EFFECT OF DRYING TEMPERATURE ON BIO-ACTIVE COMPONENTS AND PHYSICAL PROPERTIES OF LEMON PEEL

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Effect of drying temperature on bio-active components and physical properties of lemon peel

A dissertation submitted to the Department of food Technology, Central campus of Technology, Tribhuvan, University, in partial fulfillment of the requirements for the Degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Effect of Drying Temperature on Bio-active Components and Physical Properties of Lemon Peel*** presented by **Prashamsa Khanal** has been accepted as the partial fulfillment of the requirement for the **B. Tech. Degree** in **Food Technology.**

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Abstract

The study was carried out to study the effect of drying on bio-active components and physical properties of lemon peel at different temperature. Lemon of variety “madrasi baramasi” brought from local market of Dharan, Sunsari was used for the preparation of lemon peel powder. The fruit was sorted, washed and peeled. Lemon peel was submitted to oven drying at 50℃, 60℃, 70℃ till moisture content reached 5% and the dried peel was grinded to make fine powder.

Bio-active components (Antioxidant activity, Polyphenol content, Flavonoid content, Ascorbic acid, Tannin) and physiochemical analysis (Bulk density, Solubility, Oil absorption capacity and Swelling capacity) were measured on fresh (control) and dried samples. Drying lemon peel with the higher temperatures sharply increased the loss of bioactive components. The results showed that drying at 50°C indicated less reduction on bioactive component. Increase in temperature decreases the bioactive component, i.e., at 70℃ the TPC, TFC and antioxidant scavenging activity of lemon peel were found to be reduced by 31%, 61% and 26% respectively similarly, tannin and Ascorbic acid content were found to be reduced by 46% and 36% respectively. This study evaluated the physiochemical properties of peel powder which indicated that the increase in temperature increases bulk density, oil absorption capacity and swelling capacity but decreases solubility index. All analyzed samples showed that low temperature drying method is the best method for obtaining higher bioactive component.

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List of abbreviations

Abbreviation	Full form
LP	Lemon peel
TPC	Total phenolic content
TFC	Total flavonoid content
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
FAO	Food and Agriculture Organization
DPPH	2,2 –diphenyl 1-1- picryl hydrazyl
GA	Gallic acid
GAE	Gallic acid equivalent
USDA	United states Department of Agriculture
WAC	Water Absorption Capacity
OAC	Oil Absorption Capacity
SC	Swelling Capacity
LSD	Least Significant Difference
TSS	Total Soluble Solids
RSA	Radical Scavenging Activity

Part I

Introduction

1.1 General introduction

Lemon, (Citrus *limon*), small tree or spreading bush of the rue family (Rutaceae) and its edible fruit. It is one of the non-climacteric citrus fruits containing higher amount of vitamin-C and acidity. The lemon plant forms an evergreen spreading bush or small tree, 3–6 meters (10–20 feet) high if not pruned. Its young oval leaves have a decidedly reddish tint; later they turn green. In some varieties the young branches of the lemon are angular; some have sharp thorns at the axils of the leaves. The flowers have a sweet odor and are solitary or borne in small clusters in the axils of the leaves. Reddish-tinted in the bud, the petals are usually white above and reddish purple below. The fruit is oval with a broad, low, apical nipple and forms 8 to 10 segments. The outer rind, or peel, yellow when ripe and rather thick in some varieties, is prominently dotted with oil glands. The white spongy inner part of the peel, called the mesocarp or albedo, is nearly tasteless and is the chief source of commercial grades of pectin. The seeds are small, ovoid, and pointed; occasionally fruits are seedless. The pulp is decidedly acidic (Augustyn *et al.*, 2021).

Citrus fruits are highly consumed worldwide as fresh produce, juice and most often the peel is discarded as waste which contains a wide variety of secondary components with substantial antioxidant activity in comparison with other parts of the fruit (Manthey and Grohmann, 2001). While processing, it yields 50% juice, 29% peel, 20% residue and 1% seeds (Lei, 2006). These figures clearly show that a major portion of the fruit is going waste during processing, which can be utilized for many values added products. Citrus peel, the primary waste, is a good source of molasses, pectin and limonene and is usually dried, mixed with dried pulps and sold as cattle feed. Citrus peels are subdivided into the epicarp or flavedo (colored peripheral surface) and mesocarp or albedo (white soft middle layer) (Bocco *et al.*, 1998). A number of studies have recognized the presence of polyphenols, vitamins, minerals, dietary fibers, essential oils and which makes citrus a health-benefit promoting fruit. On an average, lemon peel contains Acid (g/l) 0.637 ± 0.08, pH 4.510 ± 0.01, Brix degree (%) 2, Reducing sugar (mg/ml) 0.633 ± 0.058. Lemon peel components were found to provide many health benefits, contains high amounts of Vitamin C and

calcium and thus helps in improving and maintaining bone health, contain a good amount of polyphenols that protect against several diseases. Peels have anti-cancerous properties, due to the presence of limonene, a naturally occurring chemical (Sadek, 2009).

Bioactive food components are components in foods or dietary supplements, other than those necessary to meet the basic nutritional needs, which are responsible bioactive compounds are not nutrients, even if they are contained in foods or their constituents (Weaver, 2014). These definitions opposes the view of (Guaadaoui *et al.*, 2014) who presented a definition in an envelope of 'consensus': "bioactive compounds" are essential and non-essential compounds that occur in nature, are part of the food chain, and can be shown to have an effect on human health.

Bioactive compounds including carotenoids, essential oils, antioxidants, or flavors are widely incorporated into food products in order to enhance their sensory properties or to develop their nutritional and health properties. Bioactive compounds are present in small quantities in foods, mainly in fruits, vegetables, and whole grains, and provide health benefits beyond the basic nutritional value. Bioactive compounds are molecules that can present therapeutic potential with influence on energy intake, while reducing pro-inflammatory state, oxidative stress, and metabolic disorders. Epidemiological studies indicate that high consumption of foods rich in bioactive compounds with antioxidant activity, including vitamins, phytochemicals, and mainly phenolic compounds, such as flavonoids and carotenoids, has a positive effect on human health and could diminish the risk of numerous diseases, such as cancer, heart disease, stroke, diabetes, and age-related functional decadence (Barba *et al.*, 2019).

The drying process employed affects the quality of a dried product. Drying has become a widely used way of food processing, allowing the extension of the shelf-life of fruits and by-products. Hot air drying is a process widely used in preserving fruit and vegetables, and has been used on a small scale to solve problems of excess production. One of the most important food conservation procedures by reducing water activity (a_w) is food dehydration (Ramos and Stringheta, 2004).

1.2 Statement of the problem

Lemon is mainly used by juice processing industries while the peel separated are generally wasted. Total of 50% will be loss as pomace, peel and seed after extraction of juice. Disposal of these by-products represents both a cost to the food processor and a potential negative impact on the environment (Sapkota, 2018). Lemon peel is very low in calories while high in fiber, vitamin C, and D-limonene, also contains several minerals. Flavonoids, vitamin C, and pectin in lemon peel may promote heart health by lowering blood cholesterol levels and other risk factors for heart disease (Lang, 2019). Additionally, lemon peel is found to be a good source of bioactive compounds such as polyphenols, carotenoids, vitamins, enzymes, and dietary fibers (Ajila *et al.*, 2007).

Lemon peel can be a source of valuable bioactive compounds if special technologies are used. In addition, the combined efforts of waste minimization during the production process and recovery of valuable product substantially reduce the amount of waste, as well as boost the environment profile or fruit juice processing industry. Though, lots of research has been carried out on possibility of incorporation of lemon peel to food product, which result; fresh peel has short self-life and drying can enhance stability and dry powder is easy to incorporate in different food products than fresh peel. So, it is necessary to identify appropriate technology to prepare it with minimum loss of bioactive component. The peel is being thrown out which can add environmental problem due to increased waste.

1.3 Objective

1.3.1 General objective

The general objective of the dissertation work is to study effect of drying temperature on bio-active components and physical properties of lemon peel

1.3.2 Specific objective

The specific objectives are as follows:

1. To determine the bioactive components (ascorbic acid, carotenoids, tannin, polyphenol, flavonoid and antioxidant activity) of fresh lemon peel.
2. Drying of lemon peel at different temperature (50, 60, 70℃).

3. Determination of bioactive components (ascorbic acid, tannin, polyphenol, flavonoid and antioxidant activity) of lemon peel powder.
4. To determine the physical property (Bulk density, Solubility, Oil absorption capacity and Swelling capacity) of lemon peel powder.

1.4 Significance of study

a) This study specifically determines quality parameters of raw lemon peel and the effect of various processing temperature on native quality parameters of lemon peel.

b) Better understanding of changes on bioactive components (ascorbic acid, tannin, polyphenol, flavonoid and antioxidant activity) and physical properties (Bulk density, Solubility, Oil absorption capacity and Swelling capacity) by effect of temperature.

c) This study might help in the establishment of the effective and optimized temperature for the preparation of lemon peel powder with best quality.

d) Utilization of byproduct from citrus industry, thus minimizing waste.

e) This result also can be used in scientific study for similar processing of other citrus fruits.

1.5 Limitations of the study

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

Delimitation

a) Only one species of lemon was studied.

Part II

Literature review

2.1 Lemon (*Citrus limon*)

Lemon are sour, round, and bright green citrus fruits. They're nutritional powerhouses — high in vitamin C, antioxidants, and other nutrients (Augustyn *et al.*, 2021). The botanical name of the plant is *Citrus Limon*. *Citrus Limon* commonly known as Lemon. They are a sour, and are often used to accent the flavors of foods and beverages. There are many species of lemon like the Key lime (*Citrus aurantifolia*), Persian lime (*Citrus latifolia*), desert lime (*Citrus glauca*) and kaffir lime (*Citrus hystrix*). Each of these species has unique characteristics. For instance, the Key lime is smaller, more acidic, and more aromatic than the more common Persian type (Raman, 2019). The lemon fruit is a key ingredient in certain pickles and chutneys, and lemon juice is used to flavour drinks, food, and confections. Limeade and other lemon-flavoured drinks have a flavour and bouquet quite distinct from those made from lemons. Lemon is an ingredient in several highball cocktails, often based on gin, such as gin and tonic. Freshly squeezed lemon juice is also considered a key ingredient in margaritas, although sometimes lime juice is substituted. It is also found in many rum cocktails, and other tropical drinks. The juice may be concentrated, dried, frozen, or canned. Lemon oil, from the peel of the fruit, can be obtained. Lemon extracts and lemon essential oils are frequently used in perfumes, cleaning products, and aromatherapy (Zhang, 2020).

Lemon is one of the main citrus crops, with a worldwide production of 7.3 million tons and is mainly processed to juice and lemonade. Therefore, its industrial processing generates a huge volume of byproduct, constituted essentially from solid waste (pulp, seeds, and peels) and water waste. The waste causes many economic and environmental problems in the production areas because of its fermentability. Lemon byproduct is used, most of the time, in animal feed either fresh or after ensilage or dehydration or as a fertilizer. Lemon byproduct is also rich in bioactive compounds, such as fiber (pectin), phenolic compounds (flavonoids, phenolic acids), limonoids (M'hiri *et al.*, 2018). The pulp is tender, juicy, yellowish green in color, and decidedly acid. Limes exceed lemons in both acid and sugar content (Petruzzello, 2016).

Acid lime (Citrus aurantifolia Swingle) commonly known as “Kagati” is an important commercial fruit, which has been traditionally cultivated in most of the districts of Nepal (“National Citrus Research Program,” Dhankuta-Nepal). The area coverage of lemon has been reported 16% of total fruit crops in Nepal (Dhakal *et al.*, 2005). The main production season of lemon in Nepal is September to November, but demand in the market is throughout the year (Shrestha *et al.*, 2012). Among the commercial citrus species, it comes in third rank after mandarin and sweet orange in terms of area and production. Cultivation range of lemon in Nepal is 800 m to 1400 m asl in the mid hills stretching from east to west, but potentiality of cultivation 6 range could be much wider from 125m asl terai to 1800m asl in high hills of Nepal.

Taxonomic classification of lemon

Kingdom	Plantae
Class	Dicotyledonae
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	Limon

(International, 2019)

2.1.1 Historical background and distribution of lemon

Although the mysteries of its history and origin remain unsolved, worldwide cultivation and high-demand production for citrus fruit (genus *Citrus* in family *Rutaceae*) make it stand high among fruit crops. Citrus reticulate is a native of China and Cochin China. It is widely cultivated in all subtropical regions. In India, the areas of concentrated cultivation lie in Assam, Sikkim, Central India, Punjab and Coorg. The principal tracts of cultivation in Assam are Khasi and Jaintia hills and the districts of Cacharand Kamrup, Commercial production in central region is centered in Nagpur, Bhandara, Wardha, Chindwara and Amraoti districts. In Punjab main areas are Hoshlarpur and Gurdaspur. Five indigenous cultivars are reported in Assam. These are Soh-niamtra, Sohumkait, Nagasantra, Soh-siem and Kapuratenga, extensively grown all over Assam, meghalaya and Mizoram. The important orange cultivars cultivated commercially in different parts of India include 'Nagpur orange, Khasi orange', 'Coorg orange', 'Desiorange, 'Sikkim orange', 'Butwal' and 'Emperor'. Coorg and Khasi seem to be ecological forms of the 'Nagpur' (Sapkota, 2018) .

Currently, citrus is cultivated in the subtropical and tropical regions of the world between 40° north and south latitude in over 137 countries on six continents and generates about 105 billion US dollar per year in the world fruit market (Ismail and Zhang, 2004).

In contest of Nepal, Citrus is one of the major fruits covering 25% of the total fruit area in 58 district of the country and has been recognized as high value crop by National Agriculture Perspective Plan (APP). At present, the total area of 37565 ha produces 240793 MT citrus fruits in Nepal within productive area of 24089 ha (Mora-Aguilera *et al.*, 2014). The major source of income was from citrus farming (Nrs, 43933/household/annum) followed by services (NRs.36090/household/annum) and other agricultural activities. The study also indicates that citrus farming is associated with higher income families rather than poor subsistence farmers.

2.2. Citrus Fruits Production

When most people think of citrus, the usual varieties which come on mind are lemons, limes, oranges, mandarin, grapefruit, amla, mausami, tomato, strawberry, guava, pomelos and tangerine. However, there are many different kinds of citrus fruits in the *Citrus* genus. It is one of the largest fruit crops in the world. Citrus plants are native to

subtropical and tropical regions of Asia, Island Southeast Asia, and northeastern Australia. Most citrus plants grow best in full sun but some can tolerate a little shade, and some are a bit harder than others. About 30% of citrus fruits is processed to obtain various products, mainly juice (Book). In 2010, the production of citrus fruit worldwide was estimated as 122.5 million tones with ~8.7 million hectares harvested; oranges were 50%–62% of the total area harvested and total production. However, least developed countries located in areas of Sub-Saharan Africa and Southeast Asia, which generally have the highest proportion of persons with malnutrition and micronutrient deficiencies, also have the lowest consumption of citrus (Namubiru-Mwaura and Place).

Table 2.1 Citrus fruit production in Nepal (2015/16)

S.N	Fruit crop	Productive area (Ha)	Production (Mt)	Yield (mt/Ha)
1	Mandarin	16,248	146,690	9.03
2	Sweet orange	3,443	33,558	9.75
3	Lime	3,858	27,017	7.00
4	Lemon	595	4,941	8.30
5	Others	741	6,242	8.42
	Total	24,885	218,448	8.78

(Pandey and Basnet, 2017)

2.2.1 Varieties of lemon in Nepal

Acid lime (Citrus aurantifolia) fruits are cultivated in terai, mid hill and high hill districts of Nepal. Three cultivars of lemon have been grown in terai area, i.e., acid lime (Pahade Kagati or Sun Kagati), eureka (Chasme Kagati) and natural hybrid types (Shrestha et al., 2012). Among them acid lime has high commercial value in the market due to better aroma, appropriate size and medicinal value. It is used for juice, desert, pickle and other medicinal purpose.

2.3 Physicochemical and nutritional composition of lemon

Lemon fruit ranks very high in its medicinal value in this way lemon are favorite all over the world. It is a good source of vitamin-C and other nutrients like potassium, iron, calcium, fiber, thiamine, riboflavin and vitamin B6. Natural foods, especially citrus products, have always been highly regarded as excellent sources of human nutrition. Lemon juice has many health benefits can be used for different purpose like therapeutically uses, natural antiseptic, to control asthma, headaches, pneumonia and arthritis etc.

Experimental results obtained from research work of which Ghimire (2020) utilized different varieties of *Citrus limon* species showed following Physicochemical characteristics of *Citrus limon* fruits in Table 2.3

Table 2.2 Physicochemical characteristics of *Citrus limon* fruits

Physicochemical Parameters	Value
Juice Yield (%)	23-24%
Acidity % (as citric acid)	4-5%
pH	2-3%
TSS % (°Bx)	5-6%
Vitamin-C (mg/100 g)	23-24%

Source: Ghimire (2020)

Physicochemical parameters (Juice yield, acidity, pH, ascorbic acid, TSS) were proportional to solid content in citrus juices (Gorinstein *et al.*, 2001). Juice yield of lemon is 23.5% on average. Mouei and Choumane (2014) reported the juice yield for lemon were found to be ranges from 30% to 50%. The acidity of the lemon was found to be in a range of 5-7% justified by (Mouei and Choumane, 2014). Variety and maturity level are the major factors, which influence significantly physicochemical parameters of juice. The ascorbic acid content of lemon was found to be 23-24%.

These tart, flavorful fruits contain some potassium and are rich in vitamin-C. Just 2 tablespoons of lemon juice provide a little over 15 percent of the RDA. Lemon juice contains less vitamin-C than lemon juice, with 2 tablespoons providing just 10% of the RDA. Along with supplying substantial amounts of vitamin-C, the health benefits of these fruits also rest in their fiber and phytochemicals. The peels of lemons and limes are rich in limonene phytochemicals, which seep into the juice and may help protect against cancer.10 Postharvest keeping quality is affected by preharvest and postharvest factors (such as climate, growing conditions and production practices, harvest practices, factors causing postharvest loss of quality, etc.) and postharvest handling practices and used technologies (El-Otmani *et al.*, 2011).

2.4 Benefits of Citrus Fruits

Eating citrus fruits like oranges, limes, lemons, and grapes are beneficial to the human body. Citrus fruits are a good source of fiber, necessary for adding bulk to food during digestion and preventing constipation. Citrus fruits contain antioxidants necessary for reduction of chances of contracting cardiovascular diseases. Citrus fruits and citric products like juices are a rich source of immune-boosting vitamin C. Vitamin C is good for the elasticity of your skin and reducing the severity of cold. Citrus fruits are rich in other minerals like calcium which is necessary for strong bones and potassium which is good for the functioning of the nervous system. Citrus fruits are low in calories, making them a smart choice for people seeking to lose or maintain their weight. Eating citrus fruits may help lower the risk of kidney stones in some people by raising citrate levels in urine (Asim *et al.*, 2018).

2.5 Lemon peel

During citrus processing, a large amount of citrus peel is produced, which comprises approximately 25% of the total weight of citrus fruit. Citrus peel contains several functional components, such as essential oil, pectin, carotenoids, hesperidin, and limonin, which are important raw materials in the chemical and pharmaceutical industries. Extraction of these bioactive compounds will thus enhance the value of the products from citrus peel. Therefore, citrus peel should be thoroughly studied for possible utilization. The peel is concerned, extracts from this part of the fruit were found to have a good total radical antioxidative potential (TRAP) (Gorinstein *et al.*, 2009). The utilization of functional components of citrus peel has become an important part of the citrus-processing industry.

The residues of lemon peel obtained are chosen as the raw materials. In general, the newly stripped peel should be treated within 2 h, and water content should be controlled at approximately 83% (Ghanem *et al.*, 2012). Meanwhile, no mildew, no odor, no color change, and no softening are observed. In short, timely treatments are required. Crude fiber is loss on ignition of dried residue remaining after digestion of sample. The value of ash reported in this study, lemon peel may be suitable for animal feeds (Janati *et al.*, 2012). Considering surfactant as the major component to prepare pesticide-removing agents, 1.5% pesticide-removing agent is used to soak the samples for 30 min. During soaking, stirring is not necessary and soaking duration cannot be too long. In addition, soaking should be conducted in stainless containers. The pesticide remover is cleaned with fresh water through soaking for 10 min. After drainage, the flowing water is used to rinse the samples to pH 7.0. The citrus peel is placed in a plastic box with holes to remove the water. According to different requirements, the citrus peel is cut into different blocks and filaments (Shan, 2016). Lemons come from aromatic plants and the peels are rich in essential oil that can be extracted. Lemon peels can be found as a waste product from the fruit processing industry. Lemon peel oil is one of the best essential oils to have on hand, as it can used for so many purposes (Paw *et al.*, 2020).

It is note-worthy to clarify that citrus peel, the waste product of the citrus factories can be recognized as a valuable functional food. Furthermore, the dietary fibers are indigestible and fibrous part of citrus fruits is an essential part of our diet. Because of their water retaining properties , fibers help food to pass through the gut faster and therefore have a laxative effect (Youssef, 2007).

2.6 Nutritional value of lemon peel

Despite being eaten in small amounts, lemon peels are very nutritious. One tablespoon (6 g) provides:

- Calories:3
- Carbs: 1 g
- Fiber: 1 g
- Protein: 0 g
- Fat: 0 g
- Vitamin C: 9% of the Daily Value (DV)

Lemon peel packs a high amount of fiber and vitamin C, providing 9% of the DV in only 1 tablespoon (6 g).

Additionally, it boasts small amounts of calcium, potassium, and magnesium.

D-limonene, a compound that gives lemon its characteristic aroma, is also found in the peel and may be responsible for many of this fruit's health benefits.

2.7 Composition of citrus peel

Citrus peels are comprised of two regions: the flavedo and the albedo. The flavedo consists of characteristics peel oils and pigments while the albedo is the white pithy region (Chang and Taipei, 1986). The peel contains oil sac and the oil is composed of 91-94% d-limonene and 2.0-2.1% beta-myrcene as a minor constituent (Shahidi *et al.*, 2005). Polymetholated flavones are also a class of compounds found in citrus peel and produce no negative side effects in the animal fed the polymetholated flavones containing diets (Oluremi *et al.*, 2007). The white pith on the peel contains bioflavonoids. Bioflavonoids strengthen our blood capillaries enhancing their ability to deliver blood, oxygen and nutrients to our tissue and organs. Bioflavonoids provide tonic support for the entire cardio vascular system. The high content of bioflavonoids in mandarin peel contributes to their anti-bacterial, anti-viral and anti-inflammatory properties (Shahidi *et al.*, 2005).

2.8 Constituents

Lemon peel has been found to contain fat, protein, ash, magnesium, carotenoids, dietary fiber, flavonoids and polyphenol. The mineral content in lemon peel is Sodium (Na), Potassium (K), Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Zinc (Zn), Phosphorus (P). Iron, copper, zinc and manganese play an important role in biological systems; they are essential for nutrition and are widely used in the fields of clinical medicine, environmental science and health. Thus, the analysis of these elements is clearly important. Lemon peel concentrate contains beta-cryptoxanthin, xanthophylls esters (zeaxanthin and lutein). Lemon contains limonoids, including obacunone 17 beta-D-glucopyranoside, nomilinic acid 17 beta-D-glucopyranoside, limonin, nomilin, and a limonoidglucoside mixture (Rincón *et al.*, 2005).

2.9 Flavor compounds of the lemon peel

The main flavor components of the fresh lemon are limonene, α-terpineol, 4-terpineol, neryl acetate, β-pinene, β-bisabolene, neral, citral, geranial 1,4 cineole, 1,8 cineole, p-cymene, α-bergamotene, valencene, and d-germacrene. GC-MS analysis of the flavor compounds extracted from lemon peel by using cold-press allowed identification of 54 volatile component, 25 oxygenated terpenes (11 aldehydes, 9 alcohols, 3 esters, and 2 ketones), 29 non osygenatedterpenes (12 monoterpenes and 17 sesquitepenes). (María and Gloria, 2012)

2.10 Uses for citrus peels

According to Brown (2020), the uses for citrus peels were enlisted as follows,

- Body scrub: Vitamin C is a beloved chemical exfoliant, so when paired with physical exfoliants like sugar or salt and effective body scrub.
- Fire starter: Dried citrus peels make an effective and lovely scented fire starter. Dry the peels out either at room temperature for a few days or in the oven and set them aside for your next camping trip or bonfire.
- Breath freshener: Instead of relying on chewing gum, scrape the white part off of a fresh peel and chew on it for fresh and fragrant breath.
- Bath infusion: Elevate your next bath by tossing in a few citrus peels. Essential oils found in citrus peels have been linked to increased mood.
- Drink garnish: Dried peels are a beautiful and delicious addition to cup of tea or a cocktail. Scrape away as much of the pith as you can and then dry and store the peels for your next cup.
- Household cleaner: If you love cleaning with a vinegar water solution but don't love the smell, you can add a citrus twist to your next batch. Soak citrus peels in vinegar for a week or two and then mix with equal parts water in a spray bottle.
- Lemon pepper seasoning: To make your own lemon pepper seasoning, grind dried lemon peels into a powder, and toss with salt and pepper.

- Air freshener: Mimic an essential oil diffuser by simply simmering citrus peels in a pot of water. Don't get too relaxed-you are actively using the stove so be sure to keep an eye on the pot.

- Candied peels: Candied citrus peels stay good for months, and are a wonderful addition to scones, muffins, drinks, or even as a standalone snack.

- Cocktail ingredient: Use a peeler to remove the outermost layer of the peel, allow it to soak in sugar for a few hours, and then press the peels to release the oils. This is used in drinks like an old-fashioned.

- Trash can deodorizer: Your garbage can doesn't actually have to smell like garbage. When you're done using the fruit, toss a few citrus peels at the bottom of your trash can to absorb odors.

2.11 Health benefits of lemon peel

According to Ariane Lang (2019), the health benefits of lemon peel were enlisted as follows,

a) High nutritional value: Despite being eaten in small amounts; lemon peels are very nutritious. Lemon peel packs a high amount of fiber and vitamin C, providing 9% of the DV in only 1 tablespoon (6 g). Additionally, it boasts small amounts of calcium, potassium, and magnesium. D-limonene, a compound that gives lemon its characteristic aroma, is also found in the peel and may be responsible for many of this fruit’s health benefits.

b) May support oral health: Dental cavities and gum infections are widespread oral diseases caused by bacteria like Streptococcus mutans. Lemon peel contains antibacterial substances that may inhibit microorganism growth. In one study, researchers identified four compounds in lemon peel that have powerful antibacterial properties and effectively fight common oral-disease-causing bacteria.

c) High in antioxidants: Antioxidants are plant compounds that prevent cellular damage by fighting free radicals in your body. Lemon peel is high in antioxidants, including D-limonene and vitamin C. Intake of flavonoid antioxidants like D-limonene is

linked to a reduced risk of certain conditions, such as heart disease and type 2 diabetes. Additionally, the vitamin C in lemon peel acts as a powerful antioxidant and likewise promotes immune health.

d) May promote heart health: High blood pressure, high cholesterol, and obesity are all risk factors for heart disease, which is the leading cause of death in the United States. Research suggests that compounds such as flavonoids, vitamin C, and pectin — the main fiber in lemon peel may reduce your risk. The pectin in lemon peels may also reduce cholesterol levels by increasing the excretion of bile acids, which are produced by your liver and bind to cholesterol.

e) May treat gallstones: Some studies suggest that D-limonene may help treat gallstones hard deposits that can develop in your gallbladder. In a study in 200 people with gallstones, 48% of those injected with a D-limonene solvent experienced complete gallstone disappearance, suggesting that this treatment could be an effective alternative to surgery.

2.12 Biological properties of lemon peel

2.12.1 Antibacterial properties

Antibacterial property is one of the most frequently desired properties where the growth of microorganism is controlled/eliminated by the presence of antimicrobial agents that are filled into the fiber structure. The antibacterial activity of natural products from medicinal plants is applicable for the treatment of bacterial, fungal and viral diseases. The Citrus fruits and its byproducts are of high economic and medicinal value because of their multiple uses, such as in food industry, cosmetics and folk medicine. Specifically, the Citrus peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils (Afroja *et al.*, 2017).

In an in vitro study, an ethanol extract of Citrus aurantifolia (lime) inhibited five clinical strains of Helicobacter pylori (MIC: approximately 40 mcg/mL) (Yunfeng *et al.*, 2006). The antibacterial activity of citrus essential oils was tested against human pathogenic bacteria. The oils were effective against Gram (+) and Gram (-) bacteria, with a major activity against S. aureus and E. coli. Bitter orange, lemon, and orange were effective against P. aeruginosa only at maturity (Harborne *et al.*, 2000).

2.12.2 Anticancer activity

Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression (Meshram *et al.*). Limonoids extracted from *Citrus Limon* exhibited significant growth-inhibitory effects at high concentrations (100 mcg/ml) against human breast cancer cells lines (Joshi *et al.*, 2012). For instance, it has been suggested that high dietary intake of vegetables and fruits (>400 g/day) could prevent at least 20% of all cancer cases (Gullett *et al.*, 2010). *Citrus* fruits are the most eaten fruits in the Mediterranean diet that it is known to reduce the risk of degenerative diseases, including cancer. *Citrus* fruits represent one of the most important diet sources of flavonoids whose benefits are due to many biological properties, among which the well-known antioxidant activity and the modulation of intracellular key pathways involved in degenerative processes leading to chronic pathologies such as cancer (Clere *et al.*, 2011).

Flavones extracted from the fruit peel of Citrus aurantifolia induced differentiation in mouse myeloid leukemia cells (MI), and the cells exhibited phagocytic activity in vitro. Limonoids extracted from Citrus aurantifolia exhibited significant growth-inhibitory effects at high concentrations (100 mcg/ml) against human breast cancer cells lines (MCF-7). In two in vitro studies, extracts of Citrus aurantifolia peel increased apoptopsis in human gastric cancer cells (SNU-64) and human colon cancer cells (SNU-C4) (Kim *et al.*, 2005).

2.12.3 Anti-inflamatory activities

LIM is one of the most common terpenes in nature and has been used as a flavoring agent in common food items, such as fruit juices, soft drinks, and ice cream, and in the cosmetics and pesticide industries (Hirota *et al.*, 2010). LIM has been shown to exert antiulcerogenic, gastro protective, chemo preventive, antiproliferative, insecticide, antimicrobial, and immunomodulatory effects. The biological activity of extracts of herbs has been widely studied, but few studies have evaluated the effects of essential oils obtained from plants of the genus Citrus and its constituents on anti-inflammatory activity (Arruda *et al.*, 2009). In addition, biological effects of citrus peels have been reported. Lime (Citrus aurantifolia) peel and Ponkan (Citrus reticulate Blanco) peel, which contain polymethoxy flavones, displayed anti-inflammatory activity (Huang *et al.*, 2010).

2.13 Bioactive components

Bioactive compounds are phytochemicals found in foods that are capable of modulating metabolic processes and resulting in the promotion of better health. They exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression. For instance, fruit antioxidants are commonly mixed with different macromolecules such as carbohydrates, lipids, and proteins to form the food matrix. Fruit peels and seeds have also high antioxidant activity (Lorenzo, 2020). The health benefits of citrus fruit have mainly been attributed to the high level of bioactive compounds, such as phenols (e.g., flavanone glycosides, hydroxycinnamic acids), carotenoids and vitamin C. These compounds are present in the fruit pulp and hence in the juice. But some bioactive compounds can be found in parts of the fruit which usually are not used for human food. The content of bioactive compounds depends on the species and cultivar, but also depends on the production system followed. Citrus fruits, their derivatives and their by-products (peel, pulp and oil) are reach in different bioactive compounds and its maturity, postharvest and agroindustry processes influence their composition and concentration (Duarte *et al.*, 2016)

2.13.1 Antioxidant activity

Antioxidant activity is defined "as an limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions" and primary antioxidants directly collect free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction (Lee *et al.*, 2005). There are more than 170 antioxidants from Citrus fruits that have been reported in the current literature, including vitamins, mineral elements, phenolic compounds, terpenoids and pectin (Zou *et al.*, 2016). The blanching pretreatment and high drying temperature caused the decrease in antioxidant compound (Sengkhamparn *et al.*, 2013).

Residues of citrus processing industry are sources of dietary fibre, pectin, cold pressed oils, essence, limonene, limonoids and flavonoids (Braddock and James, 1999). Lemon peel has been analyzed for few important phytonutrients such as polyphenols, carotenoids, flavonoids, vitamin, dietary fibres. The main flavonoids found in citrus species are hesperidin, narirutin, naringen, and eriocitrin. Flavonoid's content and anti-oxidant activity from the residue of lemon peel has been reported (Anagnostopoulou *et al.*, 2005). High

dietary fibre powder prepared from mandarin by product with high functional and microbial quality, as well as favorable physiochemical characteristics to be used in food formulation (Lario *et al.*, 2004).

On the basis of experiments performed by (Réblová, 2012) with phenolic acids, the existence of a relationship between the relative decrease in antioxidant activity with increasing temperature and the oxidisability of the antioxidants was found. According to this relationship, the easily oxidisable antioxidants show a decrease in antioxidant activity with increasing temperature (in comparison with their activity at a low temperature) at a slower rate than the less oxidisable ones, and maintain their antioxidant activity also at higher temperatures (in contrast to less oxidisable antioxidants)

2.13.2 Ascorbic acid

Ascorbic acid is one of the most important nutritional benefits of citrus fruits, its stability varying markedly as function of environmental conditions such as pH and the concentration of traces metal ion and oxygen. The nature of the packing can significantly affect the stability of ascorbic acid in foods. Vitamin C or ascorbic acid in citrus fruits is a water-soluble carbohydrate like substance involved in certain metabolic processes of animals. Ascorbic acid helps in the prevention of scurvy; this causes the disease which leads to the formation of spots on the skin, spongy gums and bleeding from the mucous membrane (Ayesha *et al.*, 2014).

Ascorbic acid (Vitamin C) is the only vitamin present in citrus fruits in amounts of major nutritional significance; one orange has 50mg of vitamin C, which nearly the double of the recommended daily intake. The concentration of ascorbic acid has been reported to decrease with maturity or remain constant until late in the season and then decline (Sapkota, 2018).

Abou-Arab *et al.* (2016) found that, drying of citrus peels, either by sun or oven greatly reduced the ascorbic acid concentration to less than half content of their original values (control). These results agreed with Fernández-López *et al.* (2004). This could be attributed to the fact that ascorbic acid is not stable at high temperature (Negi and Roy, 2000). Ascorbic acid is one of the substances that contribute to the antioxidant capacity in citrus juices and by products. It contributes to about 56% and about 77% of the antioxidant capacity of citrus

juice and peels, respectively (Vinson *et al.*, 2002). Ascorbic acid degradation during drying depends mainly on temperature, time and metal ions traces (Özkan *et al.*, 2004).

2.13.3 Flavonoids

Flavonoids are a diverse group of phytonutrients (plant chemicals) found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. The presence of a relatively large number of flavonoids in *Citrus* juices is a result of the many different combinations that are possible between polyhydroxylated aglycones and a limited number of mono- and disaccharides. The main flavonoids found in citrus species are hesperidin, narirutin, naringen, and eriocitrin (Gattuso *et al.*, 2007). Although flavonoids are generally regarded as non-nutritive agents, their potential role in the prevention of major chronic diseases has attracted the focus of many researchers. The citrus flavonoids include a class of glycosides, namely, hesperidin and naringin and another class of O-methylatedaglycones of flavones such as nobiletin and tangeretin, which are relatively two common polymethoxylated flavones (Chen *et al.*, 2014). The flavonoids are heat susceptible phenolic compounds therefore heat treatment during blanching cause decrease in total flavonoid content (Zhu *et al.*, 2010).

Flavonoids are known to have antioxidant activities and are used as antibiotics, anti-diarrheal, anti-ulcer and anti-inflammatory agents, and also for treatment of diseases such as hypertension, vascular fragility, allergies, and hypercholesterolemia (Bravo *et al.*, 2008). Furthermore, these flavonoids are known to possess antioxidant, anticancer, antiallergic and gastroprotective properties (Sreerama *et al.*, 2012). The dietary antioxidants are capable of blocking neuronal death in vitro and many therapeutic properties of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins (Mhiri *et al.*, 2018).

According to (Kim, 2013)20 gm of dried citrus fruit (Citrus unshiu) peel powder was refluxed for 1h in 200ml DW. The obtained extract was cooled and filtered over Whatman No. 1 paper, concentrated under vacuum at 40℃ and lyophilized. Different samples of concentrations, 50, 200, 800, 1600, and 3200 ppm (µg/ml) were prepared and analyzed for flavonoid. And the results were found ranging from 0.038 to 9.315 µg quercetin equivalent/ml of extract.

2.13.4 Polyphenol

Polyphenols are a category of compounds naturally found in plant foods, such as fruits, vegetables, herbs, spices, tea, dark chocolate, and wine. They can act as antioxidants, meaning they can neutralize harmful free radicals that would otherwise damage your cells and increase your risk of conditions like cancer, diabetes, and heart disease. Polyphenols are also thought to reduce inflammation, which is thought to be the root cause of many chronic illnesses. Citrus fruits are an important source of high-quality bioactive compounds, polyphenols and flavonoids (Muscatello *et al.*, 2018).

Phenolic compounds have a major role in growth and reproduction, providing protection against pathogens and predators besides contributing toward the color and sensory characteristics of fruits and vegetables. Phenolic content can be used as an indicator of antioxidant capacity and as a preliminary screen for any product when planned to utilize as a natural source of antioxidants in functional foods (Abdelwahab and Abouelyazeed, 2018). Phenolic antioxidants interfere with the oxidation of lipid and other molecules by rapid donation of hydrogen atoms to radicals. Phenolic compounds can be classified as free, soluble and insoluble bound phenolic compounds (Renger *et al.*, 2000). Borges *et al.* (2016) reported that derivatives of hydroxycinnamic acids such as caffeic, sinapic, p-coumaric and ferulic acid occur mainly in bound phenolic compounds of lemon, orange, mandarin and grapes. On the other hand, hydroxycinnamic acids are aromatic compounds (Bravo and Laura, 1998). Hydroxybenzoic acids (e.g. gallic acid, syringic acids), Xanthones (C6-C1-C6), Flavonoids, Isoflavonoids, Tannin(C6-C3-C6) and Bioflavonoids are the most common phenolic compounds occur in citrus fruits. The total phenolic contents of *Citrus limon* are usually higher in peels with the range of 104.2-223.2 mg Gallic acid equivalent/g. Similarly, Polyphenol content are higher in ripe peel than that of raw peel (UEDA *et al.*, 2000).

Phenolic compounds exhibit a wide range of physiological properties such as antiallergenic, anti-inflamatory, antimicrobial, antioxidant, antithrombotic, cardio protective and vasodilatory effects (Middleton *et al.*, 2000). The noticed differences in the TPC may be related to nature and characteristics of the varieties of citrus fruit. The differences in the values of TPCS for various citrus peels types may be affected by environmental conditions, the degree of fruit ripening and genetic factors (El-ghfar *et al.*, 2016).

2.13.5 Tannin

Tannins are a class of complex biomolecules of polyphenolic nature synthesized by a large variety of plants, in which they are used as antipredation or pesticide agents (Mathias *et al.*, 2016). Tannins are reported to exert physiological effects such as accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis and modulate immune responses. The anticarcinogenic and antimutagenic potential of tannins may be related to their antioxidative property which is important in protecting cellular damage including lipid peroxidation. Tannins are reported to exert physiological effects such as accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis and modulate immune responses (Chung *et al.*, 1998). Eze *et al.* (2014) , reported tannin can be reduced by employing longer blanching period of time. Tannin reduced appreciably with increased blanching period.

Tannins are the relatively high molecular weight compounds, which constitute the third important group of phenolics, may be subdivided into hydrolysable and condensed tannins. The hydrolysable tannins are esters of gallic acid (Gallo- and ellagic-tannins), while the condensed tannins are polymers of polyhydroxyflavan-3-ol monomers. A third subdivision, the phlorotannin's consisting entirely of phloroglucinol, has been isolated from several genera of brown algae (Jeffrey, 1989). Tannin, which usually gives rise to a dry, pickery, astringent sensation in the mouth, may also contain antinutritional factors (Oluremi *et al.*, 2007). Tannins have also been reported to cause damage to the intestinal tract, to be toxic after absorption from gut, and to interfere with the absorption of iron. Finally, tannins have been claimed to have carcinogenic effect (Sapkota, 2018).

According to Kim (2013) 20 gm of dried citrus fruit (Citrus unshiu) peel powder was refluxed for 1h in 200ml DW. The obtained extract was cooled and filtered over Whatman No. 1 paper, concentrated under vacuum at 40℃ and lyophilized. Different samples of concentrations, 50, 200, 800, 1600, and 3200 ppm (μg/ml) were prepared and analyzed for tannin. And the results were found ranging from 1.816 to 59.296 μg/ ml.

2.14 Physiochemical properties of lemon peel

2.14.1 Bulk density

Bulk density is a function of particle size, particle size being inversely proportional to bulk density (Onimawo *et al.,* 2012). The differences in the particle size may be the cause of various in bulk density of flours. The bulk density of powders is determined by particle density, which in turn is determined by solid density and particle internal porosity, of the particles in the container. Powders have "loose bulk density", that is, a measured density after a powder is freely poured into a container and "compact density", after it is allowed to compress by mechanical pressure, vibration, and/or impact (Sapkota, 2018). Bulk density is a measure of bulkiness of flour and an important parameter that determines the suitability of flours for the ease of packaging and transportation of particulate foods as well as for infant formulations the low bulk density flour can be used (Nelson-Quartey *et al.,* 2007).

2.14.2 Solubility

Solubility is one of the most important physiochemical and functional properties of protein concentrates. The high solubility of powder indicated potential applications in formulated food systems by providing an attractive appearance and a smooth mouth feel to the product (Kanpairo *et al.*, 2012). Solubility values ranged from 24-171% for all citrus peel samples (Abou-Arab *et al.,* 2017).

2.14.3 Swelling Capacity

Swelling capacity is the volume of expansion of molecule in response to water uptake which it possessed until a colloidal suspension is achieved or until further expansion and uptake is prevented by intermolecular forces in the swelled particle (Houssou and Ayernor, 2002). The swelling capacity of flour granules is an indication of the extent of associative forces with in the granule (Moorthy *et al.,* 1986). The variation in the swelling capacity indicates the degree of exposure of the internal structure of the starch present in the flour to the action of water (Ruales *et al.,* 1993). When starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling and solubility (Garau *et al.,* 2007).

Swelling and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains and also evidence of association bonding within the granules of sorghum starches. The higher the swelling capacity, the lower is the associative forces (Jimoh *et al.*, 2009). The extent of this interaction is influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation (Hoover and Ratnajothi, 2001). The formation of amylose – lipid complexes can restrict swelling and solubilization (Swinkels, 1985). The swelling power was defined by (Balagopalan *et al.*, 2018) as the maximum increase in volume and weight which the starch undergoes when allowed to swell freely in water.

2.14.4 Oil absorption capacity

Oil absorption capacity is attributed mainly to the physical entrapment of oils. It is an indication of the rate at which protein binds to fat in food formulations (Onimawo *et al.*, 2012). The differences in the particle size may be the cause of various in bulk density of flours. The bulk density of powders is determined by particle density, which in turn is determined by solid density and particle internal porosity, of the particles in the container. Powders have "loose bulk density", that is, a measured density after a powder is freely poured into a container and "compact density", after it is allowed to compress by mechanical pressure, vibration, and/or impact (Micha and Peleg, 1983). Fat acts a flavour retainer and increases the mouth feel of foods. Fat increases the leavening power of the baking powder in the batter and improves the texture of the baked product (Isah *et al.*, 2013). The increase in oil absorption capacity of the flour may help to maintain and improve mouth feel, if such flours are used as meat extenders etc.(Onuegbu *et al.*, 2013). Low fat absorption is highly desirable as far as flour product is concerned. This functional property determines the amount of flour to make good dough (Abou-Arab *et al.*, 2017).

2.15 Drying condition of citrus peel

According to Marey and Shoughy (2016), 5 different samples of orange and mandarin peel, fresh and dried at 40℃, 50℃, 60℃, 70℃ in oven drier. Drying was done until moisture content dropped to 5.2% for orange and 5.6% for mandarin peel were analyzed for ascorbic acid. The results for fresh orange were 55.4 ± 0.15 and for 40℃, 50℃, 60℃ and 70℃ it was found to be 51.2 ± 0.2, 40.3 ± 0.25, 30.5 ± 0.12 and 10.8 ± 0.25 respectively. For mandarin,

62.5 ± 0.1, 59.9 ± 0.22, 45.8 ± 0.22, 32.7 ± 0.02 and 13.8 ± 0.22 for fresh, 40℃, 50℃, 60℃ and 70℃ respectively. Vitamin C concentration is reported as micromoles per liter (μmol/L). Both data indicates that, increasing drying temperatures from 40 to 70 °C tends to increase the losses of vitamin C.

Part III

Materials and methods

3.1 Materials

3.1.1 Collection of raw materials

Lemon (*Citrus limon)* of variety "madrasi baramasi" at mature stage were collected and purchased in July 2021, from local market of Dharan, Sunsari.

3.2 Methodology

3.2.1 Preparation of lemon peel powder

The whole peel (flavedo and albedo) was washed with clean water and peel was removed. Four different peel powders were prepared varying the drying temperature of the peel.

Peels were then cut into small pieces ($0.5\times0.5\times0.3$ cm^3), peel was spread in trays and dried by the cabinet drier at different temperature (50℃, 60℃, 70℃). Heat was generated by heaters integrated into the side walls of the oven, and hot air was circulated among the samples by a fan. The oven temperature was controlled by a temperature control dial. After the oven reached the set point, lemon peel was placed on tray and placed in drying chamber. Samples were taken hourly to record mass and moisture loss. The drying continued until the samples reached 5 % w.b by mass balance technique. Additional samples remained in the dryer until they equilibrated at their final moisture content. The experiments were replicated three times.

The dried samples were cooled in a desiccator and then packed in low-density polyethylene bags that were heat-sealed until use. After drying at various temperatures, three replicate 100 g samples each of dried lemon peels were ground using an electric blender and sieved through 40 mesh size for analysis. The prepared LP powder was sealed in polyethylene bags to prevent moisture absorption and stored at 5 ± 1 °C for further study.

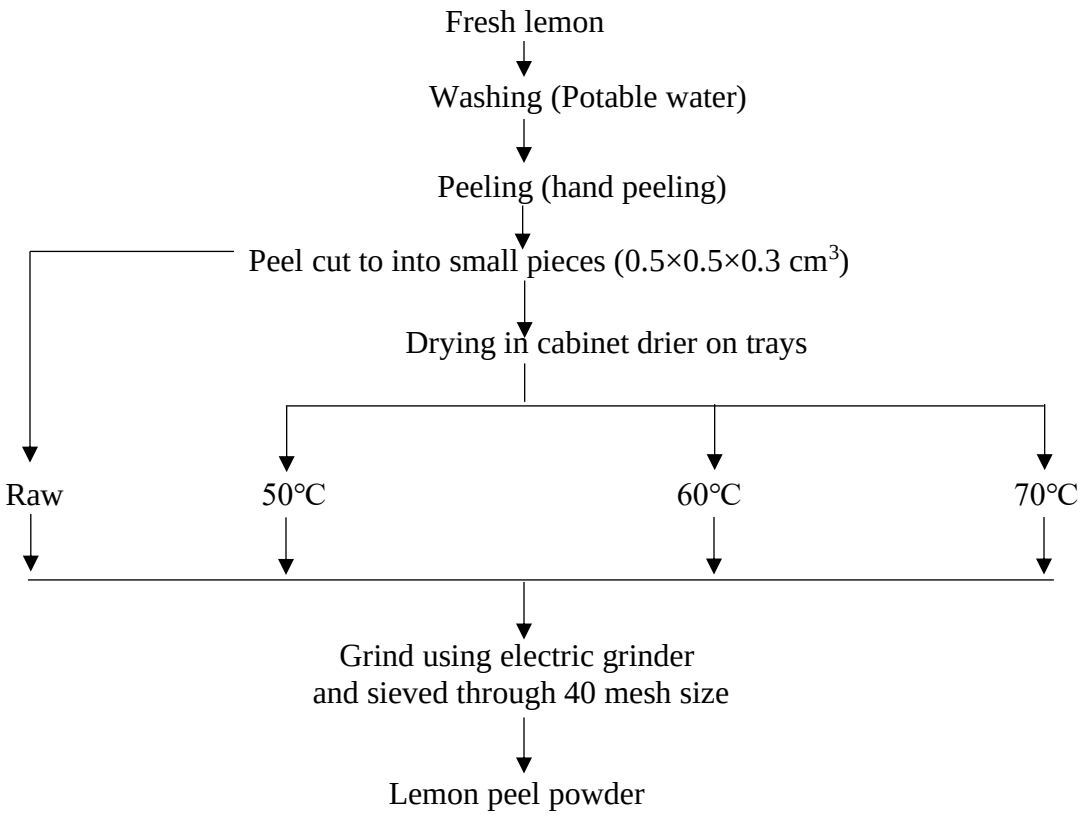

Fig. 3.1 Preparation of lemon peel powder

3.3 Extract preparation

The prepared raw peel and different temperature (50℃, 60℃, 70℃) peel was subjected to extract preparation. The phytochemical extraction was performed using aqueous extraction.

The aqueous extraction was done by taking 5 gm of the peel powder and mixing with 200 ml of distilled water in a beaker. The mixture was then be heated on a hot plate at 30º- 40 ºC and mixed with continuous stirring for 20 min. The mixture was filtered using Whatman filter paper filter and the filtrate obtained was used for the further phytochemical analysis.

3.4 Analysis of physical properties

3.4.1 Bulk density

The bulk density was determined according to the method described in Kanpairo *et al.* (2012) with some modifications. 25 g of sample was gently filled into a dried 50 ml graduated cylinder, tapped gently the cylinder for 25 times. The volume of the powder was recorded. The bulk density was calculated as following relationship.

Bulk density = Weight of powder/ Volume of powder

3.4.2 Solubility

The method of Onuegbu *et al.* (2013) was adopted for determination of solubility. Flour dispersions of samples were prepared by dispersing 1 g of flour is distilled water and made up to 10 ml. It was allowed to settle for 15 min after which 2 ml of the supernatant was transferred using a pipette into a weighed, dry Petri dish. It was evaporated to dryness and reweighed. The total soluble solids (TSS) were then calculated.

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

Where, Vs = Total supernatant/filtrate

Md = Mass of empty Petri dish

Me = Mass of petri dish plus residual solids after evaporative drying

Ms = Mass of flour sample used in the preparation of the dispersion

3.4.3 Swelling Capacity

This was determined with the method described by (Jackson, 1991) with modification for small samples. 1 g of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating,

the suspension was centrifuged at 1000 rpm for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as

Swelling power = weight of the paste / weight of dry flour.

3.4.4 Oil Absorption Capacity

The method described by (Onuegbu *et al.,* 2013) was adopted with slight modification. Each flour samples (1 g) were weighed separately and introduced into clean centrifuge tubes of known weights. Sunflower oil was mixed with the flour in each tube to make up to 10 ml dispersion. The tubes were centrifuged at 3500 rpm for 15 min. The supernatant was discarded and the tube was reweighted. The gain in mass is the oil absorption capacity.

3.5 Analysis of bioactive component

3.5.1 Total polyphenol content

Total phenolic content (TPC) in the lemon peel extracts will be determined using spectrophotometric method which was modified considerably (Abdullakasim *et al.*, 2007). The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$ aqueous solution.

The samples were thereafter incubated in a thermostat at 45 ºC for 45 min. The absorbance will be determined using spectrophotometer at wave length of 765 nm against blank. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The concentration of total phenols was expressed as µg gallic acid equivalents (GAE) per g dry weight of leaf. Gallic acid was used in the construction of standard curve and the linear range used for the calibration curve is 1000–15,000 µg GAE/L.

3.5.2 Flavonoid content

Total flavonoid content was determined using a modified aluminum chloride assay method as described in (Barek *et al.*, 2015). 2 ml of each extract solutions were pipette out in a volumetric flask (10 ml). 0.2ml of 5% $NaNO_3$ was added and stand it for 5 min. Then 0.2 ml of 5% $AlCl_3$

was added, and stand for 5 min. This is followed by the addition of 2 ml of 1N NaOH and volume was made up to 5 ml with DW. Absorbance was measured after 15 min at 510 nm against reagent Blank.

*Blank: All chemicals used for sample preparation except extract

The test results were correlated with standard curve of Quercetin (20, 40, 60, 80 and 100 µg/ml) and the total flavonoid content was expressed as mg Quercetin Equivalents (QE).

3.5.3 Tannin content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin Ciocalteu phenol reagent. 1 ml of 35% $Na2CO_3$ solution was added and then diluted to 10 ml with distilled water.

The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) was prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract (Mythili *et al.,* 2014).

3.5.4 Ascorbic acid

Ascorbic acid was determined by 2,6-dichlorophenol indophenols titration method as per (AOAC, 2005).

3.5.5 Antioxidant capacity

The total antioxidant capacity of peel extracts was analyzed according to the method described by (Klimczak *et al.,* 2007). The tubes containing peel extract (0.3 mL) and 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mMol ammonium molybdate) were incubated at 95°C for 90 min. After the mixture was cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically against a blank. The antioxidant capacity was expressed as ascorbic acid equivalents (AAE).

3.6 Physicochemical analysis of lemon peel

3.6.1 Moisture content

Moisture content were determined by using a hot air oven as per described in Ranganna (1986).

3.6.2 Crude fiber

Crude fiber content of the samples was determined by the method given in Ranganna (1986).

3.6.3 Ash content

Total ash content of the samples was determined by following the method given Ranganna (1986) using muffle furnace.

3.6.4 Oleoresin

Oleoresin of the sample was determined by following the method given in Ranganna (1986) using steam distillation methods.

3.7 Yield of peel from lemon

The percentage yield of peel was determined using weight difference of weight of whole lemons and weight of peel (mainly, epicarp and mesocarp) obtained from the lemons as,

$$\% \text{ Yield of peel from lemon} = \frac{\text{Weight of peel obtained from the whole lemons}}{\text{Weight of whole lemons}} \times 100\%$$

3.8 Data analysis

All the data obtained in this work was analyzed by the statistical program known as GenStat (GenStat Discovery Edition 12, 2009). Means were compared by Tukey HSD test at 5% level of significance to determine whether the sample were significantly different from each other and to determine which one is superior among them. The superscript was assigned to each of them in ascending order with respect to mean value. MS- Excel 2016 was also employed for the general calculations, graph and diagram construction.

Part IV

Results and discussions

This study was conducted to study the effect of drying temperature on bioactive components and physical properties of lemon peel. The lemon peel was subjected to cut into small pieces, peels were spread on trays and dried through cabinet drier at 50°C, 60°C and 70°C until its moisture level becomes 5%. The dried peels were grinded into powder using a grinder and sieved through 40 mesh size. Lemon peel were collected and analyzed for its physiochemical analysis (Bulk density, Solubility, Water absorption capacity, Oil absorption capacity and Swelling capacity) as well as functional properties (antioxidant activity, polyphenol content, flavonoid content).

4.1 Analysis of lemon Peel

4.1.1 Yield of peel from lemon

The yield of peel from lemon was found to be 14.87%.

4.1.2 Proximate analysis of fresh lemon peel

Proximate analysis refers to the quantitative analysis of macromolecules in food. The proximate composition of raw lemon peel was determined. Determined results are presented in Table 4.1

Table 4.1 Proximate analysis of fresh lemon peel

Parameter	Values
Moisture content (%)	84.23 ± 0.98
Crude fiber (% d.b.)	13.67 ± 1.43
Ash content (% d.b.)	4.5 ± 0.78
Oleoresin (% d.b.)	13.67± 2.31

Fresh lemon peel contain moisture content of 84.23% which is similar to study by Ghanem *et al.* (2012) where moisture content of lemon peel was found to be 81.23%.

The crude fibre and ash content were found to be 13.67 % and 4.5 % respectively. Similar results were found by Janati *et al.* (2012) and found to be 15.18 % and 6.26 % respectively. The oleoresin of lemon peel was 13.67 % which are in quite higher than observation of Paw *et al.* (2020).

4.1.3 Effect of drying temperature on bioactive component of Lemon Peel (LP)

The bioactive component assessed were total phenolic content (TPC), total tannin content, total flavonoid content (TFC), antioxidant activity, and ascorbic acid content.

4.1.3.1 Total phenolic content (TPC)

The degradation of phenolics during drying processes could be explained by the fact that, (1) the drying process (different temperature) might destroy some of the phenols and (2) in the dried material, all the components in the cells adhere together in the absence of water, and possibly make the extraction with solvent more difficult, and as a result overall recovery was found to be lower (Ghanem *et al.*, 2012; Li *et al.*, 2006).

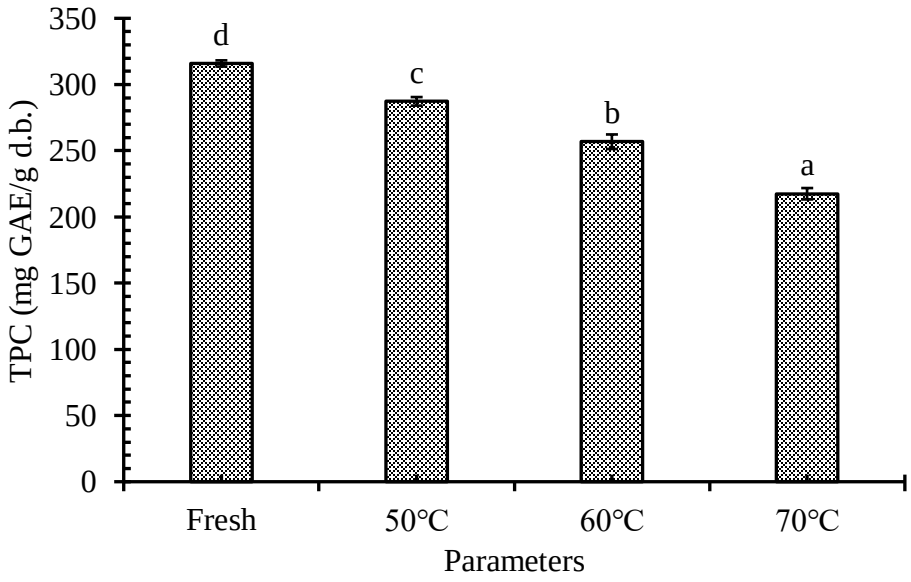

Fig. 4.1 Total phenolic content of lemon peel (TPC)

The polyphenol content of fresh was found higher (315.9 mg GAE/g) compared to another sample given in Fig. 4.1. The statistical analysis shows that polyphenol content significantly decreases ($P<0.05$) with increase in temperature from 50℃ to 70℃. Abdelwahab and Abouelyazeed (2018) illustrated that total phenolic (TPC) amount varied greatly in fresh and dried citrus peels. (Karsheva *et al.*, 2013) found that, fresh lemon peel contained highest level of TPC (3550.6 mg Gallic acid/ 100 g dry weight). The increase in drying temperature leads to a decrease in total polyphenols content due to oxidative degradation and condensation reactions.

4.1.3.2 Total tannin content

Tannins are astringent, bitter polyphenolic compounds. Citrus could serve as source of natural tannin which could be used for a variety of industrial purposes (Wollgast and Anklam, 2000).

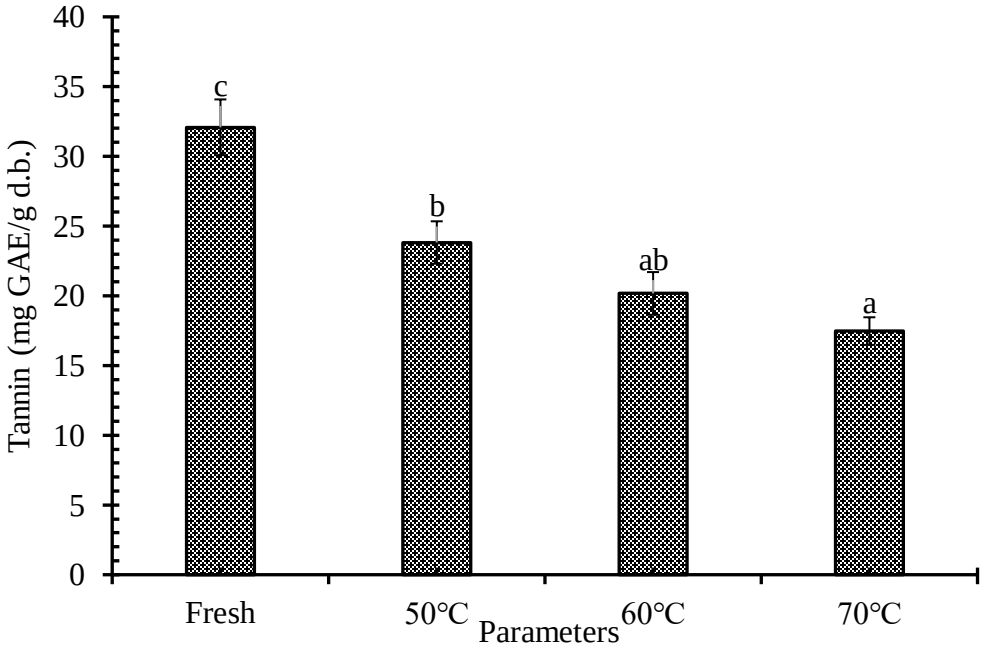

Fig. 4.2 Total tannin content of lemon peel

The content of tannin was significantly higher in fresh i.e. 32.08 ± 2.00 mg GAE per gm of dried peel in dry basis and for 50℃, 60℃ and 70℃ the tannin content were found to be 23.82 ± 1.52, 20.17 ± 1.53 and 17.47 ± 0.99 respectively, as show in Fig. 4.2 which are in accordance with the observation of (Oluremi *et al.*, 2007). From the above data we can see that, increasing temperature from 50 to 70℃ decrease tannin content significantly which may be due to higher

thermal degradation and condensation of tannins at higher temperature. So, in order to minimize loss of tannin, drying peels at low temperature is suitable.

4.1.3.3 Total flavonoid content (TFC)

Citrus plants contain a wide range of flavonoid constituents, some of which are characteristic of them. the highest concentrations of flavonoids in citrus fruit occur in the peel (Nogata *et al.*, 2006).

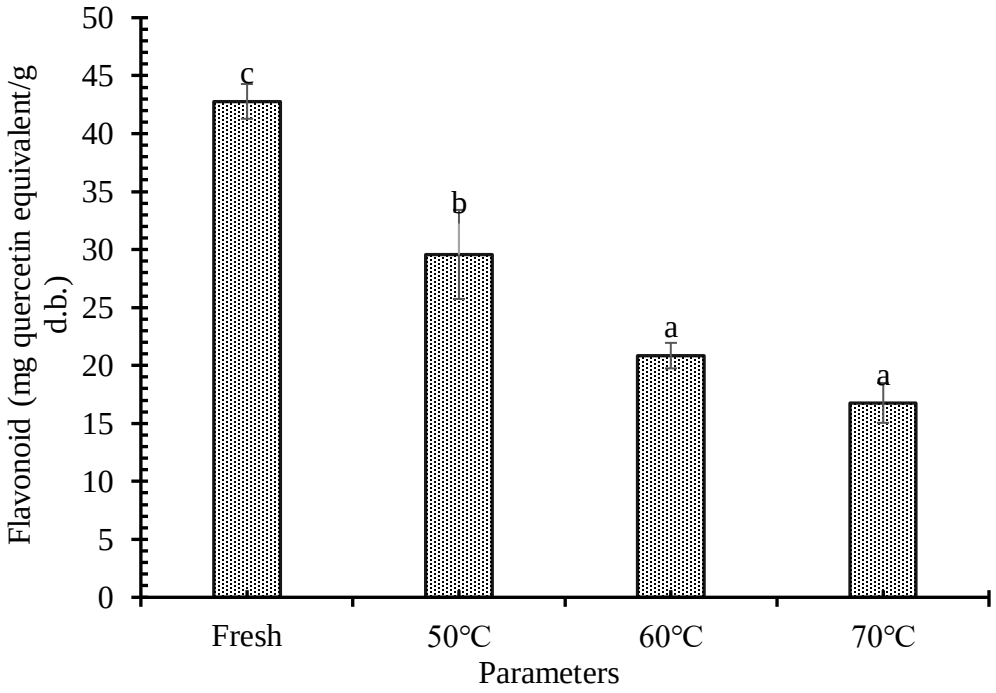

Fig. 4.3 Total flavonoid content of lemon peel (TFC)

TFCs of fresh lemon peel sample was significantly higher than the treated samples i.e., 42.78 ± 1.50 whereas, 70°C had lowest TFC i.e., 16.77 ± 1.72 µg quercetin equivalent/g of dried peel. TFC decreases from 42.78± 1.10 (fresh) to 20.58± 3.84 and 20.85± 1.10 at 50°C and 60°C, respectively. With regard TFC content, data of LP indicated that increase in temperature decreases the TFC content which is due to Flavonoids being heat sensitive degrade during hot air drying (Chaaban *et al.*, 2017). At temperature 60 and 70°C, no significant difference in flavonoid content of treated peel was found. As can be seen in Fig. 4.3 all drying conditions decreases of total flavonoids of lemon peel compared to fresh one. Similar results were found

by Mhiri *et al*. (2018) on *Citrus limon* peel cv. *Lunari*, convective dried at 60 ◦C, during 97 min. So, in order to prevent the loss or degradation of flavonoid the peels need to be dried at low temperature.

4.1.3.4 Antioxidant activity (DPPH radical scavenging activity)

The final antioxidant activity was assessed in prepared lemon peel using DPPH radical scavenging activity and UV-vis spectrophotometer at 765 nm. The data for DPPH (%RSA) of produced fermented products are given in Fig. 4.4.

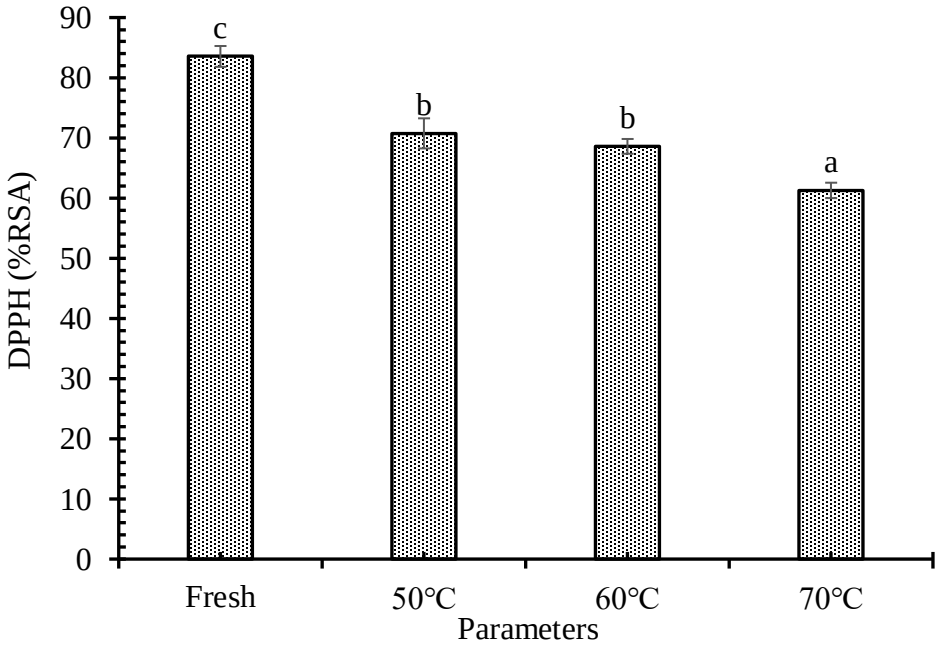

Fig. 4.4 Antioxidant activity of lemon peel

The antioxidant activity as DPPH % Radical Scavenging Activity (RSA) of sample fresh, 50℃, 60℃, 70℃ were found to be 83.54 ± 1.73, 70.75 ± 2.50, 68.59 ± 1.23 and 61.27 ± 1.28% respectively. The result in Fig 4.4 shows that for fresh peel the antioxidant activity was highest among all the samples. Antioxidant activity decreased significantly ($P<0.05$) after drying of peels and increasing temperature decreased the antioxidant activity from 50 to 70℃ as stated by Réblová (2012), although, there was no significant difference at temperature 50 and 60℃.

Jeong *et al.* (2004), also found that, antioxidant activity of citrus peel extracts gets significantly affected by heating temperature and duration of treatment on citrus peel. Polyphenol is one of the most important groups of phytochemical antioxidants in lemon fruit (Xi *et al.*, 2017). Also, ascorbic acid and its esters function as antioxidants (Cort, 1982). The effect of temperature on polyphenol and ascorbic acid in the dried lemon peel was found to be decreasing. Hence, drying peels at low temperature significantly reduce the loss of antioxidant activity.

4.1.3.5 Ascorbic acid content

The ascorbic acid content in fresh peel was found to be highest among 4 samples i.e., 83.22 ± 1.07 mg/100 g dry matter and lowest for samples dried at 70℃ i.e., 55.24 ± 3.51 mg/100 g dry matter as shown in Fig. 4.5. The data found indicates that drying and increasing the temperature significantly decreases the ascorbic acid content of the peel. But for sample dried at 50 and 60℃ there is no significant difference among the results. Similar result was found by Abou-Arab *et al.* (2016) and Fernández-López *et al.* (2004). This could be attributed to the fact that ascorbic acid is not stable at high temperature (Negi and Roy, 2000). To minimize the loss of ascorbic acid low temperature drying is suitable.

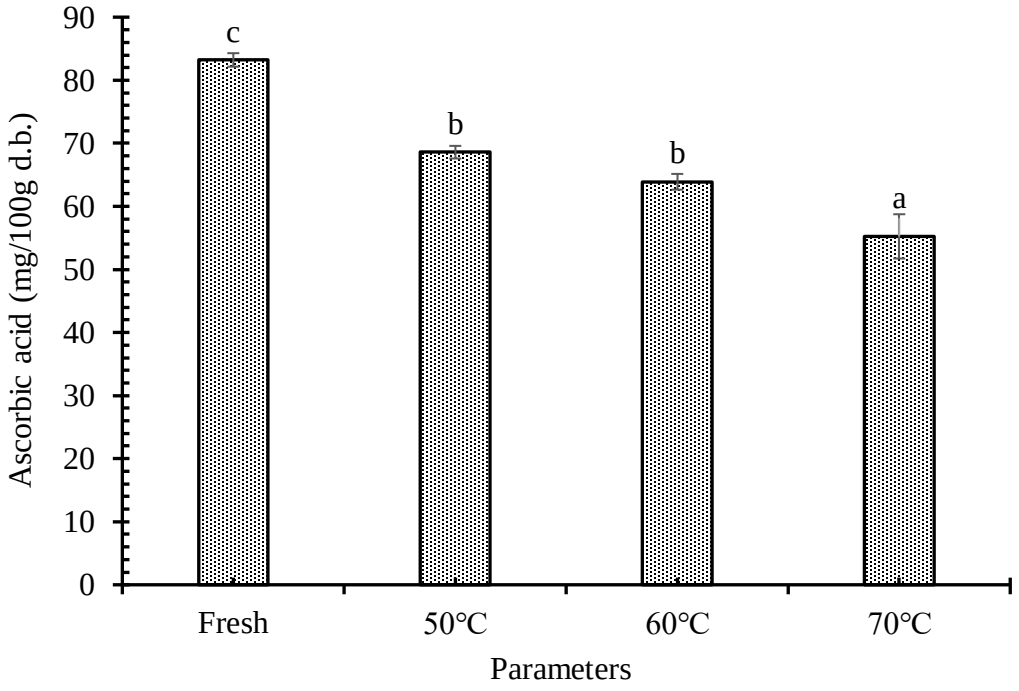

Fig. 4.5 Ascorbic acid content of lemon peel

4.1.4 Effect of drying temperature on physical properties of lemon peel

A physical property is a characteristic of a substance that can be observed or measured without changing the identity of the substance. Physical properties of lemon peel assessed were bulk density, solubility, oil absorption capacity and swelling capacity.

4.1.4.1 Bulk density

The bulk density of 70℃ had highest bulk density i.e., 0.4450±0.005 as shown in Table 4.2. At 50℃ no significant difference with control. But fresh and 60℃, fresh and 70℃, 50℃ and 60℃, 50℃ and 70℃ are statistically different whereas rest sample are not significantly different. It is seen that increase in temperature tends to increase the bulk density.

Table 4.2 Bulk Density (g/ml) of raw and different temperature LP

Parameters	Bulk Density (g/ml)
Fresh	0.3850 ± 0.005^{a}
50℃	0.4050 ± 0.005^{a}
60℃	0.4300 ± 0^{b}
70℃	0.4450 ± 0.005^{b}

All data are the mean ± Standard Deviation of triplicate. Means having similar superscripts in a column are not significantly different.

4.1.4.2 Solubility

Sample fresh had highest solubility (28.24%) significantly different to other samples. The general trend of the results shown in table 4.3 indicated that an inverse relationship was existed between the solubility temperature and the solubility index where, greater the degree of treatment used for solubility temperature, the less the value of solubility coefficient. That decrease in solubility did not only due to the degradation of pectic substances during processing but also caused by modification of structure affected to these polymers during the removal of water. These results are in agreement with those reported by (EA Abou-Arab, 2017)

Table 4.3 Solubility (%) raw and different temperature LP

Parameters	Solubility (%)
Fresh	28.24±0.11[d]
50℃	26.20±0.05[c]
60℃	21.15±0.15[b]
70℃	18.85±0.65[a]

All data are the mean ± Standard Deviation of triplicate. Means having similar superscripts in a column are not significantly different.

4.1.4.3 Oil Absorption Capacity (OAC)

70℃ had highest oil absorption capacity i.e., 3.955 ± 0.005 but 50℃ and 60℃ and 70℃ are not significantly different. The mechanism of fat absorption is mainly concerned with the physical entrapment of oil and binding of fat to the a polar chain of protein (Wang *et al.*, 1976). The oil absorption capacity of citrus peel powder may be related to their fiber content and the higher bulk densities of samples. These results were agreed EA Abou-Arab (2017).

Table 4.4 Oil Absorption Capacity (OAC) ml/g of raw and different temperature LPP

Parameters	Oil Absorption Capacity (OAC) ml/g
Fresh	1.785±0.075[a]
50℃	3.850±0.01[b]
60℃	3.920±0.01[b]
70℃	3.955±0.005[b]

All data are the mean ± Standard Deviation of triplicate. Means having similar superscripts in a column are not significantly different.

4.1.4.4 Swelling Capacity

70℃ sample had higher swelling capacity (6.765±0.185) g/g. The result in table 4.5 shows that swelling capacity increases with increase in temperature. 50℃ and 60℃ and 60℃ and 70℃ are not significantly different. That behavior of SWC may be due to fiber content of peel. The structural characteristics and the chemical composition of the fiber (its water affinity of components) is playing a significant role in the kinetics of water uptake. These results were in agreement with that reported by Garau *et al.* (2007).

Table 4.5 Swelling Capacity (SWC) g/g of raw and different temperature LPP

Parameters	Swelling Capacity (SWC) g/g
Fresh	3.600 ± 0.1^{a}
50℃	5.450 ± 0.13^{b}
60℃	6.215 ± 0.135^{bc}
70℃	6.765 ± 0.185^{c}

All data are the mean ± Standard Deviation of triplicate. Means having similar superscripts in a column are not significantly different.

Part V

Conclusion and Recommendation

5.1 Conclusions

Based on the results of this study, the primary conclusions can be summarized as follow:

1. Increase in temperature decreases the bioactive component, i.e., in 70℃ the TPC, TFC and antioxidant scavenging activity of lemon peel were found to be reduced by 31%, 61% and 26% respectively likewise, tannin and ascorbic acid content were found to be reduced by 46% and 36% respectively.

2. The drying temperature range of 50-60°C has no significant effect on bioactive components of dry lemon peel powder.

3. Physical properties of lemon peel; bulk density, OAC and SWC were increased by 15.8%, 121% and 87% respectively but solubility index was reduced with increase in temperature range of 50-70°C by 33.25%.

5.2 Recommendations

This study can be further continued with the following recommendations,

1. Low temperature can be used for better retention of bioactive component on drying lemon peel.
2. Further study can be conducted to study the changes at different drying methods and temperature ranges.

Part VI

Summary

Lemon peel is a by-product obtained from juice processing industries which is a type of citrus fruits (*Citrus limon*). Despite of potential sources of functional components and various therapeutic uses lemon peels are still not utilized in the juice industries as well as in home scale in our country. Lemon peel is a good source of bioactive compounds such as polyphenols, carotenoid, vitamins and dietary fibers.

The bioactive component of four different lemon peel powder (fresh and dried at 50, 60 and 70℃) were analyzed. The drying continued until the samples reached 5 % w.b. by mass balance technique. The bioactive component such as antioxidant activity (%), polyphenol content (mg GAE/g), Flavonoid content (mg GAE/g), Tannin (mg GA/100 g), Ascorbic acid (mg/100 g) of control, 50, 60 and 70℃ peel powder were analyzed. Also, physical properties such as Bulk Density (g/ml), Solubility (%), Oil Absorption Capacity (ml/g), Swelling Capacity (g/g) of same powder were analyzed.

The results showed that oven drying method at 50°C indicated less reduction on bioactive component. On the other hand, this study also evaluated the physiochemical properties of same samples. The results indicated that the increase in temperature increases the physiochemical properties such as Bulk density by 15.8%, Oil absorption capacity by 121% and Swelling capacity by 87% but inverse relationship was existed between the solubility temperature and the solubility index. Solubility index was reduced with increase in temperature by 33.25%.

Likewise, increase in temperature decreases the bioactive component, i.e., in 70℃ the TPC, TFC and antioxidant scavenging activity of lemon peel were found to be reduced by 31%, 61% and 26% respectively whereas tannin and Ascorbic acid content were found to be reduced by 46% and 36% respectively. All analyzed samples showed that low temperature drying method is the best method for obtaining higher bioactive component.

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Appendices

Appendix A

Equipment and utensils

i. Muffle furnace

ii. Water bath (Intake Serological Water Bath)

iii. Weighing balance

iv. Heating mantle

v. Grinder

vi. Standard sieve

vii. Cabinet dryer

viii. Refrigerator

ix. Hot air oven

x. Spectrophotometer

Chemical used

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

ii. Oxalic acid (Qualigens, Assay 99.5%)

iii. Indicators (Methyl blue, Phenolphthalein)

iv. Absolute Alcohol (Bengal Chemicals and pharmaceuticals)

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

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

vii. Folin-Ciocalteuphenol reagent (FC reagent)

viii. $Alcl_3$

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

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

Appendix B

ANOVA result for analysis for different parameter of lemon peel

Table B.1 One way ANOVA (no blocking) for Polyphenol content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	16013.68	5337.89	330.31	<.001
Residual	8	129.28	16.16		
Total	11	16142.96			

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

Table B.2 One way ANOVA (no blocking) for Flavonoid content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	1192.175	397.392	74.94	<.001
Residual	8	42.423	5.303		
Total	11	1234.598			

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

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

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	363.475	121.158	49.99	<.001
Residual	8	19.388	2.424		
Total	11	382.863			

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

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

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	773.530	257.843	82.79	<.001
Residual	8	24.916	3.115		
Total	11	798.447			

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

Table B.5 One way ANOVA (no blocking) for Ascorbic acid content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	1234.622	411.541	102.48	<.001
Residual	8	32.126	4.016		
Total	11	1266.748			

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

Table B.6 One way ANOVA (no blocking) for Bulk density

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	0.00473333	0.00157778	23.67	<.001
Residual	8	0.00053333	0.00006667		
Total	11	0.00526667			

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

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

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	204.0645	68.0215	79.15	<.001
Residual	8	6.8750	0.8594		
Total	11	210.9395			

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

Table B.8 One way ANOVA (no blocking) for Swelling capacity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	20.44622	6.81541	257.35	<.001
Residual	8	0.21187	0.02648		
Total	11	20.65809			

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

Table B.9 One way ANOVA (no blocking) for Water absorption capacity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	60.35847	20.11949	727.65	<.001
Residual	8	0.22120	0.02765		
Total	11	60.57967			

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

Table B.10 One way ANOVA (no blocking) for Oil absorption capacity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
Temperature	3	10.38482	3.46161	191.51	<.001
Residual	8	0.14460	0.01807		
Total	11	10.52942			

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

Appendix C

1. Standard curve for total phenolic determination.

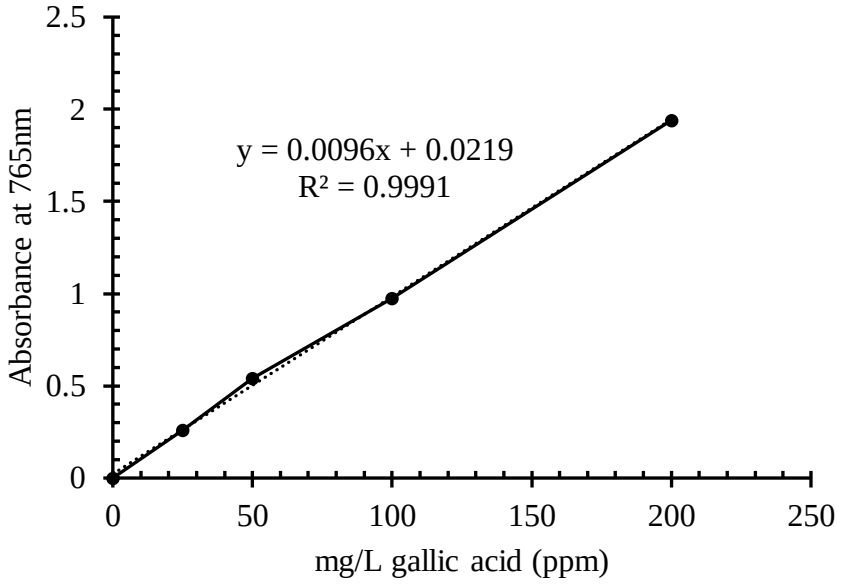


Fig. B.1 Standard curve for total phenolic content determination

2. Standard curve for Flavonoid determination.

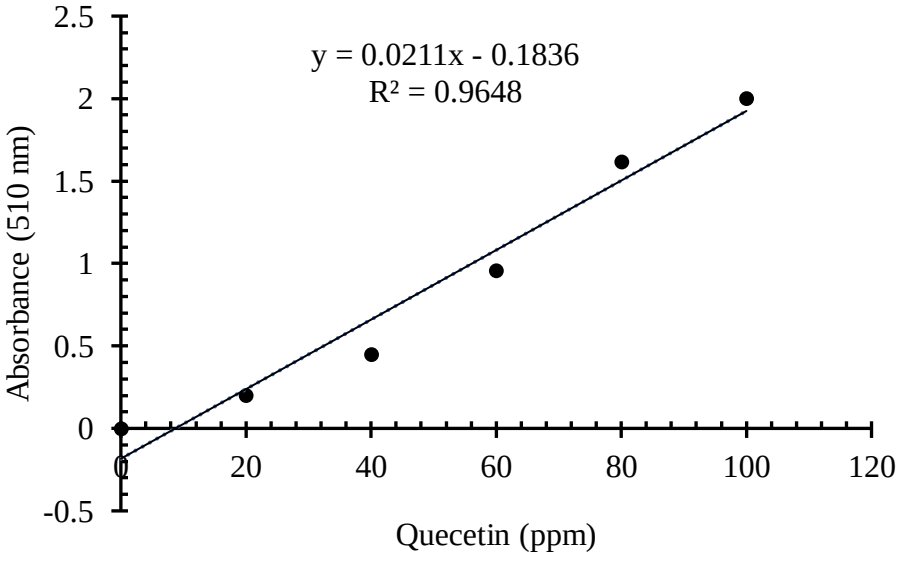


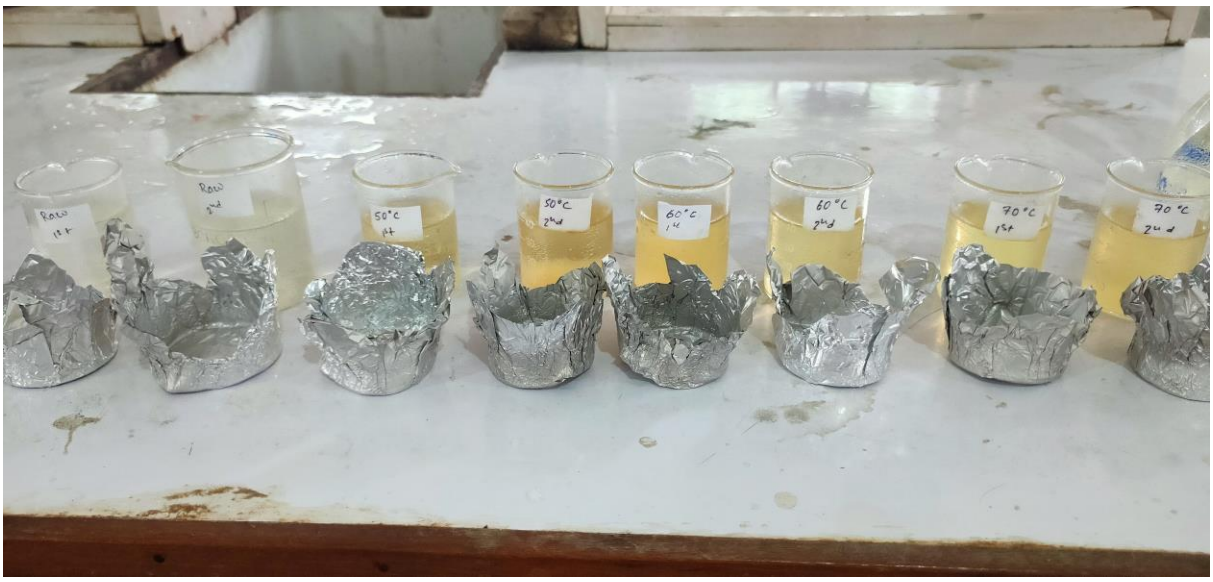
Fig. B.2 Standard curve for flavonoid determination

Photo Gallery

Fresh lemon

Drying of lemon peels

Dried lemon peel powder



Water extract of lemon peel powder (samples)