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## Flavors from Citrus Peels and their Functions in Food

Tanzeela Adeel<sup>1\*</sup>, Adnan Tariq<sup>2</sup>, Auranzaib<sup>3</sup> and Monis Akber<sup>4</sup>

<sup>1</sup>Food Safety and Hygiene Officer at Yassat Gloria Hotel, Tecom Dubai, UAE

<sup>2</sup>Assistance Manager, Quality Research and Development at National Foods Ltd Karachi, Pakistan

<sup>3</sup>Manager, QA and Development at Danpak Food Industries pvt. ltd Karachi, Pakistan

<sup>4</sup>Environment Health and Safety and Cluster Officer at Jumeira Rotana and Bujuman Arjaan by Rotana, Dubai, UAE

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#### \*For Correspondence

Tanzeela Adeel, Food Safety and Hygiene Officer at Yassat Gloria Hotel, Tecom Dubai, UAE

E-mail: tan83pk@yahoo.com

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#### ABSTRACT

The project carried out under the supervision of Dr. Shahina Mahmood who was the professor at Food Science Technology Department in the Karachi University Pakistan. We started our citrus fruits research project in September 2005, the concept project was the industry practical implementation of natural flavor of citrus fruits in different products like Candies, Yogurt and Apple Jelly and etc. for creating new taste and flavors. Citrus fruits have many useful benefits in food and cosmetic industries and the extract of citrus fruits can be used in different products and different textures.

Initially we thought of comparing the synthetic flavors with the natural flavor and how much we can replace the artificial flavor with the natural flavor at industrial level.

By applying the natural flavor in different products we found that natural flavor has strong taste which was not liked by the few students in university, even though we took proportionate amount compared to the synthetic flavor.

At industrial level the synthetic flavor was found to be cheap in cost compared to natural flavor and because of the way it has been produced very low proportionate quantity can bring required changes in the flavor of the product. Also it was imported from Thailand and costs less than locally produced natural or artificial Citric flavor.

During the midway life of our project and after doing numerous experiments we observed that the stability of the Products made with the natural citrus flavor exhibit more shelf life than the products made without citrus flavor

This was a major discovery and observation as it completely changed the course and direction of our project, we started working on the new lines on identifying and establishing the facts on the importance of having Citrus flavor in products, its benefits, impact and showcasing the positive reactions of determining the antioxidant property of flavor in oil.

## TYPES OF CITRUS FRUITS

There are several types of citrus fruits. Because of time constraint and we had to complete the project within the given time period.

We selected the lemon orange and grape fruit as it was easily available in market. It was present in market with different sizes and names i.e for grape fruit Local varieties were Mash Seedless, Duncan, Foster and Shamber mandarin, sweet orange, bitter orange, lime, lemon and rough lemon etc. <sup>[1]</sup>.

We noticed the production of citrus fruits in Pakistan was numerous and it was locally present though out the country with cheap cost. Varieties of citrus fruits were found in major cities like Sindh, Punjab, Khyber pakhtunkhwa and Baluchistan.

## EPIDERMIS OR OUTERMOST LAYER OF FRUIT

As epicuticular wax layer in platelets. Wax layer, comprised of an amorphous and soft material, is deposited slowly as fruit develops. With passage of time and exposure of the layer to air, the layer breaks up into platelets. Layer tends to be somewhat thicker around stem. Nature of layer depends upon particular variety or cultivars and also upon environmental conditions prevailing during development of the fruit.

### Flavedo

Depending upon variety, the next inner layer of the fruit will be a green, yellow, or orange coloration. The flavedo is also referred to as the sub epidermal region of the peel or rind. Interspersed throughout the flavedo are oil glands, lower portions of which extend into the next inner layer, the albedo. Cells in the flavedo that surround the oil glands are round cells and cell organelles. These include chloroplasts, which, upon maturation of the fruit, become chromoplasts with accompanying colour change. However, in some fruits, if allowed to remain on the tree over winter, the chromoplasts may revert back to chloroplasts, again causing a green colouration. The natural colour of the flavedo cells for use in enhancing the colour of citrus juice, particularly orange.

### Oil Glands

These glands are numerous and relatively fragile. It was found that the gland number between 1.9 and 2.6 glands per millimeter of flavedo surface.

### Albedo

The segmented or sections of the fruits are most familiar and obvious physical feature of a cut citrus fruit. Principal components of the section walls include the segment walls and juice sacs. The section walls (septa) of some citrus, particularly the grape fruit, contain the most limonin of any part of the fruit.

## CHEMICAL CONSTITUENTS OF CITRUS FRUITS

The composition of citrus fruit is affected by such factors as growing conditions, maturity, rockstock, variety and climate.

As with physical structure, while there are some differences between varieties in degrees, the chemical make-up of these fruits has striking similarities. The principal chemical substances present include carbohydrates, pectin, organic acids, nitrogenous compounds (amino acids etc.), lipids, (fats), pigments (carotenoids, limonin and other limonoids, flavenoids and essential oils.

### Proteins (Nitrogenous Constituents)

The nitrogen content of whole citrus fruits varies between 0.1 and 0.2% on a wet basis. The nitrogenous constituents of citrus fruit include proteins, simple peptides, amino acids, phosphatides and related substances. The proteins in citrus fruits are relatively insoluble and are found to be associated with the solid portions of the fruit, such as seeds, flavedo, a; bedo and pulp. Amino acids are found in the juice of the edible portion of the fruit and in the aqueous alcohol extractable fraction of the peel

In terms of total chemical composition of citrus fruits, nitrogenous substances represent only from 0.06% on a weight basis for lemons, 0.08% for Grape fruit and between 0.08 and 0.11% for oranges. The nitrogen present, about 70% is contained in free amino acids. Other nitrogen bearing compounds would include proteins, enzymes, nucleotides, nucleic acids, phospholipids, vitamins and inorganic forms.

The following amino acids are represented in the greatest concentration: proline, aspartic acid, arginine, gamma amino butyric acid, asparagines, glutamic acid, serine and alaninie. Other amino acids are also present such as valine phenylalanine; threoninine, leucine, methionine and lysine are sparse and sketchy.

### Carbohydrates

The principal polysaccharides of citrus are galacturonan containing pectic substances: cellulose, glucans, arabinan, and xylan. Except for cellulose most of these substances are not homogenous polysaccharides. Sugar content generally increases as

the citrus fruits commence their maturing process. Carbohydrate content of raw citrus juices are 9.2%, 8.0%, 9.0%, 11.6%, 10.4% and 9.2% in Grape fruit, lemon, lime, mandarin (tangerine), orange and tangelo respectively.

The most important factor governing sugar content is maturity, especially with the sweeter kinds of citrus. In these, the acid content slightly decreases during maturity. In oranges, tangerines and Grape fruits the soluble solids consist mainly of sugars, but in lemon and lime juice the soluble solids are mainly citric acid.

In oranges, at maturity, the reducing and non-reducing sugars are present in about equal amounts, but in less sweet fruits (lime), the reducing sugars predominate. When orange and other juices are pasteurized and canned, inversion of sucrose occurs in storage. Sugars also occur in albedo and flavedo.

### **Lipids**

Citrus fruits are very low in fat content. On a weight basis, Grape fruit, lemon, mandarin and orange contain approximately 0.2% fat, lime and tangelo, 0.1%. The citrus seeds are processed into oils, which are composed mainly of triglycerides and lesser amounts of free fatty acids. The greatest concentration of fatty acids in citrus seed oils are linoleic, palmitic and oleic acid and in smaller concentrations, stearic and linolenic acid.

### **Pectic Substances**

In addition to the soluble carbohydrates, citrus fruits contains insoluble carbohydrates that provide the structural materials and consist of roughly equal proportions of cellulose and pectin. Starch and lignin are absent. The peel is particularly rich in pectin, which may make up 20 to 40% of dry matter. In the fruit tissues, pectin is present in a water insoluble form known as protopectin. Citrus pectin is partially esterified polygalacturonides. The tissue of citrus fruits has high contents of pectic substances, and they are used as a source of commercial pectin.

During the ripening cycle of citrus fruits, insoluble protopectin of the meristematic and parenchymatous tissues changes into water-soluble pectins and pectinates, and as the fruit continue to ripen and become overripe, these products are converted into low-grade pectin and insoluble pectates, these changes being enzymatic or chemical. Pectinic substances are found essentially throughout the components of citrus fruits.

Pectin is a useful byproduct of some citrus processes and also, when present in products, contributes to their character. The organoleptic sensation described as "body" in a fruit juice can be attributed to the presence of pectin. Pectin as a naturally occurring colloidal stabilizer provides viscosity to juices.

### **Enzymes**

Pectinesterase of citrus fruits occurs in great deal in juice sacs and rag, with decreasing amount in flavedo and albedo. Pectinesterase activity is believed to be one of the principal causes of cloud instability, known as cloud loss and gelation in un-pasteurized citrus juices and frozen concentrates. Phosphatase occurs in the peel and also in solution in orange, grape fruit and lemon juices. It is inactivated by heat.

### **Organic Acids**

An organic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids. In general, organic acids are weak acids and do not dissociate completely in water, whereas the strong mineral acids do. Lower molecular mass organic acids such as formic and lactic acids are miscible in water, but higher molecular mass organic acids, such as benzoic acid, are insoluble in molecular form.

Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars<sup>[2]</sup>, which are used as the main index of maturity and one of the major analytical measures of flavor quality<sup>[3]</sup>. The main acids of citrus fruits are citric and malic acids.

### **Flavonoids**

The three kinds of flavonoids present in citrus fruits are flavanones, flavones and anthocyanins. The four main flavanones are naringenin, isosakuranetin, eriodictyol and hesperetin. The chemistry of the flavanones including their stereo qualities is quite complex. From a practical standpoint the flavanones make a major contribution to taste properties of citrus fruits but as in the case of all flavonoids, some are desirable and some are undesirable. The flavanones are very numerous in citrus fruits, occur in rather low concentrations, are very difficult to isolate and purify, common flavones include apigenin, acacetin and luteolin, occurring in the fruit as glycosides. The anthocyanins include cyanidin, peonidin, delphinidin and petunidin as complex glucosides.

### **Limonin and Related Compounds (Bitter Principles)**

The bitterness is due to limonin and isolimonin. The bitter principles are present mainly in albedo, to a small extent in seeds and slightly in the outer membrane of juice sacks. The limonin is water-soluble and is present in albedo in non-bitter form, probably as glycoside at pH 4.5 or greater. The whole family of limonin related compounds, called limonoids. Citrus fruits feature around 40 limonoids, limonin and nomilin predominating. These substances contribute to the bitter taste found in citrus particu-

larly in Grape fruit and orange juice where they occur in high concentrations. Delayed bitterness in citrus juices has traditionally been attributed to the presence of limonin. The phenomenon of delayed bitterness is due to the physical process of diffusion of limonin from the suspended solids into the juice.

### Peel Oil

In the extraction of the juices, different proportions of oil may be extracted from peel. The main constituent of oil is d-limonene. A small amount of peel oil in fresh orange juice gives it a pleasant aroma and adds to the flavour. Under certain conditions, even the small amounts of oil in canned juice may give rise to objectionable flavours upon storage. Efforts to increase the yield of the juice may result in the possible contamination of the juice with peel juice constituents, which may contribute bitterness or other off-flavours.

### Pigments

The colour of orange and tangerine juices is chiefly due to carotenes and xanthophylls. As chlorophyll in citrus peel decreases, carotenoids increase. Carotenoids are the predominant pigments of citrus fruits, the colours of which commence to show through during ripening when the chlorophyll green begins to disappear. It should be pointed out that the reddish-to-purplish colorations found in blood oranges are due to the presence of anthocyanins (a flavonoid).

### Essential Oils

**Definition:** "Essential oil represents the total odor principal of any single botanical species and is generally isolated by some physical process." OR

"They are defined as products extracted from acceptable plant material by some physical method without any physical change."

## INTRODUCTION

Essential oils are distilled volatile oil of plants materials that have strong aromatic components. These aromatic substances are made up of different chemical compounds that occur naturally in the plants. For example, alcohol, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components<sup>[4]</sup>. These aromatic characteristics of essential oils provide various functions for the plants including:

- Attracting and repelling insects.
- Protecting themselves from heat or cold.
- Utilizing the hormone in the oil as anti-bacterial agents.

Among all types of plants in the world, only about seven hundred plants are considered aromatic, and therefore, they are all significant for the production of essential oils. Besides the limited source of supply, the small amount of essential oils that are contained in each aromatic plant makes it even more valuable. Today, essential oils are primarily used in preparation of fragrances, such as soap and perfume. Although some of the chemical components of essential oils resemble "oils", essential oils are not greasy itself and they are light in weight.

However, the elevated alcohol components in essential oils make it has a higher volatility and the faster evaporation rate. In order to get the best quality and quantity of essential oils, extraction procedure seems the key-controlling step. Factors such as types of plants, chemical make-up of oil, and location of oil within the plant (root, bark, wood, branch, leaf, flower, fruit and / or seed) are all needed to be considered prior to the extraction. Choosing a proper extraction method is also important as well. In general, there are two main types of extraction methods: steam distillation and solvent extraction. Each method has its distinctive advantages over the others and its uses, however, depends upon the type of oils and the type of plants that are being extracted.

### Essential Oil Composition

The exact chemical composition of the oil is characteristic of a given plant species and the different oil constituents are synthesized by the plant during its normal growth. Research is continuing to establish the precursors and the precise bio-chemical pathways involved in their formation<sup>[5]</sup>.

**The Terpenes:** The biogenesis of the terpenes hydrocarbons and their oxygenated derivatives, which form the major fraction of essential oils proposed by Ruzicka, takes place in four steps.

The formation of C<sub>5</sub> precursor units.

Head-to-tail condensation of these units to form the primary terpenoid structure.

Cyclization.

Skeletal rearrangements involving oxidation, reduction, the shifting of the double bonds, hydroxylation and other reaction.

The terpenes may be aliphatic, alicyclic or bi-and tricyclic of varying degrees of unsaturation up to three double bonds. Diterpenes and triterpenes are rarely found in essential oils. Although the terpenes hydrocarbons from the major components of many essential oils, bear contribution toward the total flavors is small compared to that of their oxygenated derivatives. Their role is described as contributing a definite "reshness" to the overall odour. Oxygenated derivatives include alcohols, aldehydes, ketones, lactones and esters. They are the major contributors to distinctive odours and flavors.

**Benzenoid Compounds:** Another important groups of aromatic constituents of essential oils are based on benzene. Biochemical reactions directly associated with the plant's metabolism produced these compounds and they include the whole range of organic functional groups. N-Propylbenzene is considered as the precursor that is enzymatically activated. Products with many diverse functional groups attached to the benzene nucleus are formed in various stages of oxidation.

**Nitrogen or Sulphur:** Compounds containing nitrogen or sulphur. Generally these are not originally present in the oils. Plant materials containing an albuminous matter on distillation yield compounds such as ammonia, trimethylamine, hydrocyanic acid and hydrogen sulphide.

Some of the more important nitrogen compounds found in essential oils includes indole in oil of jasmine and in many citrus oils, and methyl esters of anthranilic acid in orange and lemon oil.

Sulphides occur frequently in plants, probably arising from the degradation of sulphur containing glycosides. Hydrogen sulphide is found in the distillates of certain umbelliferous fruits and diethyl sulphide is found in American peppermint oils. In onion, garlic and mustard oils, allyl sulphides are formed as products of enzymatic action.

### **Isolation and Production of Essential Oils**

Essential oils are isolated from various plant components such as leaves, fruit, bark, root, wood, heartwood, gum, balsam, berries, seeds, flowers, twigs and buds. The yield of essential oils from plants varies widely and the broad range is 0.05-18.0%. Essential oils are extracted in several different ways. Laboratory equipment that separates the oil from the fresh plant is required to obtain essential oils in their purest form. Most essential oils are produced for the perfume industry and minute amounts are used for flavor pre-packaged foods. Only about 5 percent of the essential oils produced are used in aromatherapy<sup>[6]</sup>.

### **Uses of Essential Oils**

Essential oils are extensively used as flavoring and fragrance agents. They are widely used for flavoring baked goods, snack foods, soft drinks, liquors, tobacco, sauces, gravies, salad dressings, and many such food products. Combination of essential oils are used in every fragranced product such as toothpaste, mouthwash, room freshener, paper, printing ink, paint, candles, soap and floor polish. The applications of essential oils as medicaments, disinfectants, insect repellents and insecticides are very well known from the very early times. The term aromatherapy is defined as the therapeutic uses of fragrances to cure, mitigate or prevent diseases, infections or indisposition by means of inhalation only. Fungicidal and bactericidal properties of essential oils like mint, clove, cinnamon and basil have been studied in detail and MIC (minimal inhibitory concentration) data are available for possible applications. Allelopathy of oil bearing plants is still an under investigated area with tremendous potential.

### **Storage of Essential Oils**

Most essential oil components are sensitive to light, heat, air and water. Hence they need to be stored under carefully controlled conditions. Containers should be of stain less steel, glass, aluminum or other such inert materials with appropriate stoppers. Oil containers should be filled with a minimum possible headspace so as to reduce the availability of oxygen and thus to prevent any degradation of the oil components. Direct sunlight must be avoided and containers should be stored in a cool place.

Cold pressing, or scarification, is used to obtain essential oils from the peels and seeds of citrus fruits, such as bergamot, lemon, lime, and mandarin, orange, and tangerine oils. In this process, fruit rolls over a trough with sharp projections that penetrate the peel, which pierce the tiny pouches containing the essential oils. Then the whole fruit is pressed to squeeze the juice from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the juice and is separated from the juice by centrifugation.

### **Solvent Extraction of Essential Oils**

The essential oil of citrus fruits is contained in oil sacs in the outer, colored portion of the peel of the fruit. Distillation only allows small amounts of oil to escape, but using the expression method yields much more oil. After the fruit has been washed and inspected, they proceed on to the removal of juice and oil. One method of oil extraction is the F.M.C. whole fruit extractor. The extraction of juice and oil is desirable in one machine.

In this method very little heat so it's able to produce essential oils whose fragrance would otherwise be destroyed or altered during steam distillation. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost. In this process, a chemical solvent such as hexane is used to saturate the plant material and pull out the essential oils. The plant is removed and this renders a solvent. The solvent is then boiled off under a vacuum or in a centrifugal force machine to help

separate it from the essential oils. Because the solvent has a lower boiling point than the essential oil it evaporates and the oil is left. The solvent is cooled back in to liquid and reclaimed. Along with the essential oil, the fats, waxes, and heavier oils can be extracted. This produces a substance called a concrete. Dissolving oils in to warm alcohols continues the process. The alcohol is removed under a vacuum and pure essential oil is left.

Although more cost-efficient than enfleurage, solvent extraction is more expensive than steam distillation so it is reserved for costly oils which cannot be distilled. A solvent extracted essential oil is called an absolute.

### **Uses and Yield for Citrus Oils**

Citrus oils are generally used for natural flavors, synthetic flavors, and aromatherapy. Food products that commonly use citrus oils are soft drinks, confections, baked goods, sherbets, and ices. They are also used in perfumes, cosmetics, and for the scenting of soaps.

The yield of oil obtained by cold pressing depends on fruit variety, maturity, and condition. An average lemon, for example, weighs 100-120 grams (the peel is half of this weight) and contains approximately 0.5-0.7 grams oil in the peel. For one ton of whole oranges, the yield is approximately 5 pounds of oil.

### **Packaging and Shelf Life of Citrus Oils**

Oxidation may occur between the time the oil is extracted and the time it is sealed in to a container. This is why time is important factor in order to reduce oxidation. As other essential oils, citrus oil must be kept in cold temperatures in a dark, air/water tight sealed container. The oil must also be kept free from contamination by inferior oils (deteriorated oil) or substances that might increase the rate of deterioration. Assuming that citric oil behave as other essential oils, if packed correctly the oil can last for about one year before deterioration will occur.

### **Orange (Sweet) Essential Oil Information**

The oil is extracted from the fruit of the Orange plant (*Citrus sinensis*) by cold-press method. It is also known as Portugal or china orange. Cold press oil is best to avoid chemical toxicity. Sweet and bitter orange essential oil have importance of health benefits e.g Anti depressant, Anti-inflammatory, anti septic improve digestion, anti cancer, skin treatment etc. It has vital role in human activities through aroma therapy <sup>[7]</sup>.

### **Usage of Orange Essential Oil**

Essential oils are engrossed into the body via the nose and the pores of skin and hair follicles. They enters into the body and with the help of blood Circulation they circulate though out the body. It can be used as massage oil, warm bath, body wash and room refresher for reliving sore throat.

### **Properties of the Oil**

Sweet orange oil has a sweet, fresh and tangy smell, the sweet arrange is yellow to orange in color and watery in viscosity. The shelf life is approximately 6 months.

### **Origin of Sweet Orange Oil**

Orange was originated native to china but now it abundantly cultivated in USA. Other varieties of oranges found in different countries like Europe, Asia, USA and Middle East as well. The orange has become the most commonly grown tree fruit in the world. It is a significant crop in the Far East, the Union of South Africa, Australia, throughout the Mediterranean area, and subtropical areas of South America and the Caribbean.

### **Properties**

Oranges are one of the most popular fruits around the world. It's been enjoyable as a snack or as a recipe ingredient, its juice is highly associated with good health which acts as an integral part of a healthy breakfast. Oranges are round citrus fruits with finely-textured skins that are orange in color just like their pulpy flesh.

### **Chemical Composition**

The chemical composition of orange oil varies from each other depending upon the extraction. The variety of the composition happens as a result of regional and seasonal changes as well as the method used for extraction. We used the extraction method by applying different solvent.

### **Precautions**

It is a safe non-toxic, non-irritant and non-sensitizing oil still needs care to be taken with it since it can have a phototoxic effect. It should therefore preferably not be applied before going out into sunlight for prolonged periods.

### **Uses**

In many ways orange can be used in different product and every one can get the benefits Orange pulp can be used for

preparing fresh juice which is rich in protein content. The peel of the fruit is used for making perfume and soaps. Cooking oil is extracted from its seeds. Aids in the prevention and treatment of skin diseases, catarrh, acne and stomach ache. Juice extracted from sweet orange leaves are used to control ulcers, sores etc.

### **Grape Fruit Essential Oil Information**

Grapefruit Essential Oil can be recognized to its properties as a diuretic, disinfectant, stimulant, antidepressant, antiseptic, aperitif, lymphatic and tonic substance. Grapefruit, also known as Shaddock in certain areas of the world, is a Citrus fruit, whose scientific name is *Citrus paradisi*. It is also known by two other scientific names, *Citrus racemosa* and *Citrus maxima*. Like all other citrus fruits, its Essential Oil is present in its peel and extracted [8].

### **Oil Properties**

Grape fruit essential oil has a spiky refreshing smell and is either a pale yellow or light ruby color with a watery viscosity. Like all citrus oils, Grape fruit oil should be used within six month of purchase.

### **Origin of Grape Fruit Oil**

Originally from Asia, it is now cultivated in the USA, Brazil and Israel. China is the top producer of grapefruit than USA. Grapefruit comes in many varieties, determinable by color, which is caused by the pigmentation of the fruit with respect to its state of ripeness. The most popular varieties cultivated today are red, white and pink hues, referring to the internal pulp color of the fruit. The family of flavors range from highly acidic and somewhat sour to sweet and tart.

### **Chemical Composition**

The main chemical components are  $\alpha$ -pinene, sabinene, myrcene, limonene, geraniol, linalool, citronellal, decyl acetate, neryl acetate and terpinen-4-ol.

### **Precautions**

Grape fruit oil is non-toxic, non-irritant, non-sensitizing and although listed as non-phototoxic, it can irritate the skin if exposed to strong sunlight after treatment.

### **Properties**

Some of the therapeutic properties of Grape fruit oil are cure depression and relief stress, increase appetite, antiseptic, rich in anti oxidant good for treating oily skin and acne, and disinfectant, boost immune system and stimulates brain.

### **Uses**

Grape fruit has high vitamin C content and is therefore valuable to the immune system. It helps protect against colds and flu, has a very positive effect on obesity and ensure healthy functioning of lymphatic system, help to remove excess water from the body and is therefore also great for treating cellulite.

## **EXPERIMENTS**

The experiments were carried out with dried and fresh citrus peels with different combination of solvent.

1. Dried citrus peels with Hexane solvent
2. Fresh citrus peels with Hexane solvent
3. Dried citrus peels with Methanol solvent
4. Fresh citrus peels with Methanol solvent
5. Dried citrus peels with water solvent
6. Fresh citrus peels with water solvent
7. Dried citrus peels with the combination of water and methanol solvent
8. Fresh citrus peels with water and methanol solvent
9. Dried citrus peels with edible oil.

### **Methods of Flavour Extraction from Citrus Peels (Orange, Grape Fruit And Lemon)**

**Principle:** Flavor is extracted from both dry and fresh citrus peels (orange, grape fruit and lemon) by solvent extraction method using different solvents (Hexane, methanol, water, edible oil and combination of methanol and water,) after grinding of peels with granulated sugar. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by evaporation at room temperature.

#### **Procedure for Flavour Extraction from Dried Citrus Peels**

**By Using Hexane as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any spoiled part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. Dry the peels by using a sun dryer. After complete drying the peels are grinded with an equal volume of granulated sugar. The powdered of mixture (peel and sugar) is then transferred to a flask containing hexane and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Fresh Citrus Peels**

**By Using Hexane as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any damaged or rotten part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. The peels are chopped with an equal volume of granulated sugar. The chopped mixture peel and sugar is then transferred to a flask containing hexane and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Dried Citrus Peels**

**By Using Methanol as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any rotted part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. Dry the peels by using a sun dryer. After complete drying the peels are grinded with an equal volume of granulated sugar. The powdered of mixture (peel and sugar) is then transferred to a flask containing methanol and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Fresh Citrus Peels**

**By Using Methanol as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any damaged or rotten part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. The peels are chopped with an equal volume of granulated sugar. The chopped mixture peel and sugar is then transferred to a flask containing methanol and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and methanol solvent is separated from the flavor either by using rotary evaporator. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Dried Citrus Peels**

**By Using Water as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any rotted part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. Dry the peels by using a sun dryer. After complete drying the peels are grinded with an equal volume of granulated sugar. The powdered of mixture (peel and sugar) is then transferred to a flask containing water and leaves the flask on stirrer until complete extraction. The impurities are removed by filtration and water solvent is separated from the flavor either by using rotary evaporator. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Fresh Citrus Peels**

**By Using Water as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any damaged or rotten part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. The peels are chopped with an equal volume of granulated sugar. The chopped mixture peel and sugar is then transferred to a flask containing water and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and water solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

#### **Procedure for Flavor Extraction from Dried Citrus Peels**

**By Using a Combination of Water and Methanol:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any rotted part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. Dry the peels by using a sun dryer. After complete drying the peels are grinded with an equal volume of granulated sugar. The powdered of mixture (peel and sugar) is then transferred to a flask containing a solvent (water and methanol) and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

### Procedure for Flavor Extraction from Fresh Citrus Peels

**By Using a Combination of Water and Methanol:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any damaged or rotten part of the peel. Wash the fresh peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. The peels are chopped with an equal volume of granulated sugar. The chopped mixture peel and sugar is then transferred to a flask containing a solvent (water and methanol) and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and solvent is separated from the flavor either by using rotary evaporator or by simple evaporation at room temperature. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

### Procedure for Flavor Extraction from Dried Citrus Peels

**By Using an Edible Oil as a Solvent:** Take fresh peels of citrus fruits (orange, Grape fruit and lemon). Remove any rotted part of the peel. Wash the peels to remove dust and other extraneous material. Remove the pectin layer from the peels and cut the peels into small pieces. Dry the peels by using a sun dryer. After complete drying the peels are grinded with an equal volume of granulated sugar. The powdered of mixture (peel and sugar) is then transferred to a flask containing oil and leave the flask on stirrer until complete extraction. The impurities are removed by filtration and oil is separated from the flavor by centrifugation. The flavor is then mixed with glycerin. The flavor is now ready to use in the food products.

### Utilization of Citrus Flavor in Different Food Products

**Objective:** Utilization of citrus flavor in apple jelly, sensory analysis and effect of flavor on product stability.

**Recipe:** The constituents that were used for utilization of citrus flavor in different food products are shown in **Table 1**.

**Table 1.** The constituents that were used for utilization of citrus flavor in different food products.

Constituents	Quantity
Pectin extracts	1L
Sugar	1 Kg
Citric acid	7 gm

**Procedure:** Select slightly under ripe apples. Wash and cut into small pieces. Weigh and add 1.5 L water for every Kg of the fruit. Cook for half an hour and strain through a piece of muslin cloth. Mix it with the above extract (this is called pectin extract). Pass the pectin extract through a piece of thick cloth (2 to 4 folds of muslin cloth) to make it clear. The pectin extract can also be kept overnight and the clear extract is collected by pouring without disturbing the sediment. Dissolve the required quantity of sugar in the pectin extract by heating. Boil and strain immediately through a piece of muslin cloth. Cook till the jelly starts setting. Add citric when the jelly starts setting and cook till the end point is reached. Add the citrus flavorful jelly while still hot in pre-sterilized bottles. Perform the sensory analysis. Check the stability of apple jelly after a period of time.

**Objective:** Utilization of citrus flavor in yogurt, sensory analysis and effect of flavor on product.

### Procedure

**Preparation of Milk:** Fresh cow/nagori milk may be used for making ideal quality and highly natural flavored yogurt. One to two dried skim milk was added to get 10% solid not fat (SNF). One to two percent sugar and 0.5% stabilizer such as corn starch is added to get uniform sweet and custard like texture of yogurt. The milk is then heated to 85 - 90 °C for 15 min. achieve desired results.

**Inoculation:** The milk is cooled to 40 - 42 °C temperature (light warm). The starter culture (Yogurt) is added at 2 - 3% simultaneously add the citrus flavor (orange, Grape fruit and lemon).

**Incubation:** The inoculated milk may filled in (retail) plastic cups and incubated at 40-42 °C for 3-4 hours until a firm curd was formed and pH dropped towards 4.4 - 4.3.

**Cooling:** Yogurt is cooled immediately after setting. Synergetic behavior and shelf life may be improved by cooling at temperature below 5 °C. The texture of the product will improve, after 24 hour at refrigerated temperature.

### Sensory Analysis and Stability Test

Perform the sensory analysis. Check the stability of candy after a period of time.

**Objective:** Utilization of citrus flavor in yogurt, sensory analysis and effect of flavor on product stability.

**Recipe:** The constituents that were used for the Utilization of citrus flavor in yogurt, sensory analysis and effect of flavor on product stability are shown in **Table 2**.

**Procedure:** Dissolve sugar in water. Add glucose syrup. Cook the mass at 130°C (temperature is measured by using a thermometer) for the solid content of 95 to 96%. After cooking add the citrus flavor. Perform the sensory analysis. Check the stability of candy after a period of time. Make three samples of candy and compare the results as follows (**Table 3**).

**Table 2.** The constituents that were used for the Utilization of citrus flavor in yogurt, sensory analysis and effect of flavor on product stability.

Constituents	Parts by weight
Sugar	70
Water	20
42 DE Glucose syrup	30

**Table 3.** Sensory analysis and stability test.

Sample A	Without flavor and citric acid
Sample B	With flavor and without citric acid
Sample C	Without flavor and with citric acid

### Determination of Antioxidant Property of Flavor in Oil

**Objective:** Addition of citrus peel flavor (orange, grape fruit and lemon) in the edible oil and study the antioxidant property of the flavor.

#### Theory

**Antioxidants:** Antioxidants are chemical compounds which can delay the start or slow the rate of lipid oxidation reaction in food systems.

**Minimization of Lipid Oxidation:** If a compound inhibits the formation of free alkyl radicals in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the compound can delay the start or slow the chemical reaction rate of lipid oxidation.

The initiation of free radical can be delayed by the use of metal chelating agents, singlet oxygen inhibitors, and peroxide stabilizers.

The propagation of free radical chain can be minimized by donation of hydrogen from the antioxidants and the metal chelating agents.

#### Mechanism of Antioxidants

- 1- Hydrogen donation to free radicals by antioxidants.
- 2- Formation of a complex between the lipid radical and the antioxidant radical (free acceptor).

#### Factors Affecting the Efficiency of Antioxidant

- 1- Activation energy of antioxidants to donate hydrogen should be low.
- 2- Oxidation potential should be high.
- 3- Reduction potential should be low.
- 4- Stability to pH and processing.
- 5- Solubility in oil should be high.

#### Choices of Antioxidants

Different antioxidants show substantially different antioxidant effectiveness in different fats and oils food systems due to different molecular structures. We should consider the following:

- Safety
- Antioxidant effectiveness
- Off-odor
- Off-color
- Convenience of antioxidant incorporation to foods
- Carry through effect
- Stability to pH and food processing
- Availability
- Cost
- Non absorbable, if possible.

### Application of Antioxidants to Foods

- 1- Direct addition of antioxidants to oil or melted fat.
- 2- Addition of antioxidants to the food after they are diluted in diluents.
- 3- Spraying antioxidant solution on the food or dipping food into antioxidant solution.

**Requirements:** Oil sample, Saturated potassium iodide, Acetic acid (60): Chloroform (40), Soluble starch (1%)

**Procedure:** Take oil sample in duplicates and marked as A and B. Add the citrus peel flavor in sample A. Heat both the samples for about half hour and perform the peroxide value test as follows:

Weigh 10 gm oil sample into a conical flask and add 50 ml neutralized solvent. Add 1 ml saturated potassium iodide solution and allow standing in dark for two min. Add 1 ml saturated potassium iodide solution and allow standing in dark for two minutes. A starch as indicator (red /purple color appears) and titrate with  $\text{Na}_2\text{S}_2\text{O}_3$  till the disappearance of color.

### Antimicrobial Activity of Citrus Flavor

As we know that flavor contains some essential oils, terpenoids, and aromatic compounds so some citrus flavors have also antimicrobial Activities.

There are several methods to check the antimicrobial activity of any compound.

### Methods for Testing the Efficacy of Food Antimicrobials

- *In vitro*, screening, or Exploratory Tests
- End Point Methods
- Agar diffusion
- Agar and broth dilution
- Gradient plates
- Spiral plating
- Sanitizer and disinfectant tests

### Descriptive Methods

#### Turbidimetric Assays

**Inhibition Curves:** The principle of all antimicrobial testing is to assess the degree of efficiency inhibiting or in activating a selective range of organism under specified conditions.

But we select Agar diffusion method to check activity against microorganisms because this method is easy and gives rapid results.

### Factors Affecting Antimicrobial Activity

- There are several factors which affect Antimicrobial Activity, which can be shown as follows:
- Test Microorganism
- Strain
- Inoculums size
- Cell physiology
- Culture medium in which the organism is grown
- Antimicrobial agent
- Interaction of the test compound with components of the medium
- Partial partition coefficient
- Test Medium
- pH
- Water activity
- Redox potential

- Test Procedure
- Incubation conditions
- Oxygen pressure
- Atmospheric CO<sub>2</sub> concentration
- Incubation temperature
- Equipment variability.

### Agar Diffusion Methods

**Introduction:** The agar diffusion method has probably been the most widely used method for determining of antimicrobial activity throughout the history. In this test, antimicrobial compound is added to an agar plate on a paper disk or in a “well”.

The compound diffused through the agar resulting in a concentration gradient that is inversely proportional to the distance from the disk or well. Degree of inhibition, which is indicated by a “zone” of no growth around the disk or well. It depends on the rate of diffusion of the compound and cell growth. Therefore the antimicrobial evaluated should not be highly hydrophobic because the compounds will not diffuse and little or no inhibition will be detected.

**To Run the Test:** To run the test, Petri dishes are prepared to contain a nonselective medium at a depth of approximately 4 mm. The medium is surface inoculated with a suspension containing approximately log 6.0 colony-forming units (CFU)/ml of the test microorganism. Known concentrations of antimicrobial are then added to filter paper disks, which are dried and placed on the surface of the previously inoculated plate. Alternatively, the antimicrobial may be added to wells cut in the agar with a sterile cork borer. Plates are incubated under optimum conditions for the test microorganism for 16 to 24 hours. Following incubation, plates are examined for zones of no growth indicated by halos around the disks. A control test microorganism with known susceptibility to the antimicrobial should be included.

### Terms of Agar Diffusion Method

Susceptible Microorganisms - When the zone is >30 to 35 mm in diameter.

Intermediate Microorganisms - When the zone is >20 to 30 mm in diameter.

Resistant Microorganisms - When the zone is <15 to 20 mm in diameter.

## RESULTS AND DISCUSSIONS

### Stability Test

The stability test was performed for all the products such as: Candy, Yogurt and Apple Jelly. The Products made with the citrus flavor exhibit more shelf life than the products made without citrus flavor.

### Antioxidant Activity

The extracted flavor from citrus peels show antioxidant activities in oil.

### Antimicrobial Activity

The antimicrobial activity of flavor extracted from citrus peels is given below.

### Inhibitory Zones of Extracted Flavor

The inhibitory zones of extracted flavor can be shown in **Table 4.**

**Table 4.** Inhibitory Zones of Extracted Flavor

Orange flavor extracted by methanol	= 12 mm inhibitory zone.
Orange flavor extracted by Hexane	= 14 mm inhibitory zone.
Lemon flavor extracted by Methanol	= 14 mm inhibitory zone.
Lemon flavor extracted by Hexane	= 16 mm inhibitory zone.
Lemon + Orange + Grape fruit extracted by Methanol	= 17 mm inhibitory zone.
Lemon + Orange	= 16 mm inhibitory zone.
Control in Hexane	= 9 mm inhibitory zone.
Control in Methanol	= 10 mm inhibitory zone.

## CONCLUSIONS

From the study we can conclude that the flavors that were obtained from the citrus peels can be used in a variety of foods as a flavoring agents and even due to their antibacterial properties, they can be even used for the storage and preservation purposes. These have been concluded from the tests that were done. Apart from these uses, the flavors from citrus peels can also be used for other purposes which can be concluded by the presence of different chemical constituents in them.

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