horseradish), flower (saffron), bulb (garlic and onion),fruit (star anise, cardamom, and chile pepper), and flower bud (clove) (Raghavan, 2007).

2.7.2 Basic Uses for Spices

Spices have various effects when used in foods: not only do they impart flavor, pungency, and color characteristics, they also have antioxidant, antimicrobial, pharmaceutical, and nutritional properties. In addition to these direct effects of spices, complex or secondary effects can be achieved by using spices for cooking. Such effects include salt reduction, sugar reduction, and

improved texture for certain foods. We use as an example here the role of cinnamon in texture improvement as an example of a spice's secondary function (Stuckey, 1997).

2.7.3 Essential (Volatile) Oils

Essential oils, such as oil of basil, oil of caraway, or oil of black pepper, are produced by grinding, chopping, or crushing the leaf, seed, stem, root, or bark; then cold expressing, dry distilling, or extracting through distillation (using water, steam, or steam and water) and recovering the distillate oil with a solvent. Sometimes the oil is distilled from a whole spice, such as the leaf or flower, or from a broken spice (Stobart, 1982).

Table 2.2 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	
Antibacterial effect	Preservation
Antioxidant effect	

Source: (Hirasa and Takemasa, 1998)

Depending upon the method of extraction, the nature of the volatiles can differ with the same type of spice. Essential oils are the major flavoring constituents of a spice. Each essential oil has many chemical components, sometimes even up to fifteen, but the characterizing aroma generally constitutes anywhere from 60% to 80% of the total oil. The essential oils are composed of hydrocarbons (terpene derivatives) or terpenes (e.g.,α-terpinene, α-pinene, camphene, limonene, phellandrene, myrcene, and sabinene), oxygenated derivatives of hydrocarbons (e.g., linalool, citronellol, geraniol, carveol, menthol, borneol, fenchone, tumerone, and nerol), benzene compounds (alcohols, acids, phenols, esters, and lactones) and nitrogen- or sulfur-containing compounds (indole, hydrogen sulfide, methyl propyl disulfide, and sinapine hydrogensulfate) (Rosengarten and Frederic, 1979).

Terpenes usually contribute to the aromatic freshness of a spice and can be termed floral, earthy, piney, sweet, or spicy. The oxygenated derivatives, which include alcohols, esters, acids, aldehydes, and ketones, are the major contributors to the aromatic sensations of a spice. The compounds with benzene structure provide sweet, creamy, and floral notes, while the sulfur- and nitrogen-containing compounds give the characteristic notes to onion, garlic, mustard, citrus, and floral oils. Essential oils are soluble in alcohol or ether and are only slightly soluble in water. They provide more potent aromatic effects than the ground spices. Essential oils lose their aroma with age (Guenther, 1972) .

Essential oils are very concentrated, about seventy-five to one hundred times more concentrated than the fresh spice. They do not have the complete flavor profile of ground spices, but they are used where a strong aromatic effect is desired. Essential oils are used at a very low level of 0.01% to 0.05% in the finished product. They can be irritating to the skin, toxic to the nervous system if taken internally (by themselves), and can cause allergic reactions and even miscarriages. Sometimes, alternative oils extracted from a different part of the same spice plant or from another variety are used to enhance or adulterate the more expensive essential oils, but suppliers need to meet the quality specifications that are required from manufacturers for these essential oils (Uhl and Raghavan, 1997).

2.8 Antimicrobial properties of spices

Microorganisms play a role in many different areas of the food industry. Some are utilized effectively for producing dairy products, pickles, and other fermented products, but in most cases they cause foods to become unpalatable or to spoil. An inhibitory action against microbial growth is generally expressed as antimicrobial action, which includes bacteriostatic or fungistatic action (preventing microbial growth and propagation), and many spices possess antimicrobial and/or antifungal properties. The antimicrobial properties of spices have been known and utilized for centuries. For example, cinnamon, cumin, and thyme were used in mummification in ancient Egypt, and spices were used in ancient India and China to preserve foods as well as for medicinal purposes. In ancient Greece and Rome, coriander was used to extend the preservation period for meat, and mint was used to prevent milk from spoiling. Infectious diseases, such as cholera and typhus, prevalent in the medieval period, were treated using spices, presumably for bactericidal as well as medical reasons. Research on the

antimicrobial properties of spices began in the 1880s, and mustard, clove, and cinnamon were soon proven to have antimicrobial effects. Since the early twentieth century, research on spice extracts and the essential oils of spices has been conducted in this connection (Hirasa and Takemasa, 1998).

Table 2.3 Examples of Characterizing Essential Oil Components in Some Popular Spices

Spices	**Components in 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
Pepper, black	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)

2.8.1 Antibacterial and Antifungal Properties of Spices

Several studies evaluated the antimicrobial activities of spice oils using a phenol coefficient, obtained by comparing the antimicrobial effect of each spice oil with that of phenol. Both clove oil and cinnamon oil were found to be effective in retarding microbial growth as well as oregano oil and peppermint oil also had relatively strong antimicrobial effects (Martindale, 1910). A variety of volatile oils and terpeneless oils were tested to observe their inhibitory activity against different bacteria using the filter paper disk method. Most volatile oils and terpeneless oils exhibited antibacterial activity for at least one of the microbes tested, and the volatile oils of cinnamon, cumin, dill weed, and thyme were found to exhibit relatively strong antibacterial activities. Of the bacteria tested, *Bacillus subtilis* was the most susceptible, and *Escherichia coli* was found to be relatively resistant to these spice essential oils. The same authors studied the antifungal activity of these volatile oils and terpeneless oils against 15 different yeasts and molds. Most of the spices tested were found to possess some degree of antifungal activity (Maruzzella and Lichtenstein, 1956).

The volatile oils of thyme, cinnamon, and coriander and the terpeneless oils of anise, caraway, dill, and cinnamon exhibited greatest antifungal activity, especially against *Aspergillus* and *Streptomyces* species. Another study showed that some spice oils, including those from caraway and clove, were effective against *Staphylococcus aureus* and *E.coli*. The sensitivities of various bacteria to sage, rosemary, and allspice were studied and was found that sage and rosemary had a wide range of antimicrobial activity compared to allspice: the minimum inhibitory concentration (MIC) of allspice was twice as high as those of sage and rosemary (Norman and Jill, 1991).

In addition to mace, Mori et al. reported that essential oils of celery, cinnamon, and cumin prevented slime formation on sausage. Mace extract has been also confirmed to have antimicrobial activity against *Enterobacter aerogenes, Brevibacterium* and *Achromobacter* sp., *Micrococcus flavus, B. subtilis, Leuconostoc mesenteroides,* and *Lactobacillus plantarum,* but it does not retard the growth of *Serratia marcescens* (Hadady, 1996).

Numerous studies have investigated the effectiveness of spices in retarding the growth of pathogenic bacteria and other toxin-producing microorganisms. In the 1940s, the effect of

spices against cholera, bacillary dysentery, and tubercle bacillus became clear. Dole and Knapp, observing the antimicrobial properties of various spices against eight microorganisms including *Salmonella typhi* and *Shigella dysenteriae*, found that garlic had a strong antimicrobial effect against all eight and that both onion and clove were effective against all but *B. subtilis*. *Salmonella* growth was also inhibited when this bacterium was inoculated into pre-enrichment media containing oregano. Oregano and thyme were confirmed to be effective against *Vibrio parahemolyticus* when present in growth media in powdered form at concentrations of 0.5% (Hirasa and Takemasa, 1998).

2.8.2 Antibacterial and Antifungal Properties of Chemical Components of Spices

In addition to the above research on spices and their essential oils, the antimicrobial activity of various constituents found in these spices has also been studied. Eugenol, a major component of clove and allspice (also contained in cinnamon as a secondary component), was studied by Miyao (Hirasa and Takemasa, 1998). This compound exerted complete inhibition *against Acinetobacter* sp. and yeast at a concentration of 200 ppm and against *Bacillus megaterium* and *Pseudomonas* sp. at 800 ppm. It has also been reported that both *A. flavus* and *A. versicolor* were completely inhibited by eugenol at a concentration of 250 ppm. Anetol, the major volatile compound of anise seed, and thymol, which is contained in thyme, also showed inhibitory activities against these *Aspergillus* species and against aflatoxin production, although their activities were lower than eugenol (Hitokoto *et al.*, 1980) .

2.8.3 Antibacterial and Antifungal Properties of Pungent Spices

Most pungent spices are said to have relatively strong antimicrobial properties, and this belief has been supported by numerous studies. Most antimicrobial on the glycoside sinigrine, and that of white mustard *(Sinapsis alba)* is p-hydroxybenzyl- isothiocyanate, produced by the action of the same glucosinolase on the glycoside sinalpine. Wasabi *(Wasabia japonica)* and horseradish, which also belong to the Cruciferae family, produce allyl-isothiocyanate by the action of myrosinase when their plant tissues are disrupted, and they also show strong antimicrobial activities. Many different kinds of isothiocyanate compounds exist in both types of mustard, wasabi, and horseradish as forms of glycosides, and these compounds are also said to have antimicrobial activities, although their effects are relatively small compared to the

major pungent compounds listed in the above. Most of these compounds are volatile and lose their effectiveness quickly, although their antimicrobial activities are in general, stronger than those of ginger and red pepper (Raghavan, 2000) .

Many studies have reported on how spice oils or pungent compounds inhibit the growth of many types of microorganisms. White mustard soaked in water was confirmed to be effective against various fungi existing on human skin. Miyamoto studied the antimicrobial properties of an ethanol extract of wasabi using *E.coli* . Both absorbency and sugar consumption were used as indicators of antimicrobial activities of this extract (Miyamoto, 1986).

2.9 Tejpat

Cinnamomum tamala (Tejpat) Fr. Nees., belonging to family Lauraceae, is also known as Indian Cassia and the leaves are commonly called as bay leaves. Lauraceae is an economically important family consisting mostly of trees or tree-like shrubs. The genus *Cinnamomum* is represented by about 350 species worldwide. It is native to South-east Asia, some Pacific islands and Australia, growing mainly in tropical rain forests at varying altitudes. Historically, it is one of the oldest and most used spices. *C. tamala* which is an evergreen tree up to 8m in height is also cultivated. Natural habitat is in the tropical and sub- tropical Himalayas at altitudes of 900-2500m. Due to its aroma, the leaves are kept in clothes and also chewed to disguise bad mouth odour. Its dried leaves are used as a common ingredient of Indian cooking. The leaves of this tree have a clove like taste and a faintly pepper like odor. It is also used in Indian system of traditional medicines. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhea, nausea and vomiting. Ancient literature has revealed that in the 1st century A.D., dried leaves and bark ofthis plant were prescribed for fever, anaemia and body odour. Its seeds were crushed and mixed with honey or sugar and administered to children for dysentry and cough (Edwards, 1993).

Leaves of *C.tamala(tejpat)* are widely used as a spice and also yield an essential oil on distillation. The essential oil of the leaves called tejpat oil is medicinally used carminative, anti-flatulent, diuretic and cardiac disorders. *"Ayurveda"* describes the uses of leaves of *tejpatra* in the treatment of ailments such as anorexia, bladder disorder, dryness of mouth, coryza, diarrhorea, nausea and spermatorhea. It has hypoglycemic and hypolipidemic

properties (Kar *et al.*, 2003). It is commonly used in food industry because of its special aroma. The main constituents of *C.tamala* leaves are a-pinene, camphene, myrcene, limonene, eugenol, p-cymene, methyl eugenol, eugenol acetate and methyl ether of eugenol. Eugenol(4-hydroxy-3-methoxy allylbenzene), one of the main constituents of *Cinnamomum tamala* Nees and Eberm. leaves,is used as an analgesic in dental preparations, as an insect repellent, and as a flavoring agent in foods. *Tejpat* has been used as natural food preservative (Smith *et al.*, 2002).

The leaves of *Cinnamomum tamala* are used as a spice in the food industry. The leaves have hypoglycemic and hypolipidemic properties. Essential oil of cinnamomum leaves have excellent inhibitory effect on bacteria. There are numerous studies on the composition of tejpat essential oil, however, tejpat oleoresins are not studied so vastly. Cinnamon leaf oil has a warm, spicy, but rather harsh odour, lacking the rich body of the bark oil. Its major constituent is eugenol rather than cinnamaldehyde. It is used as a flavoring agent for seasonings and savory snacks. As a cheap fragrance it is added to soaps and insecticides. The oil's high eugenol content also makes it valuable as a source of this chemical for subsequent conversion into iso-eugenol, another flavoring agent (Dighe *et al.*, 2005).

Cinnamon and cassia are often combined in the spice trade, so who knows which has been studied when it is reduced to powdered bark. Cinnamon leaves are regarded as alexeteric, aperient, balsamic, lactafuge, stimulant, tonic, and vermicidal, cassia is a folk remedy for arthritis, chills, dizziness, dysmenorrhea, goiter, headache, jaundice, lumbago, menorrhagia, nausea, phymata, postpartum, rheumatism, and snakebite. Prolonged use of tejpat is thought to improve the complexion, giving one a more youthful aspect. The bark is prepared as a tea for excessive salivation. The leaves are taken internally for rheumatism. Unani consider the bark carminative, emmenagogue, and tonic, using it for headache, inflammation, piles, and pregnancy. The bark is antiseptic, astringent, and carminative, and the EOs has demonstrated cardiovascular, hypotensive, and antiviral activities. The aqueous extract slightly inhibits the production of hemolytic plaque-forming cells, evidently with an anticomplement action, inhibiting complement-dependent allergic reactions. The major component, cinnamaldehyde, is sedative and antipyretic (Duke, 2002).

Fig.2.3: Leaves of Tejpat

2.10 Black Pepper

Among the spices, black pepper is the king. It is the most important, most popular and most widely used spice in the world. It has extensive culinary uses for flavoring and preserving processed foods and is important medicinally (P.N. Ravindran and Kallupurackal, 2001). Black pepper (*Piper nigrum*) belongs to the family Piperaceae. It is cultivated for its fruit, which is usually dried and used as a spice and seasoning. The same fruit is also used to produce white pepper and green pepper. Black pepper is native to South India, where it is cultivated extensively, and also to some other tropical regions. The fruit, known as peppercorn when dried, is a small drupe, 5 mm in diameter, dark red when fully mature, containing a single seed. Dried, ground pepper, and its variants, is one of the most common spices in European cuisine, having been known and prized since antiquity for both its flavour and its use as a medicine. The spiciness of black pepper is due to the chemical, piperine. Ground black peppercorn, usually referred to simply as ‘pepper’, may be found on nearly every dinner table in some parts of the world, often alongside table salt. Black pepper, also nicknamed as ‘black gold’ and the ‘king of spices’, is the most important and widely consumed spice in the world. Compared with many other spices, properly dried black pepper (moisture content 8–

10%) can be stored in airtight containers for many years without losing its taste and aroma. The word ‘pepper’ is derived from the Sanskrit *pippali*, via the Latin *piper* and Old English *pipor*. The Latin word is also the source of German *pfeffer*, French *poivre*, Dutch *peper* and other similar forms. ‘Pepper’ was used in a figurative sense, meaning ‘spirit’ or ‘energy’, at least as far back as the 1840s (P. N. Ravindran, 2000).

Black pepper is obtained from mature fruits of *Piper nigrum* L., a perennial woody evergreen climber, native to the evergreen forests of the Western Ghats of South India. Under cultivation pepper vines are trailed over supports – either living trees or other supports, as columns 5–6m tall and 1.0–2.0m in diameter. The climbing woody stems have swollen nodes having clinging roots at each node, which helps in anchoring the vine to the support trees (standards). Pepper plants exhibit dimorphic branching, having two different types of branches: the straight upward growing (monopodial) main stem and orthotropic shoot climbing and remaining vegetative, adhering to the support with short adventitious roots at nodes. From the axils of leaves of orthotropic shoots, lateral shoots (plagiotropic branches) grow, and they have a sympodial habit of growth, having shorter internodes and without adventitious roots. In such branches, as the growth proceeds, the terminal bud gets modified into an inflorescence (spike) and further growth is continued by the auxiliary bud. The pepper plant has a delicate root system and around 75% of the roots are confined to an area of 75 to 100 cm radius and depth (Jayasree *et al.*, 1988).

Inflorescence of pepper is a pendent spike (catkin) appearing opposite the leaf on plagiotropic branches. Spikes vary in length in cultivars, being 3–15 cm long with 50–150 flowers. The flowers are very minute, white to pale yellow in color, arranged spirally on fleshy peduncles. The species is naturally self-pollinated, and pollination is by geitenogamy. The dispersal of pollen is aided by the presence of water droplets. The fruit is a single seeded drupe, but is often called a berry, sessile, small, usually globular, having fleshy pericarp and hard endocarp. The fruits are spherical in shape in most cases, obovate in a few and oblong in others (Lin, 1994).

Fig.2.4: Black Pepper

More than hundred cultivars are known and a few of them are still popular. The traditional pepper growing tracts have their own popular cultivars. Cultivar diversity is richest in the state of Kerala. Essential oil varies from 0.4 to 7.0% while piperine from 2.0 to 7.4% among cultivars.

The quality of pepper is contributed by two components:

• piperine that contributes the pungency

• volatile oil that is responsible for the aroma and flavour.

Oleoresin of black pepper, produced by solvent extraction of dried powdered pepper, contains both aroma and pungency principles. Thus the chemistry of pepper is the chemistry of its essential (volatile) oil and piperine. The chemistry of pepper ha s been reviewed by (Narayanan, 2000) and (Parmar *et al.*, 1997).

Black Pepper is considered as an antipyretic, aromatic, carminative, rubefacient, and stimulant. Modern medicine in India utilizes black pepper as an aromatic stimulant in cholera, weakness following fevers, vertigo, coma, etc.; as a stomachic in gas and indigestion; as an alternative in arthritic diseases and paraplegia; and as an antimalarial. (Rinzler, 1990)

rationalizes, “Because pepper irritates mucous membranes, highly spiced foods may be beneficial when you have hay fever or a head cold. The spice irritates tissues inside your nose and throat, causing them to weep a water secretion that makes it easier for you to cough up mucus or to blow your nose”. Pepper root, in the form of ghees, powders, enemas, and balms, is a folk remedy for abdominal tumors. Chinese poultice the leaves onto headaches and use them for urinary calculus as well. Powdered fruits are said to alleviate “superfluous flesh.” An electuary prepared from the seed is said to help hard tumors, while a salve prepared from the seed is said to help eye indurations and internal tumors. The grain, with warm wine and egg, is said to help indurations of the stomach. A poultice, made from the pepper, salt, and vinegar, may help corns. Pepper is also poulticed, like mustard plasters, for colic, headache, parturition, puerperium, and rheumatism. A heavy dose of pepper with wild bamboo shoots is said to induce abortion. Although pepper contains the carcinogen safrole, it is at very low levels compared to sassafras. Some unidentified compounds from pepper are even more potent that pepper phytosterols at lowering cholesterol, possibly some via HMG-CoA-reductase inhibition. Black pepper also speeds up transit through the gut, more than just increasing peristalsis (Kapoor *et al.*, 2008).

Piperine is a stimulant. It is also used in synthesizing heliotropine, which has its own medicinal indications. It should not be combined with astringents, which may be rendered inert. Curcumin and piperine were also concluded to be radioprotective. Isolated piperine has an inhibiting effect on *Lactobacillus plantarum, Micrococcus specialis,* and two fecal micro-organisms (*E. coli* and *Streptococcus faecalis*). It is mutagenic with *Leptospira*; with large doses, a bactericidal effect is produced. It inhibits the ubiquitous, deadly bacterium *Clostridium botulinum*. And piperine is more toxic to houseflies than pyrethrin. A mix of 0.05% piperine and 0.01 pyrethrins is more toxic than 0.1% pyrethrin (Tainter and Grenis, 2001).

Black pepper is an essential ingredient in the Indian systems of medicines – *Ayurveda*, *Sidha* and *Unani*, and is used as a curative agent for many maladies. Pharmacological studies have substantiated many of these traditional uses. Pepper has been seen as demonstrating a number of functional properties, including: (Vijayan and Thampuran, 2000)

• analgesic and antipyretic properties

• antioxidant effects

• antimicrobial properties.

Piperine, the active ingredient in pepper, exerts substantial analgesic and antipyretic effects. found that piperine reduces inflammation in carragenin induced tests at an oral dose 50mg/kg body weight. The anti-inflammatory effect was substantiated by (V. K. Kapoor *et al.*, 1993). Piperine and its homologues get absorbed through skin, and hence are capable of acting on the subcutaneous tissues as well as on nerves and blood vessels. Pepper also has an effect on lactation by increasing milk production. Pepper oil warms the skin and brings blood to the surface, stimulating circulation. In *Ayurveda*, pepper is used in the treatment of epileptic fits and to bring about sleep. Piperine exhibited protection against penetrazole induced seizure and also against electroshock seizure. Piperine also possesses strong potentiating effect on hexobarbital induced hypnosis in mice. A compound of great interest extracted from pepper is 1-(3-benzodioxol-5yl)-1-oxo-2-propenyl-piperidide, known as antiepilepsirine, which was shown to have strong antiepileptic properties. This is used in Chinese hospitals for the treatment of epilepsy (Ebenhoech and Spadaro, 1992).

Both pepper and piperine exert liver protective action. Kaul and Kapil (1993) found that piperine reduces *in vitro* and *in vivo* lipid peroxidation and prevents depletion of GSH (Gastricsulfhydryls) and total thiols. This is a very significant property, as lipid peroxidation causes free radical production that causes tissue damage. Pepper has antioxidant activity which is attributed to the tocopherol and polyphenol contents in pepper. Supercritical carbon dioxide extracts of ground black pepper have been found superior in reducing lipid oxidation of cooked ground pork (Tipsrisukond *et al.*, 1998). The antioxidative activity of black pepper can, at least partially, be ascribed to the presence of glycosides of the flavonoids kaempherol, rhamnetin and quercetin (Vo¨ sgen and Herrmann, 1980), as well as to the phenolic amides. It was established that all the five phenolic amides present in pepper possess very good antioxidant property, which is even superior to that of the synthetic antioxidants like butylated hydroxy toluene and butylated hydroxy anisole (Nakatani *et al.*, 1986).

Addition of pepper to foods increases their keeping quality and prevents their spoilage, due to the antimicrobial properties of pepper. The essential oil of pepper is found to be inhibitory to *Vibrio cholerae, Staphylococcus albus, Clostridium diphthereae, Shigella dysenteriae,*

Streptomyces faecalis, *Bacillus* spp., *Pseudomanas* spp., etc. Pepper oil stopped the growth and aflatoxin production by *Aspergillus parasitics* at a concentration of 0.2–1%. Pepper leaf oil also exhibits antifungal activity. Pepper as well as piperine increases the bioavailability of medicaments including ampicillin and synthetic drugs as well as uptake of amino acids from food (Johri *et al.*, 1992). Piperine seems to interact with the intestinal cells so as to increase the cell permeability. Piperidine is noted as a CNS-depressant, insectifuge, spinoconvulsant and urate solvent. The amides present in pepper have been shown to have insecticidal properties (Vijayan and Thampuran, 2000).

2.11 Cardamom

Cardamom are the dried seed capsules of a small group of species or plants belonging to the family *Zingiberaceae* which contain seeds possessing a pleasant characteristic aroma and flavour. These are broadly grouped into two categories (Rao, Kumar, *et al.*, 1993; Subba, 1984):

- Small cardamom – popularly known as Chhota Elaichi (*Elettaria cardamomum*) or the true cardamom. It is also known as 'Queen of Spices'.
- Large cardamom – Bada Elaichi (*Aframomum* and *Amomum* species)

Fig 2.5: Pods and plant of cardamom

Amomum subulatum Roxb. is the greater Indian or Nepal cardamom which is also called large cardamom. It is a native of the eastern Himalayan region. The presence of several wild relatives viz., *A. delbatum*, *A. aromaticum*, *A. kinger*, *A. lingriformi*, and *A. corynostachium* and the tremendous variability within the cultivated species support the view of its origin in Sikkim (Rao, Kumar, *et al.*, 1993; Subba, 1984).

The order *Zingiberales* (formerly known as Scitamineae) to which the family *Zingiberaceae* belongs, appears to have originated as wild plants in the tropical evergreen forests. *Zingiberaceae*, the largest family of this order, is found throughout the tropics but is predominantly Asian. This family has provided important spices which are mostly aromatic, 40 genera and 900 species being recognized. The economically important species which have established themselves as aromatic spices are the genus *Zingiber* (ginger), *Curcuma* (turmeric), *Alpinia* (galanga), *Kaempferia*, all representing rhizomatous spices, and *Elettaria* (small cardamom), *Amomum* and *Aframomum* (large cardamoms) representing seed spices (Anon, 1977).

This species is cultivated in swampy places along the sides of mountain streams in Nepal, Bengal, Sikkim and Assam (eastern Himalayas) and forms one of the cash crops of eastern India. The plants are usually grown along jhoras (small springs), in moist and shady sides of mountain streams and along the hilly slopes, usually at an elevation of 765 to 1675 metres above the mean sea level. The plant is a perennial herb having subterranean rhizomes which give rise to leafy shoots and spikes. The plant matures during the third year of its growth and its height ranges from 1.5 to 3.0 m. Leafy shoot are formed by long sheath-like stalks encircling one another. The leaves are green or dark green, glabrous on both surfaces with acuminate apex. Inflorescence is a dense spike on a short peduncle bearing 40 to 50 flower buds in an acropetal sequence. The fruit is a trilocular many-seeded capsule. The capsule wall is echinated and is reddish brown to dark pink (Rao, Gupta, *et al.*, 1993).

Dried large cardamom capsules are on an average 25 mm long, oval to globose; greyish brown to dark red brown. The fruit contains 40–50 seeds, held together by a viscous sugary pulp. Though the fruits are clearly identifiable by their larger size and differences in shapes compared with small cardamom, the seeds are of nearly the same size as those of true cardamom (CFTRI, 1994). The volatile oil present in the seeds of large cardamom is one of the principal constituents responsible for providing the typical odour. The essential oil is

obtained on steam distillation of crushed seeds and yields 2.5% dark brown coloured mobile liquid with The highest volatile oil content was recorded as 3.32% in variety Golsey Dwarf, whereas the lowest was 1.95% in variety White Ramna ((Gupta *et al.*, 1984). The major constituent of large cardamom essential oil is 1,8-cineole (65–80%) while the content of terpenyl acetate is low (traces to five per cent). The monoterpene hydrocarbon content is in the range of 5–17% of which lamonene, sabeinene, the terpinenes and the pinenes are significant components. The terpinols comprise approximately five to seven per cent of the oil. The high cineole and low terpenyl acetate probably account for the very harsh aroma of this spice in comparison with that of true cardamom (Pruthi, 1993).

Of several methods available for producing essential oil, steam distillation is ideal using powdered seeds for commercial level production. The essential oil obtained by steam distillation of dry cardamom seeds ranged from 1.5 to 2.5%. An average yield of oleoresin of 4% was obtained by blending essential oil and resin fractions in the ratio of 1:1. Due to its pleasant aromatic odour, large cardamom is used for flavouring various vegetables and meat preparation in Indian dishes. It is also used as a flavouring agent in confectionery, hot or sweet pickles and in beverages. Large cardamom seed and powder are used as essential ingredients in mixed preparation and spice masala mixtures. The ripened fruits are considered to be a delicacy and are eaten raw by inhabitants of Sikkim and Darjeeling during September and October months (Gyasto *et al.* 1980, Gupta *et al.* 1984). Large cardamom is also credited with curative properties in Ayurvedic and Unani systems of medicine (Mukherjee, 1972; G. B. Singh *et al.*, 1978).

Ayurvedics use the pungent seeds for abdominal pains, biliousness, enlarged spleen, indigestion, itch, and other ailments of the head, mouth, and rectum. The seeds are credited as being alexeteric, astringent, stimulant, and stomachic, having been prescribed for abdominal diseases, biliousness, dyspepsia, rectal diseases, and vomiting. In large doses (30 grains), the seeds are taken with quinine for neuralgia. The seed decoction is gargled for gum and tooth problems. The seeds, with those of melon, are used as diuretics in kidney stones. Seeds promote elimination of bile, hence useful in liver problems. Seeds also used in gonorrhea. Unani regard the seeds as astringent, cardiotonic, hepatotonic, hypnotic, orexigenic and stomachic . The husk of the fruit (pericarp) is used for headache and “heals stomatitis” .

The use of large cardamom (fruit of *A. subulatum*), commonly known as “Heel kalan” or “Bari Ilaichi,” in the Unani system of medicine is validated in gastrointestinal disorders. A crude methanolic extract and its different fractions, viz. EO, petroleum ether (60 to 80°), ethyl acetate, and methanolic fractions, were studied in rats for their ability to inhibit the gastric lesions induced by aspirin, ethanol, and pylorus ligature. In addition, their effects on wall mucus, output of gastric acid, and pepsin concentration were recorded. The extract and its fractions of *A. subulatum,* inhibited gastric lesions induced by ethanol significantly, but not those induced by pylorus ligation and aspirin (Jafri, 2001).

2.12 Grinding and separating

Grinding herb or spice to a specified particle size using standardized sieve apertures is a normal processing activity. Grinding gives easier mixing in the final food product and aids the dispersion of flavor throughout the food. A food manufacturer will have specific particle size requirements and the processor will have to mill the herb or spice through sieves that will obtain the fineness required. For black pepper, berries are harvested when mature green, de-spiked and dried in the sun. For both black and white pepper, the quality of the product is dependent on the weather, as almost all the pepper is sun-dried. Ground pepper is obtained by grinding pepper with equipment like a hammer-mill. Cardamom capsules should be sorted into size classes and different size sieves will allow different grades to be separated as well as the separation of split and insect infested capsules. In addition to the green cardamom capsules, there is a bleached cardamom product that is creamy white or golden yellow in color. Bleaching can be undertaken with dried capsules or freshly harvested capsules (P.N. Ravindran and Kallupurackal, 2001) .

2.13 Extraction of essential oils

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

- Expression
- Hydro- or water-distillation.
- Water and steam distillation
- Steam distillation

• Solvent extraction

For each method there may be many variations and refinements and the extraction may be conducted under reduced pressure (vacuum), ambient pressure or excess pressure. The choice of extraction method will depend on the nature of the material, the stability of the chemical components and the specification of the targeted product (Douglas *et al.*, 2005).

Expression is used exclusively for the extraction of citrus oil from the fruit peel because the chemical components of the oil are easily damaged by heat. Citrus oil production is now a major by-product process of the juice industry. Distillation is the most economical method of extracting essential oil from spices and aromatic plant material. The main advantage of distillation is that it can be carried out with some very simple equipment, close to the plant production. Even in relatively remote location, large quantities of material can be processed in a relatively short time (Douglas *et al.*, 2005) .

Water distillation is the simplest of the three distillation methods. The plant material is mixed directly with water in a still pot. A perforated grid may be inserted above the base of the still pot to prevent the plant material settling at the bottom and coming in direct contact with the heated base of the base and charring. Oils from such process are commonly darker in color and have stronger still 'off-note' odors than oils produced by other methods and therefore tend to be of the lowest value. It is probably the simplest and cheapest method of extracting essential oils but the quantity of the oil has the greatest potential to be modified due to the effects of direct heating and the water contact (Douglas *et al.*, 2005).

In steam-and-water distillation, the basic still design is very similar to that of water distillation. The plant material is packed into the still pot sitting on a grill or perforated plate above the boiling water. The capacity of still pot volume is reduced but it may be possible to achieve a high packing density because the plant material is not suspended in water (Douglas *et al.*, 2005).

Advantages 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 still from the heat source.

• The effect of refluxing is minimised.

• Oil quality more reproducible.

• Faster process so more energy efficient

Steam distillation is the process of distilling plant material with the steam generated outside the still in a stand-alone boiler. As in the steam-and-water distillation system, the plant material is supported on a perforated grid above the steam inlet.

Advantages of steam distillation are as follows:

• The amount of steam and the quality of the steam can be controlled.

• Lower risk of thermal degradation as temperature generally not above 100°C.

• 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.

Flowers are generally solvent extracted and not steam distilled with the exception of rose and orange blossom. In some application, an isolate or essential oil fraction is preferred to the total oil (Douglas *et al.*, 2005) .

2.14 Research carried out on antimicrobial and preservative action of spices

1. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7 (Burt and Reinders, 2003).

2. Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria (Shabnam *et al*., 2012)

3. Effects of the zingiberaceae spice extracts on growth and morphological changes of foodborne pathogens (Oonmetta-areea, J., 2005).

4. Effect of thyme and lavender essential oils on the qualitative and quantitative traits and storage life of apple ‘Jonagold’ cultivar (Rabie *et al*., 2011).

5. Effect of volatile oil and oleoresin of anise on the shelf life of yogurt (Singh *et al*., 2010).

Part III

Materials and Methods

3.1 Material

3.1.1 Raw material collection

3.1.1.1 Collection of apple

Fresh, mature, and slightly ripe apples of Cox's Orange Pippin variety were purchased from local market of Dharan. The collected samples were carefully carried fresh to the college for detailed studies.

3.1.1.2 Collection of spices

Tejpat leaves, black pepper seeds and cardamom pods were purchased from local markets of Dharan.

3.1.2 Chemicals, glasswares and equipments

Following chemicals, glassware and equipments were used for the work as available from the laboratory at CCT, Dharan.

a) Glasswares and utensils: Petri dish, burette, pipette, test tubes, Volumetric Flask,

b) Equipments: Hot air oven, Measuring balance, Cleavenger's apparatus, Dessicator, Electronic balance, Thermometer, Heating mantle (Burner), Funnel, Muslin Cloths, Knives,

c) Chemicals: Standard ascorbic acid, Dye solution (2,6 dichlorophenol indophenol), Metaphosphoric acid (3% aqueous), Ethanol, Ascorbic acid, Fehlings Solutions.

3.2 Methods

3.2.1 Preparation of apple juice

a. Sorting/Grading

Damaged and bruised fruits were sorted out from undamaged and sound fruits.

b. **Washing**

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

c. Cutting

The fruit was then cut into small pieces with the help of stainless steel knife and the seeds were removed.

d. Juice extraction

The juice was extracted using electronic juicer. The juic was filtered using a clean muslin cloth.

3.2.2 Preparation of essential oil

The essential oils of tejpat, black pepper and cardamom were obtained by hydrodistillation using a Clevenger-type apparatus. All the essential oils were stored in refrigerator until further use.

3.3 Sample Preparation

Mature and fresh apple fruits were procured from the local market of Dharan, Sunsari . The edible fruit portion was cut into small slices and the juice was extracted by using electronic juicer. The juice was filtered through a clean muslin cloth and divided into five equal batches of 100 mL each in bottles. The control sample (I) was prepared without any additives (essential oil). Control (II) was prepared by adding 100 µL of ethanol. 10 µL of essential oil samples were predissolved in 90 µL of ethanol and then they were homogeneously mixed with three batches of apple juice. The reason for use of 10 µL of essential oil dissolved in 90 µL of ethanol was on reference to the research done by Kapoor *et al.* (2008). These samples were stored at 10 ± 2°C for 28 days. The total sugar, reducing sugar, titrable acidity, ascorbic acid, pH and microbial investigations were carried out at fixed time intervals of 7 days.

3.3.1 Determination of moisture content

The moisture determination of the fresh apple was carried out by hot-air oven method (Ranganna, 2008).

3.3.2 Determination of total and reducing sugars

Total sugar and reducing sugar was determined by Lane and Enyon's method from the process given by Ranganna (2008).

3.3.3 Determination of protein

The crude protein was determined by Kjeldahl method from the process given by Ranganna(2008).

3.3.4 Determination of titrable acidity and pH

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

3.3.5 Determination of ascorbic acid

The ascorbic acid content of apple was determined by 2, 6-Dichlorophenol-indophenol visual titration method (Ranganna, 2008).

3.3.6 Microbial Analysis

In order to determine the total microbial count and yeast and mold counts, total plate count method as described by Aneja (2010) for the examination of juice samples using the plate count agar and potato–dextrose agar, respectively, were adopted.

3.3.6.1 Total microbial count

Total microbial count was carried out using plate count agar. 1 mL of sample and 10 mL of agar were taken in petri plate and then mixed thoroughly. It was then incubated at 37°C (Aneja, 2010).

3.3.6.2 Yeast and mold count

All the experimental procedures are the same for yeast and mold counts. The only difference being that in place of nutrient agar, a potato–dextrose agar medium was used (Aneja, 2010).

3.4 Statistical analysis

The data obtained were analyzed by two ways ANOVA (no blockings) at 5% level of significance. The treatments means were compared by using LSD method (Genstat 5 Release 12.1 software programme developed by Lawes Agricultural Trust, Rothamsted Experimental

Station, 2009). Means of the data were separated whether they are significant or not by using Fisher's LSD (least significant difference) method at 5% level of significance.

Part IV

Results and discussions

The present study was carried out to find that among the spices used which one has the best effect as natural preservative on the apple juice used. For this, apple juice was extracted using juice extractor and then the juice was filtered using fine muslin cloth. The juice was divided into 5 equal batches and then essential oils were added to those juice samples. Then the samples were stored under refrigeration for 28 days and analysis was carried out at the interval of 7 days. The changes during the storage period are shown below and discussed accordingly:

4.1 Chemical composition of fresh apple fruit

Chemical composition of Apple (*Malus domestica)* was determined and the result of fresh apple is given in table 4.1

Table 4.1 Chemical composition of fresh apple

Components	Content based on edible portion of fresh fruit
Moisture (%)	85.79
Protein (% Dm)	0.1
Sugar	
-reducing sugar (% Dm)	6.66
-total sugar (%Dm)	10.49
Total acidity as malic acid (%)	0.77
Vitamin C (mg/100g)	11.76

The amount of moisture content and protein in an apple were found to be 85.79% and 0.1% respectively which were nearly similar to 85.56% and 0.26%. Vitamin C in apple was found to be 11.76 mg/100g which was a bit higher than given value 8% as determined by USDA and acidity of apple was 0.78% was nearly similar to Karadeniz and Eksi (2002). According to Karadeniz and Eksi (2002), acidity was 1.3%. The difference in result may be due to difference in method of determination and maturity of apple. The amount of reducing sugar

and total sugar were found to be 6.66% and 10.49 % respectively which were similar to Karadeniz and Eksi (2002) and USDA data source. According to Karadeniz and Eksi (2002), reducing sugar was 11.49 to 14.59%. According to USDA, 10.39% was total sugar of fresh apple.

4.2 Effect of essential oils on % total sugar during storage

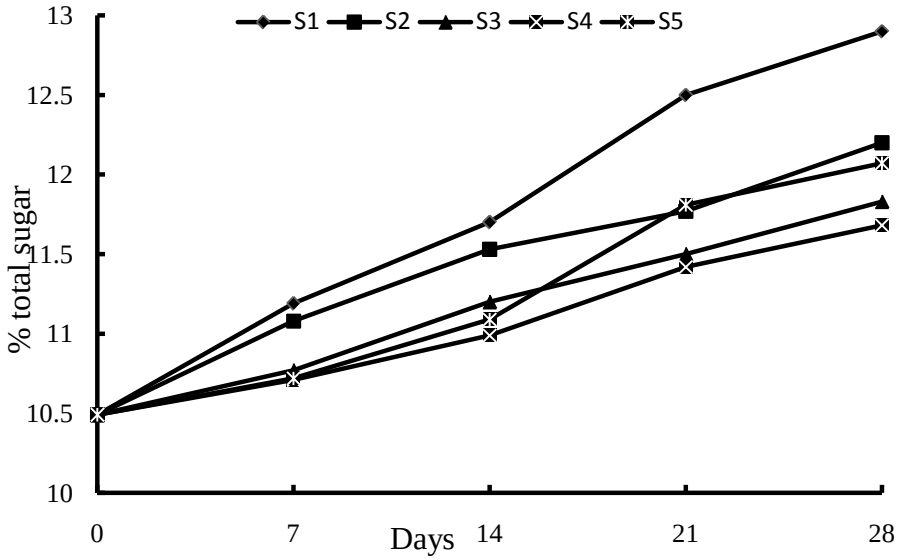

Fig.4.2: Effect of essential oil of various spices on % total sugars of apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

The % total sugar of fresh apple juice was found to be 10.49%. ANOVA showed significant results ($p < 0.05$) among different treatments and storage intervals as shown in Fig. 4.2. In fig. 4.2, on 7th day of storage, LSD showed that there is no significant difference between S1 and S2. In the same way, no significant difference was found in S3, S4 and S5 ($p \geq 0.05$). But S1 and S2 were significantly different from S3, S4 and S5. LSD showed that on 14th day ($p<0.05$), there was significant difference between samples S1 & S2, S1 & S3, S1 & S4, S1 & S5, S2 & S3, S2 & S4, S2 & S5 and S3 & S4 ($p<0.05$). Sample S3 & S5 and S4 & S5 were significantly indifferent ($p \geq 0.05$). On 21st day, on the basis of LSD, there was significant difference between S1 & S2, S1 & S3, S1 & S4, S1 & S5, S2 & S3, S2 & S4, S2 & S5, S3 & S5 and S4 & S5 ($p<0.05$). On the same day, S3 and S4 were significantly indifferent with each other ($p \geq 0.05$). All the samples were significantly different from each other on the 28th day of

storage ($p<0.05$); however, there is no significant difference between samples S2 & S5 ($p\geq0.05$).

From table A 1.2, LSD shows that there was significant change in sample S1 from 0 day to 28th day ($p<0.05$). But on the 7th and 14th day, changes were significantly indifferent and even the changes on 21st and 28th day were significantly indifferent ($p\geq0.05$). Sample S2 also showed significant change during 28 days ($p<0.05$) but the change on 14th and 21st day were significantly indifferent ($p\geq0.05$). There was significant change in samples S3, S4 and S5 during 28 days of observation on the basis of LSD table ($p<0.05$).

An increasing trend of total sugars in all treatments was observed. It is clear from the above graph that at the end of 28 days, the maximum sugar percentage was found in S1 and the minimum was noted in S4. There was continuous increase in total sugar up to 28 days of storage. The increase in total sugar might be due to hydrolysis of polysachharides like pectin, cellulose and starch and its conversion into simple sugars. Singh and Mathur (1983) observed that total sugars increased during storage in cashew apple juice. This gradual increase in total sugar percentage might be due to increase in dehydration in fruits which resulted in the concentration of juice as supported by Badshah *et al.* (1994) who found that sugar content of apples increased with storage. It shows that the total sugar increase significantly with increase in storage period.

4.3 Effect of essential oils on reducing sugar of apple juice

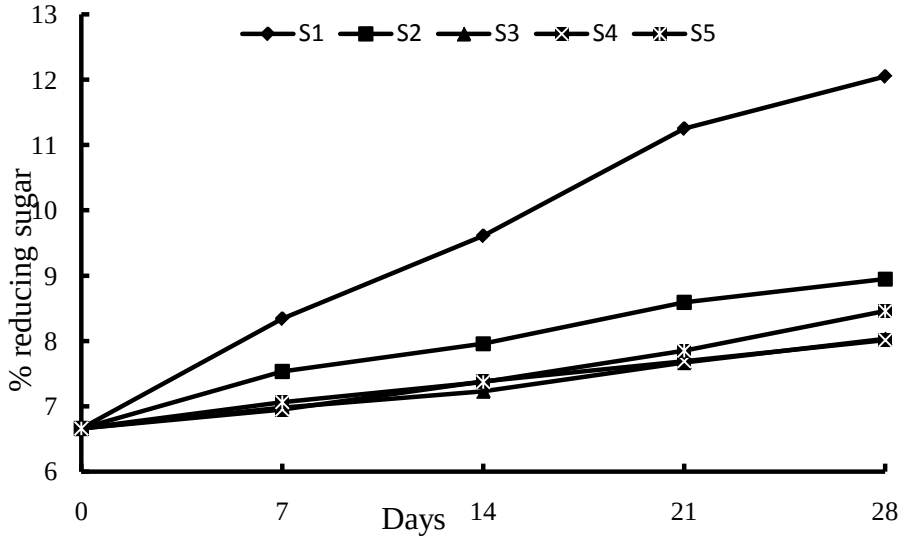

Fig.4.3: Effect of essential oil of various spices on % reducing sugars of apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

It is evident from Fig. 4.3. that statistically, all the treatments were significant ($p < 0.05$) to each other. There was a rise of percent-reducing sugar with storage period. LSD showed that on the 7th day of storage, sample S1 & S2 were significantly different from samples S3, S4 & S5 ($p< 0.05$). However, there was no significant difference in samples S3, S4 and S5. On 14th day of storage, according to LSD, samples S1 & S2, S1 & S3, S1 & S4, S1 & S5, S2 & S4, S2 & S5, S3 & S4 and S3 & S5 were significantly different from each other ($p< 0.05$). It also showed that there was no significant difference between samples S4 & S5 ($p\geq0.05$). As on 21st day, LSD gave the information that samples S1 & S2 were significantly different from each other ($p< 0.05$) and along with it, they were also significantly different from samples S3, S4 & S5 ($p < 0.05$).. Samples S3, S4 & S5 were significantly indifferent from each other on that day ($p\geq0.05$). The same result was also seen also on the 28th day of storage though there was increase in reducing sugar percentage.

The changes in reducing sugar percentage of all the samples S1, S2, S3, S4 and S5 were significantly different from each other on 0 day & 7th day, 7th day & 14th day, 14th day & 21st dat and 21st day & 28th day of observation ($p<0.05$).

The gradual increase in reducing sugars might be due to hydrolysis of polysaccharides. Moreover, it could be due to the dehydration as a result of moisture loss and decrease in acidity of fruit juice by physiological changes during storage as supported by Hussein *et al*. (2001) who found that redusing sugar increases in Golden delicious apple during storage. Comparison of treatments showed that the highest value for reducing sugar is S1, whereas the lowest value for reducing sugar is S4 although S3 also has the % reducing sugar in the same range as S4. The increase in reducing sugar content could be due to the breakdown of polysaccharides into water soluble sugar during glycolysis process. Similar changes were also observed by Mattoo *et al.* (1975) indicating that starch was completely hydrolysed into soluble sugar such as glucose, fructose and sucrose. An increase in reducing sugar with increase in

storage period could be attributed to gradual inversion of non-reducing sugars and acids into reducing sugars (Singh and Mathur, 1983).

4.4 Effect of essential oils on ascorbic acid of apple juice

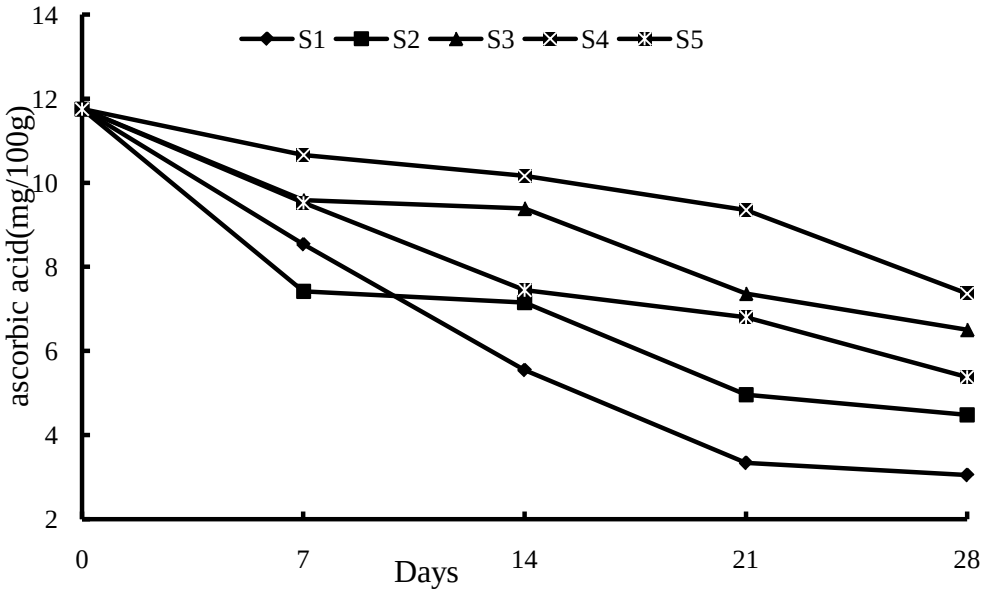

Fig.4.4: Effect of essential oil on ascorbic acid content of apple juice during storage

. S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

ANOVA showed highly significant results ($P < 0.05$) among different treatments and storage intervals as shown in Fig.4.4. Evaluation of ascorbic acid (Vit. C) is an index of the nutrient quality of fruits because as compared with other nutrients, it is much more sensitive to various mode of degradation in food processing and storage as reported by (Ozkan *et al.*, 2004). Ascorbic acid is considered an enhancer of iron absorption when it is added to the food as a natural source or in pure form (Davidson *et al.*, 2001; Hallberg *et al.*, 1986). Under storage condition, ascorbic acid decline significantly in all treatment. On 7^{th} day of storage, LSD shows that most of the samples are significantly different from each other ($p<0.05$) except samples S2 & S5 which are significantly indifferent to each other ($p\geq0.05$). Afterwards on 14^{th}, 21^{st} and 28^{th} days of storage, it can be seen that all the samples are significantly different from each other ($p<0.05$).

The change in ascorbic acid content of samples S1, S2, S3, S4 and S5 were significantly different from each other on 0 day, 7th day, 14th day, 21st day and 28th day on the basis of LSD table ($p<0.05$).

Furthermore, decrease in ascorbic acid content might be due to oxidation of ascorbic acid to dehydroascorbic acid by enzyme ascorbinase (Das and Dash, 1967). It is observed that ascorbic acid content is more in fruit juice having cardamom oil i.e. S4 than sample treated with other oils. Comparison of treatments showed that the highest value for ascorbic acid content was found in S4 followed by S3, and the lowest for S1. The possible reason may be that cardamom essential oil (S4) and pepper oil (S3) delayed the oxidation of ascorbic acid to dehydroascorbic acid by enzyme ascorbinase to some extent(Das and Dash, 1967). Enzymes like cytochrome oxidase, ascorbic acid oxidase and peroxidase are responsible for the oxidation of ascorbic acid and subsequent loss of Vitamin C potency (Nagy, 1980). Incorporation of air into juice during extraction has also been recognized as the cause of ascorbic acid loss (Farnworth *et a*l., 2001). Data regarding storage intervals showed that in all the treatments, ascorbic acid contents decreased as the storage period was prolonged.

4.5 Effect of essential oils on titrable acidity of apple juice

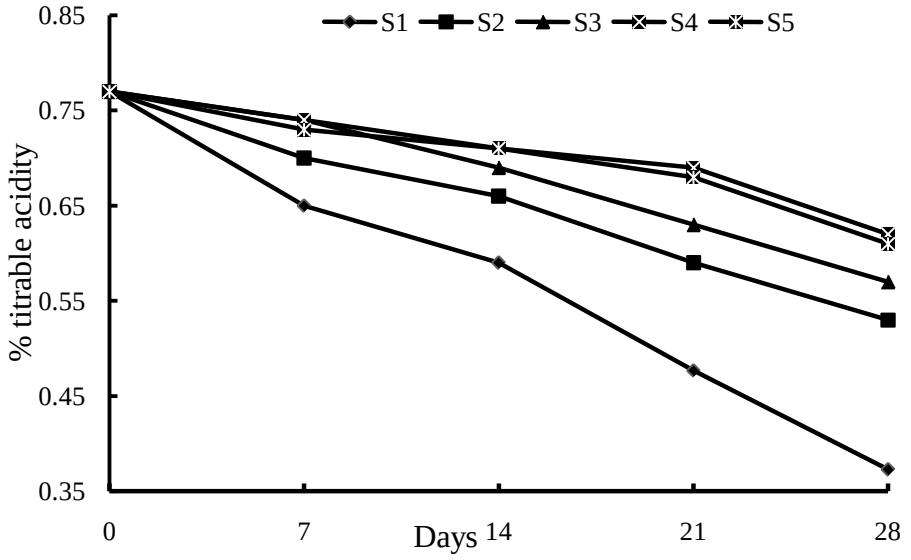

Fig.4.5: Effect of essential oil on % titrable acidity on apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

Change in titrable acidity of apple juice upon storage with various treatments for 28 days is shown in Fig.4.4. ANOVA showed highly significant results ($p< 0.05$) among different treatments. From the graph, it is clear that under storage condition, decrease in acidity occurs upto 28 days of storage period. LSD showed that on 7^{th} day of storage, samples S1, S2, S3 S4 & S5 are significantly different from each other ($p<0.05$) except sample S3 & S5 which are significantly indifferent from each other ($p\geq0.05$). On 14^{th} day, all the samples are significantly different from each other according to LSD information ($p<0.05$). The lowest level of acidity can be seen in control sample i.e. S1 and the highest can be observed in S4. Similar outcomes can be seen on 21^{st} and 28^{th} days of storage.

The changes in titrable acidity of sample S1 were significantly different on 0 day, 7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day from each other ($p<0.05$). Similarly, the change in titrable acidity of sample S2, S3 and S5 were significantly different on 0day, 7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day from each other ($p<0.05$). There was significant difference in titrable acidity change on 0day & 7^{th} day and 7^{th} day & 14^{th} day ($p<0.05$). On 14^{th} day and 21^{st} day, there was no significant difference in titrable acidity of sample S4 ($p\geq0.05$). The change on 28^{th} day was significantly different from changes on other days ($p<0.05$).

Decrease in titrable acidity might be due to release of acids by decomposition, hydrolysis, oxidation or fermentation which modifies hydrogen ion concentration and consequently, decreases food acidity. Similar observations have been recorded for kinnow by (Panesar *et al.*, 2000) and for orange juice (Goyle and Ojha, 1998). The decline in acidity could be due to conversion of acid into sugar and salts (Ruttner *et al.*, 1975).

4.6 Effect of essential oils on pH of apple juice

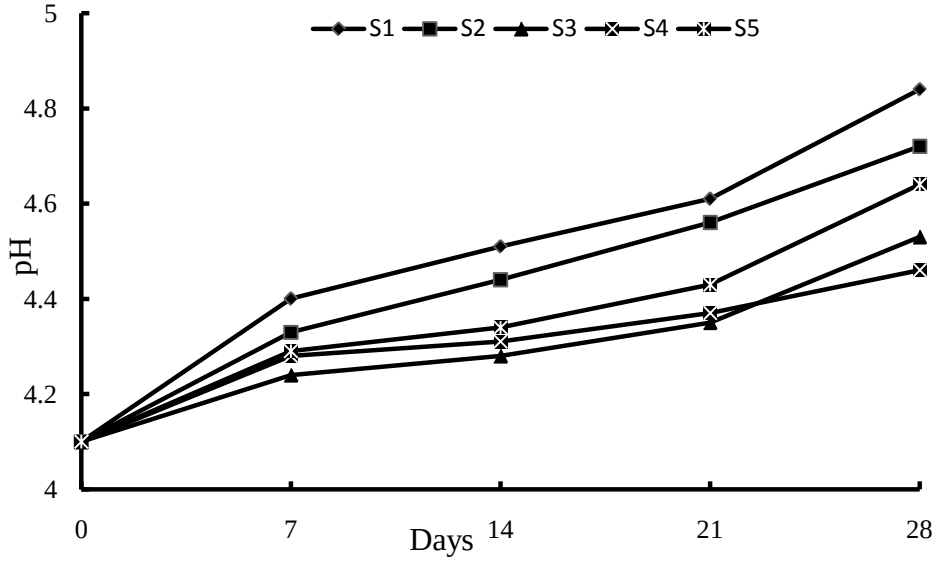

Fig.4.6: Effect of essential oil on pH of apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

The change in pH during storage condition is shown in Fig.4.6. It can be seen that the pH increases gradually according to decrease in titrable acidity. It is evident from above graph that all the treatments affect the pH of the samples significantly ($p< 0.05$). On 7^{th} day of storage, LSD showed that sample S1 & S2 are significantly indifferent with each other ($p\geq0.05$). Samples S2, S3, S4 & S5 showed no significant difference among each other ($p\geq0.05$) but S1 is significantly different from S3, S4 & S5 ($p<0.05$). LSD showed highly significant difference among all the samples on the rest of 14^{th}, 21^{st} and 28^{th} days ($p<0.05$). An increasing trend of pH in all treatments during storage was observed. At the end of the experiment i.e. on the 28^{th} day, maximum pH value was found in S1 followed by S2 and minimum pH values were noted in S4 as shown in the graph. This may be due to the higher concentration of essential oil which causes comparatively less increase in acidity and other biochemical changes resulting in less increase in pH. Furthermore, gradual increase was observed in pH during storage.

From LSD table, it can be seen that pH change in sample S1 on 0 day is significantly different from 7^{th}, 14^{th}, 21^{st} and 28^{th} days ($p<0.05$). There was no significant change in pH on 7^{th}, 14^{th} and 21^{st} days ($p\geq0.05$). On 28^{th} day, the change in pH was significantly different from

other days ($p<0.05$). LSD showed that there was significant difference in pH of Sample S2 on 0 day and 7th day ($p<0.05$). The changes in pH on 7th day & 14th day, 14th day & 21st day and 21st day & 28th day were significantly indifferent from each other ($p\geq0.05$). pH change in Sample S3 was significantly different from 0 day than 7th day ($p<0.05$). The change on 7th day was significantly indifferent from change on 14th day ($p\geq0.05$) and similarly, there was no significant difference in pH change on14th day and 21st day ($p\geq0.05$). There was significant difference in pH change on 21st day and 28th day of sample S3 ($p<0.05$). Similar observations as that of Sample S3 were seen on Sample S4 during 28 days ($p<0.05$). On sample S5, there was significant difference in pH change on 0 day and 7th day ($p<0.05$). There was no significant difference on pH change on 7th day, 14th day and 21st day of observation ($p\geq0.05$). The change on 28th day was significantly different ($p<0.05$).

The increase in pH may be due to a decrease in acidity and increase in total sugar content (Baruah and Mohan, 1985). pH increase might be due to decrease in acidity through biochemical changes within fruit juice durin storage (Khalid, 1974). It is evident that the pH value of all the samples under refrigerated condition significantly increased during storage.

4.7 Effect of essential oils on total microbial count of apple juice

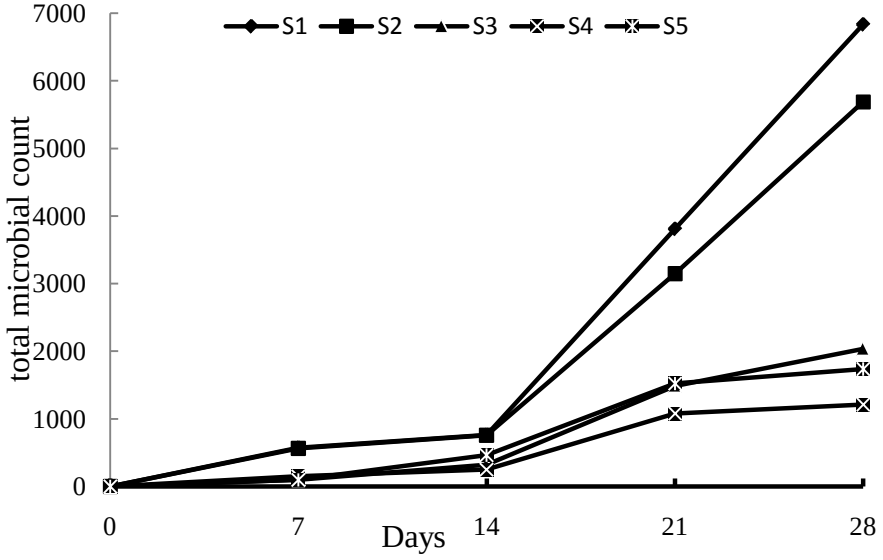

Fig.4.7: Effect of essential oil microbial count of apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

In all the samples, microbial loading had increased with increasing storage time. However, beneficial effects of addition of essential oil of various spices on the microbial growth were observed. On 28 days of observation, all the samples showed significant different with each other. These additives were able to delay the growth of microorganisms in juices. The cardamom essential oil was found to be most effective in controlling microbial growth. Other essential oil was also found to be very effective. The activity of essential oil is due to the presence of higher percentage of monoterpene hydrocarbons, which may be responsible for controlling the microbial growth as explained by (Ahmed *et al.*, 1993), which acts synergistically. Kapoor *et al.* (2008) also mentioned the synergistic action of various phenolic compounds, piperine, flavonides and several other constituents, which inhibits the growth of these microbes. Nevertheless, it is difficult to give a definite explanation for all results recorded. Microbial increase might be due to the germination of spores as the effect of preservatives decreased gradually. Bacterial groth is eliminated to a very lare extent by acidity, bacterial spores will not germinate in juice with pH less than 4.5 and vegetative cells of pathogenic bacteria will not grow at ph less than 4.0(Smelt *et al.*. 1982). At the end of storage duration, microbial growth showed higher increase which might be because of temperature of storage and modification in pH of the juice. This increase in microbial load is supported by Suaad and Eman (2008), who studied that there is presence of different species of bacteria in supposedly bacteria-free commercially available fruit juices over time. The increase may be partly due to high moisture content in fruit juices which has been found to promote the growth of yeast and bacteria (MacRae *et al.*, 1993). Moreover, the inhibition of growth of microbes can be attributed to the synergistic action of various phenolic compounds present in the essential oils. Hence, according to these data, preservative order of essential oil could be:

S4 > S3 > S5 > S2 > S1

4.8 Effect of essential oil on yeast and mold counts of apple juice

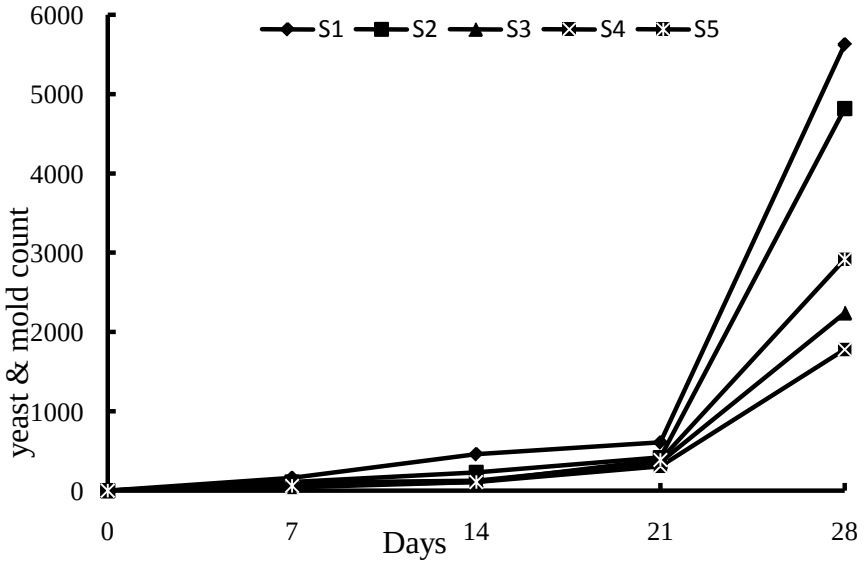

Fig.4.8: Effect of essential oil on yeast and mold count of apple juice during storage

S1- Control I S2- Control II S3- Black pepper oil S4- Cardamom oil S5- Tejpat oil

The yeast and mold counts increase with prolonged storage period. This might be because of external contamination and also the growth of yeast and molds already present in the juice. This increase in yeast and mold, and their microbial contamination may also be due to the growth of acid-producing bacteria. A lot of work has been reported on the antimicrobial efficiency of essential oils (Burt and Reinders, 2003). They have been used as flavoring agent in hot food and beverages and due to their versatile content of antimicrobial compounds, they possess natural agent for food preservation (Conner, 1993). The antimicrobial activity of essential oils is assigned because of phenolic compounds (Karapinar and Aktung, 1987). It would increase the flavor as well as the juice quality.

From above graph, it is clear that microbes, yeast and mold are increased as the storage time increases. This may be due to the contamination of juice during storage and also the growth of yeasts and molds already present in juice. 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 toward the later stages of storage, control sample showed significantly higher ($P > 0.05$) growth of yeasts and molds as compared to the other treated samples. This may be due to

exponential growth of microbes during log phase. In fact, cardamom oil had shown least proliferation of spoilage microbes. The control of growth of spoilage microorganisms in apple juice by the addition of cardamom essential oil might be due to the major components, say, 1,8- cineol (43.7%), a-terpineol (9.5%), terpinene-4-ol (3.2%), spathulenol (2.7%) and a-pinene (1.6%), respectively (Singh *et al.*, 2004). Moreover, the inhibition of growth of microbes can be attributed to the synergistic action of various phenolic compounds present in cardamom oil. Cardamom essential oil showed the most preservative effect as compared to black pepper and tejpat essential oil.

PART V

Conclusions and Recommendations

5.1 Conclusions

As stated in the objective, essential oils of black pepper, tejpat and cardamom were extracted. The effects of essential oils on apple juice were studied on the basis of total sugar, reducing sugar, pH, titrable acidity, ascorbic acid and microbial change. Comparing the effects of the essential oils on apple juice, the best essential oil was determined for preservative purpose among the three oils. As per the objectives and my study, following conclusions were drawn:

1. Significant changes were observed in different parameters undertaken when compared to the control sample.
2. During observation, % total sugar, % reducing sugar, pH, total microbial count and yeast & mold count were increased whereas ascorbic acid content and titrable acidity decreased in the control sample.
3. Apple juice containing cardamom oil was found to be superior to other essential oils (i.e. black pepper and tejpat).There was no significant change in ascorbic acid content in the sample containing cardamom essential oil during the storage. Total microbial count and yeast & mold count were inhibited to greater extent.

5.2 Recommendations

Based on the result obtained, following recommendations could be made:

1. Preservative properties of oleoresins and essential oils of different spices for different fruit juices could be studied.
2. Sensory evaluation and enzymatic changes could be studied.

Part VI

Summary

Nepal is an agricultural country; most of the economy depends upon farming. Varieties of crops are grown throughout the country. Among the various agricultural commodities, horticulture crops play a significant role in the economic growth of the country. Fresh agricultural commodities are great source of vitamins and minerals. They are very susceptible to spoilage i.e. have very short shelf life resulting in loss of freshness and nutrients. Because of this, food safety is a fundamental concern for both consumers and food industries. Different preservation methods have been applied to fruits and their products for lengthening their freshness and storage period. Certain chemical preservatives have been used for preservation and storage of fruits and their products. Pure apple juice is a colorless and virtually odorless liquid. Within seconds of its expression from the fruit, however, it undergoes a sequence of enzymatic changes to produce the color and aroma we are familiar with. Raw juice can be protected from microbiological degradation for few days under refrigerator. Application of conventional chemical preservatives such as benzoic acid and sorbic acids and sulfites to fruit juice is an alternative. As these chemical preservatives are imported. For developing countries, preservation should be inexpensive, simple and reliable. Different spices of preservation importance are grown within the country. Essential oils possess remarkable preservative properties. Since ancient times, herbs and spices have been used for preventing food preservation. At the end of the last century, antimicrobial activities of spices had already been examined and heir oils are well known to retard microbial spoilage. The present work was carried out to preserve apple juice using essential oil of spices. Along with this comparison between spices was carried out on the basis of their preservation properties. The chemical composition of fresh apples (*Malus domestica*) was determined. The value of moisture content, acidity, total sugar, reducing sugar, pH, protein, and vitamin C were found to be 85.79%, 0.77%, 10.49%, 6.66%, 4.1, 0.1% and 11.76% respectively.

The spices (i.e. black pepper, tejpat and cardamom) were ground into powder. The spice powder was then used for essential oil extraction. The essential oils of spices were extracted by hydrodistillation using Clevenger-type apparatus. All the essential oils were stored under refrigeration until further use. Apples were washed to remove dust, adhered impurities etc. and

cut into small pieces. The juice was extracted using electronic juicer and filtered through clean muslin cloth. Five equal batches of apple juice were prepared. Control sample was prepared without any additives i.e. essential oil. Control sample II was prepared adding 100 µL of ethanol. 10 µL of each essential oil sample i.e. black pepper, tejpat and cardamom was predissolved in 90 µL of ethanol and then homogeneously mixed with other three samples of juice. Those samples were then kept under refrigeration for 28 days and changes were examined at the interval of 7 days.

The experiment carried out for 28 days showed certain changes in the samples. In reference to the control sample, samples treated with essential oils were somehow preserved. The notations S1, S2, S3, S4 and S5 are for control sample with no additives, control sample with ethanol, sample with black pepper oil, sample with cardamom oil and sample with tejpat oil respectively. At the end of 28 day, % total sugar, % reducing sugar, % ascorbic acid, pH, % titrable acidity, toal microbial count and yeast and mold count of Sample S1 were 12.9, 12.05, 3.05, 4.84, 0.373, 6832 and 5623 respectively and that of sample S2 were 12.2, 8.95, 4.48, 4.72, 0.53, 5690 and 4817 respectively. The % total sugar and % reducing sugar of Sample S3, S4 and S5 were 11.83, 11.68, 12.07, 8.03, 8.01 and 8.46 respectively. Ascorbic acid content of sample S3, S4 and S5 were 6.51, 7.37 and 5.38 respectively and that of pH and % titrable acidity were 4.53, 4.46, 4.64, 0.57, 0.62 and 0.61 respectively. The total microbial count and yeast % mold count of sample S3, S4 and S5 were 2033, 1210, 1737, 2240, 1780 and 2917 respectively. In the case of total sugar, reducing sugar and titrable acidity, essential oils had somewhat same kind of effect. Cardamom essential oil showed the most preservative effect on ascorbic acid, pH, total microbial count and yeast & mold count of the sample. From overall observation, it can be seen that cardamom oil had the most preservative action on the sample.

References

Ahmed, V., Baqai, T. F. and Ahmed, R. (1993). A tigogenin pentasaccharide from Cestrum diurnum. Phytochemistry (34), 511-515

Anon. (1977). In Encyclopaedia Britannica Macropaedia (15th ed.). Vol. 19.

Anonymous. (2008a). "Apples Keep Your Family Healthy". Washington State Apple Advertising Commission. Retrieved from http://www.bestapples.com/healthy. [Accessed 22 October, 2012].

Anonymous. (2008b). For decreased risk of colon, prostate and lung cancer: "Nutrition to Reduce Cancer Risk". The Stanford Cancer Center (SCC). Retrieved from http://cancer.stanford.edu/information/nutritionAndCancer/reduceRisk/. [Accessed 23 November, 2012].

Anonymous. (2008c). Origin, History of Cultivation. University of Georgia. Retrieved from http://web.archive.org/web/20080121045236/http://www.uga.edu/fruit/apple.html. (Last update January 21, 2008). [Accessed 10 October, 2012].

Badshah, N., Haroon, U. R. and Safi, S. (1994). Role of calcium in prolonging the shelf life of apples. *Sarhad J. Agric.* **10** (6), 639-645.

Baruah, P. J. and Mohan, N. K. (1985). Some aspects of development physiology of sapida fruit. *J. Res. Assam Agric. Univ.* **6** (1), 59-61.

Bhurtyal, P. R., Regmi, K. R. and Sapkota, R. P. (1996). NARC: status and prospectus. Presented at 1st National workshop on horticultural research. Lumle horticultural research centre.

Boyer, J., Liu, R.H. and Liu, R.H. (2004). "Apple phytochemicals and their health benefits" (5 ed.). Vol. 3. Cornell University, Ithaca, New York 14853-7201 USA. Department of Food Science and Institute of Comparative and Environmental Toxicology. p. 1.

Retrieved from http://www.nutritionj.com/content/3/1/5. [Accessed 24 November, 2012].

Bradley, Marshall, F., Martin and L., D. (2009). The Organic Gardener's Handbook of Natural Pest and Disease Control. Rodale, Inc.

Burt, S. A. and Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. *Letters in Applied Microbilogy*. **36**, 162-167.

CFTRI. (1994). "Studies on post harvest technology, product development and diversification of end uses of large cardamoms". Mysore. Central Food Technological Research Institute. p. 90.

Cha, A., Graves, V. and Shea, T. B. (2006). Apple juice concentrate maintains acetylcholine levels following dietary compromise. *Journal of Alzheimer's Disease* **9**(3), 287-291.

Choudhary, P. (1999). Nutritional and microbiological quality of sweet orange (Mosambi) juice sold in Udaipur city. Unpublished M.Sc. Dissertation. Rajasthan Agricultural Univ., India.

Conner, D. E. (1993). Naturally occurring compounds. In P. Davidson and A. L. Branen (Eds.), *Antimicrobials in Foods* (pp. 441-468). New York: Marcel Dekker.

Correa de Souza, M.C., Banassi, M.T. and Meneghel, F.A. (2004). Stability of unpasteurized and refrigerated orange juice. *Braz. Arch. Biol. Technol.* **47**, 391-397.

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

Das, R. C. and Dash, J. (1967). The effect of wax emulsion, 2, 4-D and 2, 4, 5-T on storage behaviour of Mosambi fruits (Citrus sinensis L). *Proc. Int. Symp. Subtrop. Trop. Hort New Delhi*. 104-107.

Davidson, L., Walczyk, T., Zavaleta, N. and Hurrell, R. F. (2001). Improving iron absorption from Peruvian school breakfast meal by adding ascorbic acid. *Am. J. Clin. Nutr.* **73**, 283-287.

Desmond and Andrew. (1994). "The World Apple Market". Haworth Press.

Devkota, L. N. (1999). Decidious Fruit Production in Nepal. *In*.). pp. 66-67. Bangkok, Thailand. Regional Office for Asia and the Pacific.

Dighe, V. V., Gursale, A. A., Sane, R. T., Menon, S. and Patel, P. H. (2005). Quantitative determination of Eugenol from Cinnamomum tamala Nees and Eberm leaf powder and polyherbal formulation using Reverse Phase Liduid Chromatography. *Chromatographia*. **61**, 433-446.

Dorantes, L., Colmenero, R., Hernandez, H., Mota, L., Jaramillo, M. E., Fernandez, E. and Solano, C. (2000). Inhibition of growth of some food-borne pathogenic bacteria by Capsicum annum extracts. *Int. J. Food Microb.,*. **57**, 125-128.

Douglas, M., Heyes, J. and Smallfield, B. (2005). "Herbs, Spices and Essential Oils". United Nations Industrial Development Organization &Food and Agriculture Organization of the United Nations.

Duke, J. A. e. a. (2002). "CRC Handbook of Medicinal Plants" (2 ed.). CRC Press. Boca Raton.

Ebenhoech, A. and Spadaro, O. (1992). Antiepilepsirine: A New Chinese Anticonvulsant Herb Drug. *J. Eco. Tax. Bot.* **16**, 99-102.

Edwards, D. M. (1993). The marketing of non-timber forest products from the Himalayas: The Trade betweenEast Nepal and India. . Presented at Rural Development Forestry Network Paper. Overseas Development Institute, London. pp. 1-21.

Eissa, H. A., Nadir, A. M. and Ibrahim, K. E. (2003a). Effect of anti-browning and anti-microbial constituents of some spices on the quality and safety of apple juice. *A.M.S.E. France*. **64** (2), 42-57.

Eissa, H. A., Nadir, A. M. and Ibrahim, K. E. (2003b). Effect of anti-browning and anti-microbial constituents of some spices on the quality and safety of apple slices. *A.M.S.E. France*. **64** (4), 15-33.

Ejechi, B., Souzey, J. and Akpomedaye, D. (1998). Microbial stability of mango juice preserved by combined application of mild heat and extracts of two tropical spices. *J. Food Protect.* **61**, 725-727.

El-Assi, N., Huber, D. and Breeht, J. (1997). Irradiation induced changes in tomato fruit and pre-carp firmness, electrolyte efflux and cell wall enzyme activity as influneced by ripening stage. *J. Amer. Soc. Hort. Sci.* **122**, 100-106.

Ferree, Curtis, D. and Warrington, I. J. (1999). "Apples: Botany, Production and Uses". CABI Publishing.

Forney, C.F., Kalt, W. and Jordan, M.A. (2000). The composition of strawberry aroma is influnced by cultivar, maturity and storage. *Hort. Science.* 35,1022-1026.

Goyle, A. and Ojha, P. (1998). Effect of storage on vitamin C, microbial load and sensory attributes of orange juice. *J. Food. Sci. Technol.* **35**, 346-348.

Guenther, E. (1972). The Essential Oils. *In:* "History Origin in Plants Production Analysis" (Vol. 1). (R. E., Ed.). Malabar, Florida. Krieger Publishing.

Gupta, P. N., Nagni, A., Misra, L. N. and Nigam, M. C. (1984). Gas chromatographic evalutaion of the essential oils of different strains of *Amomum subulatum* growing wild in Sikkim. *Soundrdruck aus Perfumerie und Kosmetic*. **65**, 528-529.

Hadady, L. (1996). "Asian Health Secrets". Crown Publishers. New York.

Hallberg, L., Brune, M. and Rossander, L. E. o. a. a. o. i. a. f. d. t. o. m. S. w. a. a. r. f. a. s. a. a. g. i. d. a. i. (1986). Effect of ascorbic acid on iron absorption from different types of meals. *Hum. Nutr.* **40A**, 97-113.

Hendel. (2012). "The Book Of Genesis: A Biography". Princeton University Press.

Hirasa, K. and Takemasa, M. (1998). Spice Science and Technology. New York, USA: CRC Press.

Hitokoto, H., Morozumi, S., Wauke, T., S., S. and Kurata, H. (1980). Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Appl. Environ. Microbiol.* **39**, 811-822.

Hussein, M.A., Mahdy, E.L. and Ibrahim, T.K. (2001). Effect of Calcium chloride and Gibberellic acid treatments on Anna and Dorest Golden apples during storage. *J. Agr. Sci.* **32**(2), 185-200.

Jafri, M. A. (2001). Evaluation of the gastric antiulcerogenic effect of large cardamom (fruits of *Amomum subulatum Roxb*). *J. Ethnopharmacol*. **75** (2-3), 89-94.

Javed, S., Mishtaq, S., Khokhar, I., Ahmad, R. and Haider, M.S. (2012). Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria. *African Journal of Biotechnology*. **11**(94), 16065-16070.

Jayasree, S., Wahid, P. A. and Kamalam, N. V. (1988). Absorption of soil applied radio phosphorus by black pepper vines and support trees in relation to the root activities. *J. Plantation Crops*. **16**, 73-87.

Oonmetta-areea, J. (2005). Effects of the zingiberaceae apice extracts on growth and morphological changes of food borne pathogens. Ph.D. in Food Technology. Suranaree University of Technology.

Johri, R. K., Thusa, N., Khajura, A. and Zutshi, U. (1992). Piperine mediated changes in permeability of rat intestinal epithelial cells. *Biochem. Pharmacol*. **43**, 1401-1407.

Juniper, B. E., Mabberley, D.J. . (2006). "The Story of Apple". Timber Press.

Kapoor, Singh, B. and Singh, G. (2008). Essential oils and oleoresins of Cinnamomum tamala (Tejpat) as natural food preservatives for pineapple fruit juice", . *J. Food Process. Preserv.* **62** (32), 719-728.

Kapoor, V. K., Chawla, A. S., Kumar, M. and Kumar, P. (1993). Search for anti-inflammatory agents. *Indian Drugs*. **30**, 481-493.

Kar, A., Choudhari, B. K. and Bandyopadhyay, N. G. (2003). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of Ethnopharmacology*. **84** (1), 105-108.

Karadeniz, F. and Eksi. (2002). A study on total sugar of golden delicious variety of apple. *J. European Food Research and Technology*. 159-160.

Karapinar, M. and Aktung, S. E. (1987). Inhibition of food born pathogen by thymol, eugenol, menthol and anethole. . *J. Agric. Food Chem.* **4**, 161-166.

Kaul, I. B. and Kapil, A. (1993). Evaluation of liver protective potential of piperine-an active principle of black pepper. *Planta Medica*. **59**, 413-417.

Kharel, G. P. and Hashinaga, F. (2004). "Principles of Food Preservation". Shibundo Co. Ltd. Kagoshima, Japan.

King, A. D., Bolin, H.R. (1989). Physiological and microbiological storage stability of minimally processed fruit and vegetables. *Food Technol.* **143** (2), 132-135.

Kumari, V. (1995). Nutritional and microbial quality of sugar cane juice sold in Udaipur City. Unpublished M.Sc. Dissertation. Rajasthan Agricultural Univ., India.

Lal, G. and Siddappa, G. S. (1960). "Preservation of fruits and vegetables". Publication and Information Division of Indian Council of Agrucultural Research. New Delhi.

Lawson, W. (2006). Apple and apple juice contains antioxidants that protect cells throughout the body, particularly the brain and heart. *Psychology Today*. Retrieved from 2010-06-19. [Accessed 7 November, 2012].

Lea, A. G. H. (1994). Apple juice. *In:* "Non-carbonated Fruit Juices and Fruit Beverages" (2nd ed.). (P. Ashurst, Ed.). pp. 182-225. Glasgow. Blackie and Sons.

Lee, K. W., Kim, Y. J., Kim, D. O., Lee, H. J. and Lee, C. Y. (2003). Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem.* . **51** (22), 6516-6520.

Lee, K. W., Lee, S. J., Kang, N. J., Lee, C. Y. and Lee, H. J. (2004). Effects of phenolics in Empire apples on hydrogen peroxide-induced inhibition of gap-junctional intercellular communication. **21**, 1-4.

Leistner, L. (1994). Application of hurdle technology in developing countries. *In:* "Food Preservation by Combined Processes". (L. L and G. L, Eds.). pp. 95-98. Brussels, Belgium. Commission of the European Community.

Lin, R. I. (1994). Pharmacological properties and medicinal use of pepper (*Piper nigrum L.*). In G. Charalambous (Ed.), *Spices, Herbs and Edible Fungi*. Amsterdam: Elsevier Science B.V.

Maher, T. (2011). Antioxidants Found in Apples May Extend Lifespan. *Journal of Agricultural and Food Chem.*

Marth, E.H. 1978. *Standards Methods for the Examination of Fruits and Dairy Products,* 14th Ed., American Public Health Association, Washington, DC.

Martindale, W. H. (1910). *Perfum. Essent. Oil Rec.* **1**, 266.

Maruzzella, J. C. and Lichtenstein, M. B. (1956). *J. Am. Pharm. Assoc. Sci. Ed.* **45**, 378.

Mattoo, A. K., Murata, T., Pantastico, E. B., Chachin, K., Ogata, K. and Phan, C. T. (1975). Chemical changes during ripening and senescence, in post harvest physiology, handling utilization of tropical and sub-tropical fruits and vegetables [Newsletter]. AVI Publishing, Westport, CT. 13-15.

Miyamoto, T. (1986). Antimicrobial properties of an ethanol extract of Wasabi using *E.coli J. Antibact. Antifung. Agents*. **14**, 517.

Mukherjee, D. K. (1972). Large cardamom. *World Crops*. **25** (1), 31-33.

Nakatani, N., Inatani, R., Ohta, H. and Nishioka, A. (1986). Chemical constituents of pepper and application to food preservation. Naturally occurring anti-oxidative

compounds. *Environ. Health Perspect.* **67**, 135-147.

Narayanan, C. S. (2000). Chemistry of Black Pepper. *In:* "Black Pepper". (P. N. Ravindran, Ed.). pp. 143-162. Harwood Academic.

Norman and Jill. (1991). "The Complete Book of Spices". Viking Studio Books, div. Penguin Books. New York.

Ozkan, M., Kirca, A. and Cemeroglu, B. (2004). Effect of hydrogen peroxide on the stability of ascorbic acid during storage in a various fruit juices. *Food Chem*. **88**, 591-597.

Panesar, P. S., Sharma, H. K. and Rai, R. (2000). Preservation of kinnow juice. *Indian Food Packer*. **54** (3), 79-81.

Parmar, V. S., Jain, S. C., Bisht, K. S., Jain., R., Taneja, P., Jha, A., Tyagi, O. D., Prasad, A. K., Wengel, J., Olsen, C. E. and Boll, P. M. (1997). Phytochemistry of the genus Piper. *Phytochemistry*. **46**, 597-673.

Phillips, J. P. (1866). A Pembrokeshire Proverb. Notes and Queries (Oxford University Press). Retrieved from http://nq.oxfordjournals.org/cgi/reprint/s3-IX/217/153-d. (Last update February 24, 1866). [Accessed 12 October, 2012].

Potter, D., Eriksson, T., Evans, R. C., Oh, S. H., Smedmark, J. E. E., Morgan, D. R., Kerr, M., Robertson, K. R., Arsenault, M. P., Dickinson, T. A. and Campbell, C. S. (2007). Phylogeny and classification of Rosaceae. *In:* "Plant Systematics and Evolution" (Vol. 266).). pp. 5-43.

Pruthi, J. S. (1993). "Major Spices of India- Crop Management and Post Harvest Technology ". ICAR Publications. New Delhi.

Rabiei, V., Shirzadah, E., Angourani, H.S. and Sharafi, Y. (2011). Effect of thyme and lavender essential oils on the qualitative and quantitative traits and storage life of apple 'Jonagold' cultivar. *J. Medicinal Plants Research.* **5**(23), 5522-5527.

Raghavan, S. (2000). Spices: Tools for Alternative or Complementary Medicine. *Food Technology*.

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

Ranganna, S. (2008). "Handbook of Analysis and Quality control for fruit and vegetables products" (2nd ed.). Tata McGraw Hill Publishing Co. Ltd. New Delhi.

Rao, Y. S., Gupta, U., Kumar, A. and Naidu, R. (1993). A note on large cardamom (Amomum subulatum Roxb) Germplasm collection. *J. Spices and Aromatic Crops*. **2** (1&2), 77-80.

Rao, Y. S., Kumar, A., Chatterjee, S., Naidu, R. and (1993). Large cardamom *(Amomum subulatum Roxb)*- A review. *J. Spices and Aromatic Crops*. **2** (1&2), 1-15.

Ravindran, P. N. (2000). Black Pepper. *In:* "Introduction. In. Ravindran, P. N.".). pp. 1-23. Harwood Academic.

Ravindran, P. N. and Kallupurackal, J. A. (2001). Black Pepper. *In:* "Handbook of henbs and spices" (Vol. 1). (K. V. Peter, Ed.). Washington DC, USA. Woodhead Publishing Ltd.

Rinzler, C. A. T. C. B. o. H., Spices and Condiments, Facts on File, New York, 1990. (RIN). (1990). "The Complete Book of Herbs, Spices and Condiments ".New York.

Rosengarten and Frederic, J. (1979). "The Book of Spices". Pyramid Communications. New York.

Ruttner, H. P., Koblit, W. and Rust, D. (1975). Gluconeogenesis in the ripening barriers of grape (Vitis vinifera L). *Indian J. Hort.* **113** (4), 319-323.

Sharma, R. (2005). "Improve your health with Apple, Guava, Mango". Diamond Pocket Books (P) Ltd.

Singh, A. and Goswami, T. K. (1996). Controlled atmosphere storage of fruit and vegetable. *J. Food Sci. Technol*. **43** (1), 1-7.

Singh, G., Kapoor, I.P.S. and Singh, P. (2010). Effect of volatile oil and oleoresin of anise on the shelf life of yoghurt. *J. Food Processing and Presevation.* ISSN 1745-4549.

Singh, G., Marimithu, P., Catalan, C. and Lampasona, M. P. (2004). Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract *J. Sci. Food Agric.* **84**, 1874-1878.

Singh, G. B., Pant, H. G. and Gupta, P. N. (1978). Large cardamom-a foeign exchange earner from Sikkim. *Indian Farming*. **21**, 3-6.

Singh, K. and Mathur, P.B. (1983). Studies in cold storage of cashew apple. *Indian J. Hort.* **40**, 115-121.

Smelt, J.P.P.M., Ratjes, J.G.M., Crowther, J.S. and Verrios, C.T. (1982). Growth of toxin formation by Clostridium at low pH values. *J. Appl. Bacteriol.* **31**, 525-529.

Smith, R., Adams, T., Doull, J., Feron, V., Goodman, J. and Marnett, L. (2002). Safety assessment of allylalkoxybenzene derivatives used in flavoring substance- methyl eugenol and estragole. *Food and Chemical Toxicology*. **40**, 851-870.

Spanos, G. A. and Wrolstad, R. E. (1992). Phenolics of apple, pear and white grape juices and their changes with processing and storage. *J. Agric. Food Chem.* **40**, 1478-1487.

Speck, M.L. 1976. *Compendium of Method for the Microbiological Examination of Foods,* American Public Health Association, Washington, DC.

Stobart, T. (1982). "Herbs, Spices and Flavorings". Milton Peter Corp. with The Overlook Press. Woodstock, NY.

Stuckey, M. (1997). "The Complete Spice Book". St. Martin's Press. New York.

Subba, J. R. (1984). Agriculture in the hills of Sikkim. *Sikkim Science Society*. 286.

Tainter, D. R. and Grenis, A. T. (2001). "Spices and Seasonings: A Food Technology Handbook". John Wiley and Sons Inc. New York.

Tipsrisukond, N., Fernando, L. N. and Clarke, A. D. (1998). Antioxidant effects of essential oil and oleoresin of black pepper in ground pork. *J. Agric. Food Chem.* **46**, 4329-4333.

Uhl and Raghavan, S. (1997). Spices & Spice Extractives].

Vijayan, K. K. and Thampuran, A. (2000). Pharmacology, toxicology and clinical applications of black pepper. *In:* "Black Pepper". (P. N. Ravindran, Ed.). pp. 455-466. Harwood Academic.

Vo¨ sgen, B. and Herrmann, K. (1980). Flavonglykoside von Pfeffer (Piper nigrum L.), Gewu¨rznelken (Syzygium aromaticum L.) und Piment (Pimenta dioica L.). *Z Lebensmittel Untersuch Forsch*. **170**, 204-207.

Wikipedia. (2013). Apple. Wikimedia Foundation, Inc. Retrieved from http://en.wikipedia.org/wiki/Apple. (Last update August 1, 2013). [Accessed 12 August, 2013].

Appendices

Appendix A

* Similar alphabets a, b, c, d and e indicate not significantly different within same day.

** Similar alphabets m, n, o, p and q indicate not significantly different within same sample.

Table A 1.1 Changes in total sugar with variance in sample

Sample → / Days ↓	S1	S2	S3	S4	S5	LSD
0	10.49±0.597523	10.49±0.160935	10.49±0.16093	10.49±0.160935	10.49±0.160935	-
7	$11.19\pm0.210079_{a}$	$11.08\pm0.131149_{a}$	$10.77\pm0.12013_{b}$	$10.71\pm0.132035_{b}$	$10.72\pm0.098489_{b}$	0.1976
14	11.7 ± 0.1^{a}	11.53 ± 0.141892^{b}	11.2 ± 0.1^{c}	10.99 ± 0.030551^{d}	$11..09\pm0.121655^{e}$	0.1539
21	12.5 ± 0.1^{a}	11.77 ± 0.152753^{b}	11.5 ± 0.1^{c}	11.42 ± 0.036056^{c}	11.81 ± 0.056862^{d}	0.1286
28	12.9 ± 0.1^{a}	12.2 ± 0.1^{b}	11.83 ± 0.05773^{c}	11.68 ± 0.085049^{d}	12.07 ± 0.058595^{e}	0.1316
Grand Mean	11.486	11.413	11.158	11.059	11.236	

Table A 1.2 Changes for total sugar with variance in day

Days → / Sample ↓	0	7	14	21	28	LSD
S1	$10.49\pm0.597523_{m}$	$11.19\pm0.210079_{n}$	$11.7\pm0.1_{n}$	$12.5\pm0.1_{o}$	$12.9\pm0.1_{o}$	0.5342
S2	$10.49\pm0.160935_{m}$	11.08 ± 0.131149^{n}	$11.53\pm0.141892_{o}$	11.77 ± 0.152753^{o}	12.2 ± 0.1^{p}	0.2528
S3	$10.49\pm0.1609348_{m}$	$10.77\pm0.1201388_{n}$	$11.2\pm0.1_{o}$	$11.5\pm0.1_{p}$	$11.83\pm0.057735_{q}$	0.2503
S4	$10.49\pm0.160935_{m}$	10.71 ± 0.132035^{n}	$10.99\pm0.030551_{o}$	$11.42\pm0.036056_{p}$	$11.68\pm0.085049_{q}$	0.1870
S5	$10.49\pm0.160935_{m}$	10.72 ± 0.098489^{n}	$11.09\pm0.121655_{o}$	$11.81\pm0.056862p$	$12.07\pm0.058595q$	0.1944
Grand Mean	10.49	10.893	11.303	11.799	12.138	

Table A 1.3 Changes in reducing sugar with variance in sample

Sample Days ↓	S1 →	S2	S3	S4	S5	LSD
0	6.66±0.03	6.66±0.03	6.66±0.03	6.66±0.03	6.66±0.03	-
7	8.34±0.11536^{a}	7.53±0.168028^{b}	6.98±0.100167^{c}	6.95±0.091652^{c}	7.06±0.07^{c}	0.1193
14	9.61±0.167033^{a}	7.96±0.073711^{b}	7.23±0.095044^{c}	7.38±0.045826^{d}	7.85±0.092916^{d}	0.1306
21	11.25±0.377492^{a}	8.59±0.046188^{b}	7.67±0.086217^{c}	7.69±0.070238^{c}	7.85±0.072111^{c}	0.3738
28	12.05±0.529371^{a}	8.95±0.065064^{b}	8.03±0.177764^{c}	8.01±0.09^{c}	8.46±0.085049^{c}	0.5269
Grand mean	9.851	7.935	7.314	7.341	7.478	

Table A 1.4 Changes in reducing sugar with variance in day

Days → Sample ↓	0	7	14	21	28	LSD
S1	6.66±0.03$_{m}$	8.34±0.11536$_{n}$	9.61±0.167033o	11.25±0.377492$_{p}$	12.05±0.529371q	0.5547
S2	6.66±0.03$_{m}$	7.53±0.168028$_{n}$	7.96±0.073711o	8.59±0.046188$_{p}$	8.95±0.065064q	0.1646
S3	6.66±0.03$_{m}$	6.98±0.100167$_{n}$	7.23±0.095044o	7.67±0.086217p	8.03±0.177764q	0.1976
S4	6.66±0.03 m	6.95±0.091652 n	7.38±0.045826 o	7.69±0.070238$_{p}$	8.01±0.09$_{q}$	0.1272
S5	6.66±0.03 m	7.06±0.07 n	7.85±0.092916 o	7.85±0.072111	8.46±0.085049$_{q}$	0.1334
Grand mean	6.66	7.371	7.907	8.608	9.103	

Table A 1.5 Changes in pH with variance in sample

Sample → / Days ↓	S1	S2	S3	S4	S5	LSD
0	4.1±0.1	4.1±0.2	4.1±0.1	4.1±0.1	4.1±0.2	0.2063
7	4.4±0.1^{a}	4.33±0.017321^{a}	4.24±0.01^{b}	4.28±0.02^{b}	4.29±0.032146^{b}	0.0933
14	4.51±0.01^{a}	4.44±0.01^{b}	4.28±0.01^{c}	4.31±0.01^{d}	4.34±0.026458^{e}	0.02629
21	4.61±0.01^{a}	4.56±0.01^{b}	4.35±0.017321^{c}	4.37±0.01^{d}	4.43±0.01^{e}	0.01975
28	4.84±0.017321^{a}	4.72±0.02^{b}	4.53±0.01^{c}	4.46±0.01^{d}	4.64±0.01^{e}	0.02977

Table A 1.6 Changes in pH with variance in day

Days → / Sample ↓	0	7	14	21	28	Grand Mean
S1	4.1±0.1$_{m}$	4.4±0.1$_{n}$	4.51±0.01$_{n}$	4.61±0.01$_{o}$	4.84±0.017321p	0.1165
S2	4.1±0.2$_{m}$	4.33±0.017321$_{n}$	4.44±0.01op	4.56±0.01pq	4.72±0.02q	0.1645
S3	4.1±0.1$_{m}$	4.24±0.01$_{n}$	4.28±0.01$_{no}$	4.35±0.017321$_{o}$	4.53±0.01p	0.0838
S4	4.1±0.1$_{m}$	4.28±0.02$_{n}$	4.31±0.01$_{no}$	4.37±0.01$_{o}$	4.46±0.01p	0.0842
S5	4.1±0.2$_{m}$	4.29±0.032146$_{n}$	4.34±0.026458$_{n}$	4.43±0.01$_{n}$	4.64±0.01$_{o}$	0.1666

Table A 1.7 Changes in ascorbic acid with variance in sample

Sample → / Days ↓	S1	S2	S3	S4	S5	LSD
0	11.76±0.138684	11.76±0.138684	11.76±0.138684	11.76±0.138684	11.76±0.138684	-
7	8.54±0.105987^{a}	7.42±0.058595^{b}	9.59±0.157162^{ce}	10.66±0.111355^{d}	9.53±0.045826^{e}	0.2177
14	5.55±0.09609^{a}	7.15±0.147309^{b}	9.39±0.136504^{c}	10.17±0.065064^{d}	7.45±0.02^{e}	0.1968
21	3.34±0.101489^{a}	4.96±0.078102^{b}	7.37±0.226053^{c}	9.35±0.110151^{d}	6.8±0.160935^{e}	0.2739
28	3.05±0.020817^{a}	4.48±0.225019^{b}	6.51±0.216333^{c}	7.37±0.072342^{d}	5.38±0.066583^{e}	0.2509
Grand mean	6.449	7.151	8.923	9.861	8.183	

Table A 1.8 Changes in ascorbic acid with variance in day

Days → / Sample ↓	0	7	14	21	28	LSD
S1	11.76±0.138684$_{m}$	8.54±0.105987$_{n}$	5.55±0.09609$_{o}$	3.34±0.101489$_{p}$	3.05±0.020817$_{q}$	0.1827
S2	11.76±0.138684$_{m}$	7.42±0.058595$_{n}$	7.15±0.147309$_{o}$	4.96±0.078102$_{p}$	4.48±0.225019$_{q}$	0.2587
S3	11.76±0.138684$_{m}$	9.59±0.157162$_{n}$	9.39±0.136504$_{o}$	7.37±0.226053$_{p}$	6.51±0.216333$_{q}$	0.3259
S4	11.76±0.138684$_{m}$	10.66±0.111355$_{n}$	10.17±0.065064$_{o}$	9.35±0.110151$_{p}$	7.37±0.072342$_{q}$	0.1877
S5	11.76±0.138684$_{m}$	9.53±0.045826$_{n}$	7.45±0.02$_{o}$	6.8±0.160935$_{p}$	5.38±0.066583$_{q}$	0.1859
Grand mean	11.76	9.146	7.943	6.363	5.358	

Table A 1.9 Changes in titrable acidity with variance in sample

Sample → / Days ↓	S1	S2	S3	S4	S5	LSD
0	0.77±0.01	0.77±0.01	0.77±0.01	0.77±0.01	0.77±0.01	-
7	0.65±0.01^{a}	0.7±0.01^{b}	0.74±0.01^{ce}	0.74±0.01^{d}	0.73±0.01^{e}	0.01929
14	0.59±0.01^{a}	0.66±0.01^{b}	0.69±0.01^{c}	0.71±0.01527^{d}	0.71±0.01^{e}	0.01649
21	0.477±0.020817^{a}	0.59±0.01^{b}	0.63±0.00577^{c}	0.69±0.01^{d}	0.68±0.01^{e}	0.02119
28	0.373±0.01527^{a}	0.53±0.01^{b}	0.57±0.01^{c}	0.62±0.02^{d}	0.61±0.01^{e}	0.01479
Grand mean	0.5720	0.65	0.6787	0.7067	0.70	

Table A 1.10 Changes in titrable acidity with variance in day

Days → / Sample ↓	0	7	14	21	28	LSD
S1	0.77±0.01$_{m}$	0.65±0.01$_{n}$	0.59±0.01$_{o}$	0.477±0.020817$_{p}$	0.373±0.01527$_{q}$	0.02530
S2	0.77±0.01$_{m}$	0.7±0.01$_{n}$	0.66±0.01$_{o}$	0.59±0.01$_{p}$	0.53±0.01$_{q}$	0.01819
S3	0.77±0.01$_{m}$	0.74±0.01$_{n}$	0.69±0.01$_{o}$	0.63±0.00577$_{p}$	0.57±0.01$_{q}$	0.01694
S4	0.77±0.01$_{m}$	0.74±0.01$_{n}$	0.71±0.01527$_{o}$	0.69±0.01$_{p}$	0.62±0.02$_{q}$	0.02486
S5	0.77±0.01$_{m}$	0.73±0.01$_{n}$	0.71±0.01$_{o}$	0.68±0.01$_{p}$	0.61±0.01$_{q}$	0.01819
Grand mean	0.77	0.7120	0.6727	0.6120	0.5407	

Table A 1.11 Changes in for total microbial count with variance in sample

Sample → S1 / Days ↓	S1	S2	S3	S4	S5	Grand Mean
0	0	0	0	0	0	-
7	573 ± 2.5166^{a}	563 ± 2.5166^{b}	102 ± 2.6457^{b}	154 ± 64.117^{b}	102 ± 1.5275^{b}	53.48
14	762 ± 1.5275^{a}	758 ± 2.0816^{a}	320 ± 2.0816^{b}	252 ± 2.0816^{c}	462 ± 1.5275^{d}	3.812
21	3810 ± 55.6776^{a}	3147 ± 50.332^{b}	1487 ± 15.2752^{c}	1078 ± 2.6457^{d}	1525 ± 5^{e}	53.06
28	6832 ± 12.58^{a}	5690 ± 10^{b}	2033 ± 152.75^{c}	1210 ± 10^{d}	1737 ± 51.316^{e}	123.6
Grand mean	2395.1	2031.5	789	538.8	765.2	

Table A 1.12 Changes in total microbial count with variance in day

Days → / Sample ↓	0	7	14	21	28	Grand Mean
S1	0_{m}	$573\pm2.5166_{n}$	$762\pm1.5275_{o}$	$3810\pm55.6776_{p}$	$6832\pm12.58_{q}$	46.50
S2	0_{m}	$563\pm2.5166_{n}$	$758\pm2.0816_{o}$	$3147\pm50.332_{p}$	$5690\pm10_{q}$	41.84
S3	0_{m}	$102\pm2.6457_{n}$	$320\pm2.0816_{o}$	$1487\pm15.2752_{p}$	$2033\pm152.75_{q}$	124.9
S4	0_{m}	$154\pm64.117_{n}$	$252\pm2.0816_{n}$	$1078\pm2.6457_{o}$	$1210\pm10_{o}$	124.9
S5	0_{m}	$102\pm1.5275_{n}$	$462\pm1.5275_{o}$	$1525\pm5_{p}$	$1737\pm51.316_{q}$	124.9
Grand mean	0	298.5	510.93	2209.3	3500	

Table A 1.13 Changes in yeast and mold count with variance in sample

Sample → / Days ↓	S1	S2	S3	S4	S5	LSD
0	0	0	0	0	0	-
7	160 ± 1^{a}	109 ± 1^{b}	101 ± 1.5275^{c}	42 ± 1.5275^{d}	62 ± 1^{e}	2.280
14	459 ± 1^{a}	231 ± 3.055^{b}	124 ± 1^{c}	108 ± 2.0816^{d}	110 ± 1.5275^{d}	3.141
21	608 ± 6.244^{a}	418 ± 1.5275^{b}	361 ± 3.214^{c}	306 ± 5.29^{d}	387 ± 3.5118^{e}	6.449
28	5623 ± 50^{a}	4817 ± 104.08^{b}	2240 ± 52.9^{c}	1780 ± 32.9^{d}	2917 ± 76.376^{e}	133.9
Grand mean	1875.4	1115.0	565.2	447.1	695.2	

Table A 1.14 Changes in yeast and mold count with variance in day

Days → / Sample ↓	0	7	14	21	28	LSD
S1	0_{m}	$160\pm1_{n}$	$459\pm1_{o}$	$608\pm6.2449_{p}$	$5623\pm50_{q}$	41.01
S2	0_{m}	$109\pm1_{n}$	$231\pm3.055_{o}$	$418\pm1.5275_{p}$	$4817\pm104.08_{q}$	84.7
S3	0_{m}	$101\pm1.5275_{n}$	$124\pm1_{o}$	$361\pm3.214_{p}$	$2240\pm52.9_{q}$	43.16
S4	0_{m}	$42\pm1.5275_{n}$	$108\pm2.0816_{o}$	$306\pm5.29_{p}$	$1780\pm32.9_{q}$	27.21
S5	0_{m}	$62\pm1_{m}$	$110\pm1.5275_{n}$	$387\pm3.5118_{o}$	$2917\pm76.376_{p}$	62.22
Grand mean	0	94.60	206.53	416.07	3475	

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