EFFECT OF STORAGE TEMPERATURE ON THE PHYSICOCHEMICAL, NUTRITIONAL AND MICROBIOLOGICAL PROPERTIES OF COLD-PRESSED ORANGE JUICE

By

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ABSTRACT

EFFECT OF STORAGE TEMPERATURE ON THE PHYSICOCHEMICAL, NUTRITIONAL AND MICROBIOLOGICAL PROPERTIES OF COLD-PRESSED ORANGE JUICE

Chin Man Fai

Orange, scientifically known as *Citrus Sinensis*, is valued for its juice products and has a high-water content. This delicious fruit has a tantalizing flavor, a fragrant smell, and an eye-catching flesh hue. It also contains a ton of phytochemicals and antioxidants, which have a variety of positive effects on human health. Orange juice is a preferred beverage for many owing to its accessibility. Nevertheless, there is limited information available on the influence of temperature changes on the nutritional value and health-promoting qualities of juice. This study aims to examine the impact of storing cold-pressed orange juice at two different temperatures, room temperature (24°C) and refrigerator cold (4°C), for 12 days on its physicochemical (total soluble solids, color, browning index, pH, titratable acidity, and viscosity), nutritional (ascorbic acid content, total phenolic content, and total antioxidant capacity), and microbial properties. According to the results of the study, orange juice stored at 4°C had no detrimental effects on its quality, nonetheless, orange juice stored at 24°C significantly decreased in pH, viscosity, and total soluble solids.

Likewise, at 24°C, titratable acidity significantly increased and nutrients like ascorbic acid and phenolic content degraded more quickly. The proliferation of microorganisms can have a negative impact on the overall quality of the juice, leading to its significant deterioration of orange juice as a result of the higher temperature (24°C). Therefore, freshly cold-pressed orange juice should be refrigerated at 4°C for optimum nutritional value preservation and shelf life.

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DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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APPROVAL SHEET

This final year project report entitled **"EFFECT OF STORAGE TEMPERATURE ON THE PHYSICOCHEMICAL, NUTRITIONAL AND MICROBIOLOGICAL PROPERTIES OF COLD-PRESSED ORANGE JUICE"** was prepared by CHIN MAN FAI and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Dietetics at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **CHIN MAN FAI** (ID No: **20ADB03024**) has completed this final year project report entitled "EFFECT OF STORAGE TEMPERATURE ON THE PHYSICOCHEMICAL, NUTRITIONAL AND MICROBIOLOGICAL PROPERTIES OF COLD-PRESSED ORANGE JUICE" under the supervision of Dr. Tan Chin Xuan from the Department of Allied Health Sciences, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project report in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(CHIN MAN FAI)

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LIST OF ABBREVIATIONS

°Brix	Degree Brix
BI	Browning Index
CFU	Colony Forming Units
CUPRAC	Cupric Reducing Antioxidant Power
DCPIP	2,6-Dichlorophenolindophenol
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid
HORAC	Hydroxyl Radical Antioxidant Capacity
M	Molarity
M.E	Equivalent Weight
mP.a.s	Millipascal-Second
N	Normality
n	Number
NFC	Not From Concentrate
PCA	Plate Count Agar

PG	Polygalacturonase
PMG	Pectin Methylesterase
rpm	Revolutions Per Minute
RSA	Radical Scavenging Activity
RTD	Ready To Drink
TA	Titratable Acidity
TE	Trolox Equivalents
TPC	Total Plate Counts
TRAP	Total Radical-Trapping Antioxidant Parameters
TSS	Total Soluble Solids

CHAPTER 1
INTRODUCTION

1.1 Research Background

Citrus Sinensis, or orange, is a member of the *Rutaceae* or *Rue* family and is well-recognized as the world's primary fruit crop grown in many different countries with tropical climates. The biggest orange-producing nations include Brazil, China, Japan, Mexico, Pakistan, the United States, and various Mediterranean countries. Oranges consist of two unique anatomical regions: the pericarp (peel or rind) and the endocarp or pulp that contains juice sac glands. Orange skin is made up of the epidermis and epicuticular wax, along with numerous small oil glands that produce a unique aroma. The pericarp of the orange consists of two parts: the outer flavedo or epicarp, which comprises mainly parenchymatous cells and cuticles, and the albedo or mesocarp underneath the flavedo, which consists of tubular-like cells that are tightly packed into the intercellular space. Within the orange, there is normally a pleasant pulp and many to countless seeds. The orange pulp is normally made up of 11 segments of a juice sac that range in flavor from sour to sweet (Ould Yerou et al., 2017).

According to Statista research, cold-pressed orange juice (64.4 million liters) is being consumed the most in Malaysia, followed by apple juice (24.4 million liters), grapefruit juice (18.2 million liters), and grape juice (9.6 million liters) in the year

2022 (Statista, 2022). Fresh fruit juices are aqueous liquids collected from fruit tissue, which are normally extracted manually or mechanically (Kaddumukasa et al., 2017). Numerous studies indicate a correlation between diets rich in fruits and vegetables and reduced risk factors for major chronic illnesses in humans, including cardiovascular disease, several types of cancer, and age-related degeneration (Khaksar et al., 2019). Pure (100%) fruit juices are strongly recommended by health promotion standards and dietary requirements. Ruxton et al. (2009) concluded that pure fruit juices (without any additional components, such as sweeteners) preserve the key health-promoting elements of whole fruit (Khaksar et al., 2019).

Pediatricians suggested fruit juice from a health standpoint as an additional supply of water and a source of vitamin C for healthy newborns and youngsters when their diets grew to incorporate solid meals with higher kidney solute levels (Ibrahim, 2016). Fruit juices support a healthy diet in a variety of ways, including maintaining a healthy gastrointestinal system and battling infections and disorders (Ibrahim, 2016). Fruit juice has the capacity to increase immune system activity, delay the effects of aging, prevent malignancies, speed up cellular repair and metabolism, cleanse the body, maintain blood pressure, lessen inflammation, and reduce cholesterol levels (Ibrahim, 2016). Due to these nutritional advantages, customers now regularly drink fresh fruits and vegetable juices, which has a favorable influence on their health (Kaddumukasa et al., 2017). Although its shelf life stored under refrigerators has been known to be short, freshly extracted juice can be drunk right away or kept refrigerated for later use (Kaddumukasa et al., 2017).

Cold-pressed fruit juices are extracted from the fruit at a very low speed, cold-pressed extractors. First crushed and then pressed the fruit. This method of extraction almost eliminates heat production while maintaining the fruit juice's nutritious value (Khaksar et al., 2019). The raw fruit juice preserves a ton of nutrients during the cold-pressing process for maximum health benefits and a wonderful flavor. The two-stage cold-press juicing procedure employs a hydraulic cold pressing to produce nutritional juice from fruits and vegetables. This is because there is no high speed or heat involved, oxidation is reduced and the juice's integrity, texture, flavor, and quality are preserved. The juice's shelf life can be increased by two to three times using this procedure. The fruit is first chopped, chewed, and then ground into a soft pulp by the machine. The pulp is then sandwiched between two icy solid surfaces. The pulp is subjected to extremely high hydraulic pressure by the machine, which squeezes and extracts the juice steadily while leaving the fiber behind. Fruit juice that has been cold-pressed is very rich in vitamins, minerals, and enzymes. It has almost minimal pulp remnant, making it easy to digest (Khaksar et al., 2019).

1.2 Problem Statements

Fruits may be utilized more effectively by being juiced since the juice can be transported and stored more readily. However, the extraction procedure may result in microbial contamination, which may shorten the extracted fruit juice's shelf life. Thermal processing techniques, such as pasteurization, can be employed to increase the shelf life of extracted fruit juice by reducing the extent of microbiological contamination and preventing other enzymatic and non-enzymatic processes that contribute to spoilage. However, because not all microbes and enzymes are eliminated by pasteurization, the juice's quality may vary while it is being stored (Jerry and Bright, 2019). Some of these modifications could have an impact on the juice's flavor and color, which would lower the juice's sensory acceptability. During storage, several bioactive substances, such as ascorbic acid, may also degrade, lowering the juice's nutritional value. It is also possible for bacteria to revive and thrive while being stored. As a result, changes that take place during storage are often affected by the storage temperature (Jerry and Bright, 2019).

The skin and cell walls of minimally processed fruits and vegetables are exposed, and the liquid contents are easily polluted by environmental air and microbes (Kaddumukasa et al., 2017). Freshly extracted juice derived from fruits and vegetables is very prone to deteriorating physicochemical properties, which causes customers to reject the product. Juice production requires a lot of human manipulation, which has been shown to contaminate the final product with microbes (Kaddumukasa et al., 2017). The harmful or spoilage bacteria might come from healthy food handlers or other sources (Ruxton et al., 2009). Food handlers have transitory bacteria, especially *Staphylococcus aureus*, living on their throats, nasal passages, hair, and skin (Ruxton et al., 2009). When no protective clothing is worn when handling food, bacteria will get into the fruit juice during manufacturing (Ruxton et al., 2009). Moreover, bacteria are also transferred into fruit juice as a result of frequent hand immersion in fruit juice, which causes pain and skin damage that leads to sores (Ruxton et al., 2009). Particularly when food workers use their bare hands, wounds are attractive breeding grounds for bacterial infections. These viruses come into touch with juice when food workers prepare it with their bare hands. When damp hands are used during food preparation, physical contact facilitates the spread of bacteria (Ruxton et al., 2009). Additionally, bacteria are spread by direct contact with human or animal excrement or indirectly through polluted water, soil, or processing equipment, which can cause the juice to deteriorate (Ruxton et al., 2009). On an even more severe side, contamination may also be hazardous to the consumer's health.

Fruit juice biodegradation is further influenced by physicochemical factors such as storage temperature, pH, total soluble solids, color, titratable acid, total phenolic content, and ascorbic acid level (Kaddumukasa et al., 2017). The development and proliferation of bacteria in food items are facilitated by high temperatures, which increase metabolic processes, degradation, and spoiling of the food items and reduce storage stability or shorten the shelf life of the fruit juice. In order to prevent the growth of bacteria, spore germination, and potential toxin production to lethal levels, food items need to be kept in the refrigerator at 0 – 4°C (Kaddumukasa et al., 2017). The pH should be raised from 6 to 7 for mesophilic bacteria to flourish at their best. Increased bacterial populations cause metabolic byproducts to build up, which in turn causes fruit juices to biodegrade and possibly deteriorate. Due to exposure to temperature variations during storage, essential elements including antioxidants, vitamins A, C, and E, and phytonutrients are damaged, resulting in shorter shelf life for the food product. Consumers will not purchase food products that have degraded owing to deteriorative reactions that reduce shelf life, like, microbial spoilage, the development of bad tastes, or changes in color, texture, or appearance (Kaddumukasa et al., 2017). Based on the current understanding and available information, there is no information on the nutritional quality of freshly cold-pressed orange juice that takes into account storage conditions.

The impact of storage conditions, particularly temperature and time period, on the nutritional quality of fruit juices is another significant aspect related to the quality of fruit juices. Some bioactive substances, like vitamin C content, total phenolic content, and total carotenoid content, may degrade during storage, which is crucial for the quality of fruit juices. The shelf life or expiry date of beverages is typically a finite time span during which juices retain most of their physicochemical features. However, it is unclear at this time how storage conditions impact bioactive qualities like antioxidant capacity and how long they will remain stable. A few researches have examined the impact of storage conditions on the antioxidant capacity and bioactive components of various fruit juices in order to solve this problem (Khaksar et al., 2019). Based on the information currently available to us, it appears that there is no information on the nutritional value quality of freshly cold-pressed orange juice that takes into account storage conditions.

1.3 Significance of Study

The purpose of this study is to identify the ideal storage conditions (temperature and time period) for cold-pressed orange fruit juices that would successfully preserve their original vitamins and sufficient levels of organic acid and serve as the foundation for public recommendations. Additionally, the results of this study will raise consumer knowledge of the bioactive compound and antioxidant content of fruit juices as well as the impact of room temperature and refrigerated storage on the quality of the orange fruit juice beverage.

1.4 Objectives

1.4.1 General Objective

This study, therefore, seeks to investigate the effects of physicochemical, nutritional, and microbiological properties of freshly cold-pressed orange juice (*Citrus Sinensis*) during storage at room temperature (24°C) and refrigerator cold (4°C) for 12 days.

1.4.2 Specific Objectives

The specific objectives are as follows:

i. To determine the physicochemical changes (total soluble solids, color, pH, and titratable acidity) of the cold-pressed orange juices during storage at room temperature (24°C) and refrigerator cold (4°C) for 12 days.

ii. To determine the nutritional changes (ascorbic acid content, total phenolic compound, and total antioxidant capacity) of the cold-pressed orange juices during storage at room temperature (24°C) and refrigerator cold (4°C) for 12 days.

iii. To determine the microbiological changes of the cold-pressed orange juices during storage at room temperature (24°C) and refrigerator cold (4°C) for 12 days.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of Fruit Juice and Its History

Fruit juice is a natural liquid that is extracted mechanically from ripe fruits without the use of heat or a solvent. It is defined as an extractable fluid content of the fruit cells or tissues (Rajauria and Tiwari, 2018). According to the Food and Drug Administration (2004), the definition of fruit juice is an aqueous liquid that is extracted from one or multiple fruits, as well as any purees made from the edible sections of those fruits (FDA, 2004). Juicing has been a healthy practice for thousands of years, despite the misconception held by some that it is merely another diet trend. Juice consumption by humans has been documented as long back as 150 B.C. (Garden of Flavor, 2021). Writings from an Israelite tribe that crushed figs and other fruits to get juices from them may be found among the Dead Sea Scrolls. These people thought that fruit juice had healing properties and provided them with strength. This theory is supported by contemporary science, and fruit juice is widely consumed nowadays due to its high antioxidant, potassium, and vitamins C, E, and K content (Juicernet, 2022).

Juicing was founded in the 1930s when Dr. Norman Walker created the first commercial juicer, although mashing or grinding fruits for their therapeutic benefits is an age-old tradition. The juice was extracted by the enormous yet powerful

Norwalk machine using a hydraulic press (Juicernet, 2022). This device made juicing broadly accessible, made juicing more easily accessible to customers as well as consumers, and increased demand for freshly squeezed fruit juice (Garden of Flavor, 2021; Juicernet, 2022).

During the next decades, juicing machines are constantly developing all around the world. Masticating, centrifugal processors, and juicers for both domestic and commercial usage were all developed in the 1950s. In the late 1900s, juicing became more popular (Garden of Flavor, 2021). The health advantages of juicing grew as juice equipment became more widely available (Juicernet, 2022). Professional dietitians and entrepreneurs of juicing businesses started promoting fresh fruit juice and all of its advantages to human health (Juicernet, 2022). Juicing pioneers like Dave Otto, who founded the Beverly Hills Juice Club in 1975, gravitated toward California, the state where the Norwalk was invented (Garden of Flavor, 2021). Early juice bar franchises like Jamba Juice, which began in the 1990s as local juice bars grew, helped juice bars into the national mainstream (Juicernet, 2022).

As for cold press juicing, its roots may be found in cider and juice pressing, both of which have been there since the Roman era. Despite being manufactured for many years, cold-pressed fruit juice became increasingly popular during the juice "cleansing" trend, growing into a larger industry in the early 21st century (Betty Hallock, 2013). Hydraulic pressure has recently taken the position of hand and foot press, and several American and European manufacturers now produce cold-pressed juice. These presses function by sandwiching two metal plates with a cotton bag containing fruit that has been "chipped." Just below the squeezing region, there is a stainless-steel bowl where the juice is collected. Afterward, the cold-pressed juice is then packaged and made ready for human consumption (Cruz The Juice Ltd, 2019).

2.2 Type or Varieties of Oranges

The sweet orange (*Citrus sinensis*) is a type of cultivar of the citrus fruit and is a cross between the mandarin (*Citrus reticulata*) and the pomelo (*Citrus maxima*). Orange trees may yield both bitter and sweet types of orange fruit. Orange fruits are renowned for having high vitamin C content, whereas orange oil for household cleaning and orange zest for salad dressing are just a few uses for orange peels. Oranges have their roots in a variety of places, including southern China, portions of Southeast Asia, and even sections of India. Today, orange trees may be found all over the world in both subtropical and tropical regions. The top three orange-producing countries worldwide are Brazil, China, and India. Florida and California have the most industrial orange orchards in the United States (Mashama Bailey, 2022). Around 1450, Italian traders or Portuguese traders brought the orange fruit to the Mediterranean. To a certain extent, oranges were mostly used for therapeutic purposes, however, affluent nobles quickly discovered how delicious and aromatic the fruit was and began consuming it for themselves (Amy Grant, 2021).

Sweet oranges (*Citrus sinensis*) and bitter oranges (*Citrus aurantium*) are the two fundamental types of oranges. There are four categories of sweet oranges, each with its characteristics: Blood or pigmented orange – There are two varieties of blood oranges: pale blood oranges and deep blood oranges. *Citrus sinensis* naturally mutated to produce blood oranges. The rich deep red color of the whole fruit is due to high levels of anthocyanin. Blood orange is a rather sour orange compared to other popular types, yet it has a distinctive flavor that resembles oranges with raspberries (Mashama Bailey, 2022; Edible Arrangements News, 2022). October through May is when the blood orange is in season, but February and early March are when it is at its best (Bailey Fink, 2022). The orange fruit cultivars in the blood orange group include Tarocco, Scarlet Navel, Sanguinelli, Moro, and Maltese (Amy Grant, 2021). Navel orange – The most popular orange available in supermarkets and of significant commercial importance is the navel orange. Because of its sweet flavor, low acidity, high vitamin C content, and seedlessness, this common orange is adored by many consumers. Navel oranges are distinguished by their thick skins and pith. The navel orange's flesh is delicious whether added to fruit salads or simply eaten by itself (Mashama Bailey, 2022). From November through June, navel oranges are in season, with January and February being the prime months (Bailey Fink, 2022). The Bahia, Cara cara, California Navel, Dream navel, and Late Navel, or Washington are the most prevalent forms of navel oranges (Amy Grant, 2021). Common orange – The common orange is grown worldwide and comes in a variety of forms. Around two-thirds of oranges produced worldwide are common oranges, and the majority of those common oranges are used to make orange juice (Ryan King et al., 2021). There are dozens of different types of common oranges, but Hamlin, Hart's Tardiff Valencia, and Valencia are the most popular (Amy Grant, 2021). Acid-less orange – Acid-less oranges possess a very mild flavor taste because they are lacking acid. Early-season fruit known as acid-less oranges also goes by the names of Sweet oranges and Lima oranges. The thick peel, somewhat light-colored flesh, and some seeds are all present (Dan Nosowitz, 2018). They have relatively little acid, which serves as a preservation measure, making them unsuitable for juicing. They are often not grown in great numbers (Amy Grant, 2021). South America and the Mediterranean area are both common places to find acid-less oranges (Mashama Bailey, 2022). There are bitter oranges, which include: Seville orange (*Citrus aurantium*) is cultivated in Southeast Asia. Even though the thick skin of these oranges is practically never consumed raw, it is a key component of orange marmalade and serves as the rootstock for the sweet orange tree (Mashama Bailey, 2022). Only the months of December through early February are when these oranges are in season (Bailey Fink, 2022). Bergamot orange (*Citrus Bergamia Risso*) is primary produced for its peel in Italy. Oranges are rarely consumed whole because of their very strong bitter and acidic flavor. However, their distinctive yellow-green peel is what makes Earl Grey tea special and is also a component of fragrances (Mashama Bailey, 2022). From November through January, bergamot oranges are in season (Bailey Fink, 2022). Trifoliate orange (*Poncirus trifoliata*) is a little orange and is employed as the rootstock for sweet orange plants. The downy fruit that trifoliate oranges produce is often employed to produce marmalade. Northern China and Korea are their home countries (Amy Grant, 2021).

Figure 2.2.1: Sweet Orange (*Citrus sinensis*)

Figure 2.2.2: Mandarin Orange (*Citrus reticulata*)

Figure 2.2.3: Pomelo Orange (*Citrus maxima*)

Figure 2.2.4: Bitter Oranges (*Citrus aurantium*)

Figure 2.2.5: Blood or Pigmented Orange

Figure 2.2.6: Navel Orange

Figure 2.2.7: Common Orange

Figure 2.2.8: Acid-less Orange

Figure 2.2.9: Bergamot Orange (*Citrus Bergamia Risso*)

Figure 2.2.10: Trifoliate Orange (*Poncirus trifoliata*)

2.3 Orange Juice

Among the many citrus juices, orange juice is the most widely consumed. It is a liquid extract made by squeezing or reaming oranges from the fruit of the orange tree (Sahar et al., 2019). Orange juice is marketed in three main ways: as a frozen concentrate that is mixed with water after purchase; as a reconstituted liquid that has been concentrated and then mixed before purchase; or as a single unconcentrated beverage known as Not From Concentrate (NFC). The final two kinds are additionally referred to as Ready To Drink (RTD) juices (Sahar et al., 2019).

According to the findings from the UK National Diet and Nutrition Survey, a large number of adults (69%) and adolescents (92%) do not consume the recommended 5 servings of fruits and vegetables each day (University of Cambridge et al., 2018). One part of the fruit is equal to a tiny 150 mL glass of 100% fruit juice, which can help individuals come closer to the 5 A Day goal. Studies show that fruit juice consumers are 42% more likely than non-drinkers to reach the 5 A Day requirement for fruit and vegetables (University of Cambridge et al., 2018).

The public is still unclear about the pureness of 100% fruit juice, whether it has additional sugars, and even how much to drink. A recent poll of health professionals revealed that 63% of those polled believed that 100% of fruit juice contains additional components including sugar, colorings, and preservatives, which is false (Benton and Young, 2019). The European Directive rigorously regulates 100% fruit juice, therefore nothing may be added or subtracted from the fruit juice. This comprises water, sugar, colorants, stabilizers, taste enhancers, and preservatives. As a result, if a packaging container is labeled “100% orange juice,” it will only contain pure orange juice derived from entire oranges (European Commission, 2012). Contrary to common opinion, just 1 – 2 medium-sized oranges are required to generate one 150 mL glass of 100% orange juice, and the juice’s natural sugars, water content, minerals, and vitamins will correspond to those of the original fruits (Ulla Ringblom, 2017).

Pure orange juice is well known for being a good source of ascorbic acid, but less is known about the other important nutrients it contains. In addition to providing folate and potassium, a modest 150 mL glass of 100% orange fruit juice also includes citrus flavonoids including hesperidin. Some groups in the United Kingdom were found to be deficient in nutrients like potassium and folate, which are necessary for the human body’s basic activities (University of Cambridge et al., 2018). Fruits are known to be a dietary source of polyphenols like flavonoids and carotenoids, and 100% fruit juices also contain significant amounts of these organic plant pigments. Hesperidin is a specific kind of citrus flavonoid found in 100% orange juice. According to recent research (Morand et al., 2011), hesperidin may have a favorable effect on endothelial function in human beings and may thus play a role in the cardiovascular health of 100% orange juice.

2.4 Physicochemical Analysis

The physicochemical analysis is a technique for determining the physical and chemical properties of the component that enables the identification of the interactions between system components, through an examination of the connections between a substance's qualities and composition (Zlomanov et al., 2013). The discipline of chemistry that studies how the composition and characteristics of matter interact is known as "physicochemical analysis." Material is made up of several interlocking particles that share particular properties, including composition, particle size, structure, and the type of chemical bonding. The substance's qualities are determined by these features (Zlomanov et al., 2013).

The many types of particles that make up a material are referred to as their composition. For instance, a crystal of sodium chloride is composed of sodium and chloride ions that occupy cationic sites (Na^+) as well as anionic sites (Cl^-). In addition to atoms and ions, the component particles can also be molecules, such as Iodine crystals consisting of I_2 molecules, while ice is composed of H_2O molecules., as well as other types of particles (Sinton, 2004). The ordered arrangement of atoms within molecules is referred to as chemical structure (Sinton, 2004).

The composition and structure of a crystal influence its properties, like the lattice energy and chemical, electrical, and optical characteristics or properties. Distinct lattice energies and, thus, various characteristics (such as melting temperature, boiling temperature, and hardness) are defined by the distinct spatial configuration of identical particles. For example, the carbon atoms in graphite and diamond (Zlomanov et al., 2013).

Most frequently, the following physicochemical properties of fruit juices are taken into account when determining the fruit juice quality: pH, titratable acidity, total soluble solids, dry matter contents, ash content, crude protein, ascorbic acid, total sugar, reducing sugar, and total soluble solids/titratable acidity ratio (Nonga et al., 2014). However, in this research, the physicochemical parameters used to assess the quality of fruit juice include total soluble solids, pH, color, viscosity, and titratable acidity.

2.4.1 Total Soluble Solids

An essential factor in determining the quality of fruit juice is the total soluble solids. The combined impact of maturational phases and ripening circumstances has a considerable impact on the total soluble solids content (Nonga et al., 2014). The number of soluble solids in liquid is indicated in total soluble solids. Total soluble solids value has an impact on fruit flavor since it might reveal the fruit's amount of sweetness. Total sugar content, together with a minor amount of amino acids, soluble proteins, and other organic compounds, make up the majority of the total soluble solid.

The liquid must be extracted from the fruit in a laboratory setting in order to determine the total soluble solids of fruit juice. A refractometer is typically used to measure total soluble solids. The fruit juice extract is applied to the detector and measured. The speed of light through the sample and the vacuum speed are compared to determine the value of the total soluble solid, which is given as °Brix. The amount of total soluble solids in the solution increases as the solution becomes more concentrated, which results in a decreased rate of light penetration (Hadiwijaya et al., 2020).

Based on the study done by Eng Keng et al. (2015), the total soluble solids content of fresh orange fruit juices from 5 different brands was examined. The result showed that Australian Citrus, Kangara, MFC, Unifrutti, and Vitor fresh squeezed orange juice had total soluble solids contents of 10.6 ± 0.0°Brix, 11.3 ± 0.1°Brix, 11.3 ± 0.1°Brix, 9.2 ± 0.0°Brix, and 10.6 ± 0.0°Brix, respectively (Eng Keng et al., 2015).

2.4.2 pH

The pH scale ranges from pH 0 to pH 14, and it is used to determine the concentration of hydrogen ions in solutions as well as the intensity of acidity or alkalinity. Lower pH levels are considered acidic, whereas higher pH values are alkaline or caustic. A pH of 7 is regarded as neutral (Swyngedouw et al., 2008).

The measurement of a fruit juice beverage's pH offers crucial information about its uniformity, safety, and quality. Since pH variation in fruit juice may have a significant impact on the flavor, freshness, and shelf-life of the finished fruit juice product, it is one of the characteristics that are most commonly examined during the fruit juice beverage quality inspection. However, pH has a role in beverage extraction as well. For example, since enzyme activity is highly pH-dependent, controlling this parameter increases yields and improves the distinctive flavor. In addition, managing pH controls the development of both good and unwanted microbes. Fruit juice is one of the many beverages whose pH has to be controlled (Aadil et al., 2019).

A digital pH meter is being used to measure the fruit juice's pH. Using an electric pH meter to assess a solution's acidity or alkalinity by looking at the activity of hydrogen ions. The two essential components of a pH meter include a reference electrode and a pH-responsive electrode that is connected to a voltmeter. Typically, a glass pH-responsive electrode and a silver chloride electrode serve as the reference. The two electrodes operate as a battery in a solution, with the electric potential (charge) generated by the glass electrode immediately influenced by the hydrogen-ion activity in the solution. The voltmeter measures the difference in potential between the glass and reference electrodes. Consequently, the pH electronic meter displays the decimal pH reading of the solution (Teresa et al., 2006).

Due to their relative abundance in organic acid, fruit juices have a pH range of 2 to 5, which has a low pH (Hossain et al., 2010). Fruit juices include a combination of organic acids that contribute to their overall acidity; the composition of these organic acids varies depending on the kind and stage of ripeness of the fruit (Nonga et al., 2014).

2.4.3 Color

Due to its sensory qualities, one of the most well-liked fruit juices worldwide is orange juice, which is consumed extensively across the globe. Color has been cited as affecting customer acceptability among the qualitative aspects valued by consumers. The perception of a food's taste can then be altered by visual cues, like a food's color, by changing the gustatory and olfactory qualities, and/or by influencing the perception of flavor across all senses. For decades, scientists have been examining how color affects food acceptance as well as taste, odor, and flavor (both theoretically and practically). Based on the research done by Fernández Vázquez et al. (2014), the researchers looked at a larger range of colors (using red and green food coloring) and qualities (taste, sweetness, flavor, and sourness), as well as predicted and actual liking before consuming orange fruit juice. This study provided insight into the relationship between minute differences in orange fruit juice color (reddish to greenish hues) and perceived taste intensity, sweetness, and sourness, as well as the predicted and actual customer preference for orange fruit juice (Fernández Vázquez et al., 2014).

The term “color measurement” refers to the process of spectrally quantifying the amount of light that is emitted, transmitted, or reflected by a color sample. Fruit juice was subjected to a color analysis using a color-measuring tool called a colorimeter or color meter. This tool can locate a color in the color space by measuring the values of red, green, and blue, which are stated as L*a*b* values (Korifi et al., 2013). On a scale ranging from 0 to 100, the color value “L*”, which gauges lightness, is quantified. The color value “a*” quantifies red color (positive values) to green color (negative values), and the color value “b*” measures yellow color (positive values) to blue color (negative values) (Ferrández Vázquez et al., 2014).

In general, the food sector may utilize color assessment as an objective tool, much quicker and easier than chemical analysis, to assess changes in fruit juice’s visual quality (Cefola et al., 2012). Fruit juice may undergo both enzymatic and non-enzymatic browning, hence the browning index (BI), which may depict the true brown color, may be regarded as a crucial technical metric (Cefola et al., 2012). One of the most prevalent markers to determine the browning of sugar in fruit juice products is the browning index (BI), which is defined as the purity of the brown color present in the fruit juice product (Lunadei et al., 2011). Knowing the color value of the fruit juice item will enable a thorough characterization of its color and a more accurate assessment of its quality (Lunadei et al., 2011). Several researchers have already used the browning index to evaluate the degree of browning in various fruit and vegetable juices (Cefola et al., 2012).

2.4.4 Viscosity

When a fluid is subjected to shear stress, its ability to resist deformation is measured by its viscosity (Wong and Wong, 2013). It is commonly understood as the flow behavior or pouring resistance of a substance. Viscosity is a measure of the resistance to internal flow in a fluid and can be considered a measure of fluid friction. The final viscosity of a substance plays a crucial role in its processing stage (Maheshwar, 2018). Understanding how temperature and concentration affect the viscosity of fruit juice drinks is crucial information. It helps to maintain control over the flow and temperature of the heating media to ensure the continuous flow and gelling of the fruit juice product. Additionally, it aids in calculating the rate of heat transfer, energy consumption with increasing concentration, and other important factors. These considerations are critical in the mixed flow system of fruit juice production (Shaziya and Jayashree, 2015). Additionally, viscosity is an important indicator of fruit juice quality that influences how it tastes and how well it can suspend its solid component throughout the duration of the product’s shelf life (Santos et al., 2018).

Based on the study done by Bull et al. (2004), two types of fresh orange fruit juice were analyzed for viscosity. The result showed that Valencia and Navel fresh squeezed orange juice were having a viscosity of 5.3 mP.a.s and 4.7 mP.a.s, respectively (Bull et al., 2004).

2.4.5 Titratable Acidity

Titratable acidity is used to determine the overall acid content in a food item. Organic acids such as citric, lactic, tartaric, and acetic are commonly found in food products. Nonetheless, inorganic acids like phosphoric and carbonic, formed by carbon dioxide in solution, can also contribute to acidification in food. These acids play a crucial role in influencing the pigmentation, microbiological stability, flavor (tartness), and color (via their effect on pigments like anthocyanin or other pH-influenced pigments), and maintaining quality, organic acids have an impact on the flavor of food (resulting from the various chemical sensitivity of food ingredients to pH) of the food product.

Titratable acidity is calculated by neutralizing the acid in a specified quantity (weight or volume) of a food product using a standardized base, such as sodium hydroxide (NaOH), a potent base that is frequently employed in the measurement of titratable acidity. The target pH or the color shift of a pH-sensitive dye, commonly phenolphthalein, serves as the titration's endpoints. The titratable acidity is calculated using the amount of titrant employed, the normality of the base, and the volume (or weight) of the sample and is represented as the predominant organic acid as g citric acid/100g of juice (Nielsen, 2017b).

Based on the study done by Eng Keng et al. (2015), the total soluble solids content of fresh orange fruit juices from 5 different brands was examined. The result showed that Australian Citrus, Kangara, MFC, Unifrutti, and Vitor fresh squeezed orange juice had titratable acidity of 0.97 ± 0.01%, 0.74 ± 0.01%, 1.01 ± 0.01%, 0.81 ± 0.01%, and 0.86 ±0 .01%, respectively (Eng Keng et al., 2015).

2.5 Nutritional Analysis

The technique of assessing the nutritional value of food products is called nutritional analysis (Fairulnizal et al., 2020). The goal of this research is to identify the bioactive substances that are present in food. Bioactive molecules are described as nutrients and non-nutrients present in foods of both plant and animal origin. These bioactive compounds offer physiological benefits beyond traditional nutritional functions (Cinthia Baú Betim Cazarin et al., 2021). Terpenes and terpenoids (about 25,000 different types), alkaloids (about 12,000 different types), and phenolic compounds (about 8000 different types) make up the majority of bioactive substances (Antolak and Kregiel, 2017).

Fruit juices contain vitamins, minerals, trace elements, phytochemicals including flavonoids and polyphenols, and other nutrients that have been linked to a variety of health advantages. The majority of the time, it appears that these fruit juice chemicals work by altering gene activity. Fruit juice has powerful illness risk-lowering qualities and promotes excellent health when consumed as part of a well-balanced diet. Alternative medicine is therefore in great demand for a variety of ailments, including chronic inflammation, osteoarthritis, diabetes mellitus, hypertension, and aches and pains in the muscles (Bhardwaj et al., 2014).

The ascorbic acid concentration, total phenolic content, total carotenoid content, total flavonoid content, total anthocyanin content, and total antioxidant capacity are the most frequent nutritional analyses or bioactive qualities of fruit juice. Ascorbic acid content, total phenolic content, and total antioxidant capacity are the nutritional/bioactive characteristics employed in this study to evaluate the nutritional content of fruit juice.

2.5.1 Ascorbic Acid Content

Ascorbic acid, commonly referred to as Vitamin C, is a type of water-soluble vitamin that is found in various foods (vegetables and fruits) and living organisms. Ascorbic acid is crucial for the synthesis of collagen, the uptake of iron, the activation of the immune system, as well as osteogenesis, and wound healing. Additionally, it functions as a potent antioxidant that fights illnesses brought on by free radicals (Danet et al., 2008). However, too much ascorbic acid can irritate the stomach, and vitamin C’s metabolic byproduct, oxalic acid, can harm the kidneys (Danet et al., 2008). A labile chemical, ascorbic acid is quickly broken down by enzymes and oxygen in the environment. Overheating, light, and heavy metal cations can all hasten its oxidation (Danet et al., 2008). Due to its potential for change during extraction and storage, the ascorbic acid level in food and drinks serves as a significant indication of quality that must be carefully checked (Danet et al., 2008).

The ascorbic acid content may be determined using a variety of analytical techniques. Volumetric procedures, such as titration with an oxidant solution like dichlorophenol indophenol (DCPIP), potassium iodate, or bromate, are examples of traditional approaches (Danet et al., 2008). However, the indophenol technique, the approved method of analysis for ascorbic acid determination of fruit juice, was utilized in this study to evaluate the ascorbic acid concentration of fruit juice (Nielsen, 2017a). The 2,6-dichloroindophenol indicator dye is used in the titration technique to employ the indophenol method to measure the ascorbic acid content of fruit juice products. The 2,6-dichloroindophenol indicator dye is converted to a colorless solution by ascorbic acid. Upon titration of a sample containing ascorbic acid with dye, any unreacted dye will produce a rose-pink color in the acidic solution. The titer of the dye can be determined by using a standard ascorbic acid solution. The dye and volume for the titration are used to add to the food sample in the solution, to determine the ascorbic acid level present in the fruit juice sample (Nielsen, 2017a).

According to the research done by Oluwatoyin Tirenioluwa Fatunsin (2015), Abushusha, and Sayed (2017), and Sharma et al. (2019) the researchers discovered that freshly extracted orange juice typically contains 20 – 80 mg of ascorbic acid/100 ml.

2.5.2 Total Phenolics Content

Chemically speaking, phenolic compounds are described as substances that include aromatic rings that have been hydroxylated, with the hydroxyl group being directly connected to the phenyl group, substituted phenyl group, or even another aryl group (Swanson, 2003). Phenolic compounds can be easily separated into flavonoids and non-flavonoids into several sub-classes (Singla et al., 2019). Depending on the number of phenol units present in their molecular structure, the presence of substituent groups, and/or the kind of connection between phenol units (Singla et al., 2019).

The most significant components of various fruit juices are flavonoids and non-flavonoids, which can influence lipid metabolism, boost antioxidant capacity and reduce cholesterol absorption. The majority of phenolic compounds are isomers, derivatives, or isoflavones of phenolic acids, catechins, lignins, flavones, and flavonols. The activation of natural defense mechanisms and the modification of cellular signaling pathways are two ways that dietary phenolic compounds may provide indirect protection (Bhardwaj et al., 2014). Citrus juice (orange and lemon) contains carotenoids, for example, β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene as well as limonoids, hesperidin, narirutin, naringin, flavanones, flavones, and flavonols (Bhardwaj et al., 2014). These flavonoids have demonstrated several positive properties, including anti-inflammatory, antioxidant, antiviral, antihypertensive, anticarcinogenic, and antiviral (Mahmoud et al., 2019).

The modified vanillin test, the Folin-Denis assay, the Prussian blue test, and the Folin-Ciocalteu assay are a few of the assay techniques that are most frequently used to determine total phenolic compounds in food products (Keskin ai et al., 2012). The Folin-Ciocalteu test, which is the recognized technique of analysis for determining the total phenolic compounds of fruit juice, was employed in this study to evaluate the total phenolic compounds of fruit juice (Kupina et al., 2018).

The Folin-Ciocalteu reagent, a complex combination of hetero-poly phosphotungstate-molybdate, is used to remove phenols from their natural environments in order to create a blue-colored complex. The number of reactive phenolic chemicals present in the sample directly relates to how intense the blue color is. By measuring the sample solution's absorbance at 765 nm, using gallic acid as a reference, and comparing the results to a calibration curve, the phenol concentration is ascertained. The technique may measure the total amount of polyphenols, which are represented as mg of gallic acid equivalent/100 mL (mg GAE/100 ml) of sample juice (Kupina et al., 2018).

Based on the study done by Wern et al. (2016), the researchers discovered that the average total phenolic content of freshly extracted orange juice is 60.02 ± 12.07 mg GAE/ml.

2.5.3 Total Antioxidant Capacity

The natural sources of antioxidant phytochemicals derived from plants, particularly fruit juices, have garnered a lot of attention lately. Fruit juices include a variety of antioxidants, including carotenoids, tocopherols, ascorbic acid, and polyphenols. Vitamin C functions as a potent antioxidant, preventing the human body from oxidative stress, and aiding in cell growth and repair as well as immunity to infections and illnesses. It has been demonstrated that vitamin C lowers levels of C-reactive proteins, is a sign of inflammation, and protects against cancer, infection, a malfunctioning immune system, and the risk of cardiovascular disease in human beings (Bhardwaj et al., 2014).

Plasma concentrations of natural antioxidants tend to rise after consuming fruit juices (especially orange, pomegranate, and cranberry). A different scavenging of free radicals mechanism is present in each antioxidant vitamin. The orange color of orange and tangerine juice is attributed to several carotenoids, including α-carotene, zeta antheraxanthin (yellowish), violaxanthin (yellowish), β-citraurin (reddish orange), and β-cryptoxanthin (orange). Lycopene is responsible for the red or pink hue of some varieties of pigmented grapefruit juice, and anthocyanins. These substances all function as antioxidants in the human body. Orange, lemon, and lime juices are citrus fruits, and their juices are a significant source of bioactive chemicals, including phenolic compounds and antioxidants like ascorbic acid, which are crucial for human nourishment (Bhardwaj et al., 2014). There are more than 170 distinct phytochemicals in orange juice, including more than 60 flavonoids, most of which have been found to have antioxidant, anti-inflammatory, and antitumor benefits (Bhardwaj et al., 2014).

The total antioxidant capacity of foods and plant extracts has been measured using a variety of analytical techniques, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, the FRAP (ferric reducing antioxidant power) method, the ORAC (oxygen radical absorption capacity) assay, the TRAP (total peroxyl radical trapping antioxidant parameter) assay, the CUPRAC (cupric reducing antioxidant power) assay, and the HORAC (hydroxyl radical antioxidant capacity) assay (Pisoschi and Negulescu, 2012; Pisoschi et al., 2009) However, in this study, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was utilized to analyze the fruit juice to determine its overall antioxidant capability. This is due to the fact that the antioxidant potential of fruit juices and fruit extracts was determined using the spectrophotometric technique with DPPH.

The delocalization of the electron over the whole molecule makes DPPH• (2,2-diphenyl-1-picrylhydrazyl) a persistent free radical. In contrast to the majority of free radicals, DPPH• does not dimerize. A purple tint with a maximum absorption band at around 520 nm is caused by the delocalization of the DPPH• molecule. The violet hue of DPPH• is lost when it interacts with a hydrogen donor, leading to the generation of the reduced form (DPPH). Therefore, the decline in absorbance is directly related to the antioxidant content. The proportion of orange fruit juice's antiradical activity (%) on the DPPH radical in comparison to the control is how the outcome is represented (Pisoschi and Negulescu, 2012).

Based on the study done by Wern et al. (2016), the researchers discovered that the average total phenolic content of freshly extracted orange juice is 302.74 ± 71.53 μmol TE(Trolox Equivalents)/100 mL (Wern et al., 2016).

2.6 Microbiological Analysis

Microbiological analysis involves the use of different scientific techniques such as biological, biochemical, molecular, or chemical methods to detect, identify, or count microorganisms in various substances such as food, drinks, environmental, or clinical samples. The main purpose of this analysis is to identify and treat harmful bacteria that cause illness and food spoilage (Hngaro et al., 2014). To keep food safe and avoid deterioration, microbiological testing is crucial. This involves finding microorganisms in food items that could be harmful to human health and looking into food poisoning outbreaks to figure out what caused them and stop them from happening again (Bintsis, 2017).

Fruits are often covered in a microflora throughout harvest and postharvest processing, which includes shipping, storage, and processing. Fruit juices include this microflora (Aneja et al., 2014). These substances act as a nourishing environment for various microorganisms, including bacteria and fungi like molds and yeasts that can thrive in acidic conditions. Prior to processing, yeasts dominate the flora of fruits due to their tolerance to acidity. *Candida*, *Dekkera*, *Hanseniaspora*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces* are some of the most well-known genera. The filamentous fungi that are most often isolated from fresh fruits and juices include *Penicillium*, *Byssochlamys*, *Aspergillus*, *Paecilomyces*, *Mucor*, *Cladosporium*, *Fusarium*, *Botrytis*, *Talaromyces*, and *Neosartorya*. Lactic acid, as well as acetic acid bacteria, have been identified from fruit juices among other microorganisms (Aneja et al., 2014).

Several factors affect the deterioration of fruit juice, including pH, oxidation-reduction potential, water activity, nutrient availability, the presence of antimicrobial agents, and competing microorganisms. Among these parameters, the pH and water activity have the most significant impacts on juice spoilage. The microbial spoilage of juice can result in a loss of clarity, the development of unpleasant flavors, the production of carbon dioxide, changes in color, texture, and appearance, and product degradation (Aneja et al., 2014). The bacterial genera *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Zymomonas*, and *Zymobacter* are among the most frequently reported ones. *Pichia*, *Candida*, *Saccharomyces*, and *Rhodotorula* are yeast genera that are frequently found and are in charge of causing juice spoiling (Aneja et al., 2014). Fruit juice spoiling has also been linked to a number of common molds, including *Penicillium sp.*, *Aspergillus sp.*, *Eurotium*, *Alternaria*, *Cladosporium*, *Paecilomyces*, and *Botrytis* (Aneja et al., 2014).

The total amount of microbes in fruit juice may be counted using a variety of different techniques. Pour plate, surface spread plate, surface drop, and petrifilm methods are among the several enumeration techniques. However, the pour plate method was employed in this study to count all the microorganisms present in the fruit juice (Adams and Moss, 2008). This is due to the pour plate method's ability to separate and count live bacteria and fungus from suspension or liquid samples (Sanders, 2012).

In the pour plate method, a set volume of inoculum (usually 1 ml) from a sample or broth is carefully transferred to the center of a sterile Petri dish using a sterile pipette. The inoculated plate is then filled with a molten agar (about 15 ml) that has been cooled down and mixed well. Once the agar has solidified, the Petri dish is flipped over and incubated at 37°C for 24 – 48 hours. This method is widely used to cultivate microorganisms and is suitable for counting colony-forming units (CFU) (Sood et al., 2011).

Microbial growth can occur both within and on the surface of the agar medium, leading to the formation of visible colonies. These colonies can be of varying sizes and may appear either as discrete entities or merge to form confluent growth. After incubation, the colonies are counted using a colony counter, and each colony is referred to as a colony-forming unit (CFU) (Sood et al., 2011).

Based on the study done by Nayik et al. (2014), the researchers discovered that the average total number of colonies of freshly extracted orange juice is $3.30 \times 10^5 \pm 0.36$ CFU/mL (Nayik et al., 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Sodium Hydroxide (NaOH), Phenolphthalein, Acetic acid (CH_3COOH), Ascorbic acid, 2,6-dichlorophenol sodium salt (DCPIP), Metaphosphoric acid (HPO_3), Sodium Bicarbonate ($NaHCO_3$), Folin-Ciocalteu's phenol reagent, Sodium Carbonate (Na_2CO_3), Gallic acid (3,4,5-trihydroxybenzoic acid), DPPH (2,2-diphenyl-2-picrylhydrazyl), Methanol (CH_3OH), Plate count agar (PCA) and Peptone water.

3.2 Analytical Procedure

3.2.1 Orange Collection

The orange fruits (*Citrus Sinensis*) were bought from a neighborhood store in Kampar, Malaysia. Based on resemblance in size, ripeness, and color, the fruits were chosen. Orange fruits that were distorted and damaged were excluded from the sampling.

3.2.2 Method of Processing Fruit Juice

Oranges were thoroughly cleaned with tap water before being dried with tissues. They were then sliced, skinned, and weighed before being extracted by the cold-pressed juice extractor to produce fruit juice. Filtering was then done to get rid of the fibrous debris and seeds before the storage condition and future analysis.

3.2.3 Juice Storage

The prepared fruit juices were put into a 50 mL falcon tube bottle and covered with aluminum foil to keep them from being exposed to light while being subjected to the storage condition. 400 mL (8 falcon tubes) of orange juice were divided and kept at room temperature (24°C) and refrigerator cold (4°C) respectively. On day 0 and day 12, all orange juice under various storage conditions was examined. For comparison reasons, freshly made cold-pressed orange juice was utilized as the benchmark.

3.2.4 Total Soluble Solids

According to Mohamad Salin et al. (2022), total soluble solids (TSS) in orange juice were measured using a digital refractometer (Atago PAL-3, Tokyo, Japan). Orange juice's total soluble solids were measured in °Brix. Juice samples were applied in the amount of 2 drops to the refractometer's transparent prism. Each orange juice's °Brix value was recorded in triplicate.

3.2.5 pH

A pH meter (Eutech pH 700, Waltham, MA, USA) was used to measure the orange juice's pH. As referred to by Mohamad Salin et al. (2022), 10 mL of orange juice was put in a beaker, and the pH of each sample of juice was measured in triplicate.

3.2.6 Color

By using a colorimeter (Konica Minolta CM-600d, Osaka, Japan), the juices' colors were analyzed. Approximately 5 mL of orange juice sample was placed on the mini petri dish and a colorimeter was used to perform color analysis. Three color characteristics were assessed and represented as L*, a*, and b* values, including brightness (L*), redness/greenness (a*), and yellowness/blueness (b*). Each orange juice's color value was recorded in triplicate (Khaksar et al., 2019). The browning index was determined using the equation (Ismail et al., 2021) below:

$$\text{Browning index} = [100\,(Z - 0.31)] / 0.172$$

$$\text{where } Z = (a^* + 1.75L^*) / (5.645L^* + a^* - 0.3012b^*)$$

3.2.7 Viscosity

A viscometer (Brookfield DV2T, Berwyn, IL, USA) was used to analyze the orange juices' viscosity. A beaker containing 10 mL of orange juice was utilized for the viscosity analysis, which was conducted at a 100-rpm speed for one minute. The viscosity of orange juice was measured in triplicate for each orange juice and expressed as mP.a.s.

3.2.8 Titratable Acidity

Based on Olorunjuwon et al. (2014), titratable acidity was assessed. 1 milliliter of fruit juice was homogenized in 20 milliliters of distilled water before being filtered through Whatman No. 1 filter paper. To ascertain the end point of phenolphthalein, 2 to 3 drops of phenolphthalein were added to the filtrate as an indicator and titrated against 0.05M sodium hydroxide. Titratable acidity was estimated using the following formula and represented as g citric acid/100mL of juice:

$$TA = (MNaOH \times mL\ NaOH \times M.E) / mL\ juice\ sample$$

Where: TA = Titratable acidity

MNaOH = Molarity of NaOH used

mL NaOH = amount (ml) of NaOH used

M.E = Equivalent factor of citric acid = 192.124mg

3.2.9 Ascorbic Acid Content

The indophenol technique was also used to test the ascorbic acid level. Approximately 2 mL of orange juice was combined with 5 mL of a metaphosphoric acid-acetic acid solution as a stabilizing agent. The mixture was then titrated using a solution of 2,6-dichlorophenol indophenol. For about 15 seconds, there was a faint pink tint. The ascorbic acid concentration was calculated using the volumetric dye quantity that was measured during the titration (Nielsen, 2017a). The results were presented as mg ascorbic acid/100mL orange juice.

Titer = F = mg ascorbic acid in the volume of standard solution titrated**/(average mL dye used to titrate standards − average mL dye used to titrate blank)

**mg ascorbic acid in volume of standard solution titrated = (mg of ascorbic acid/50mL) × 2mL

mg ascorbic acid/mL = $(X - B) \times (F/E) \times (V/Y)$

where: X = mL for sample titration

B = average mL for sample blank titration

F = titer of dye

E = mL assayed

V = volume of the initial assay solution

Y = volume of sample aliquot titrated

3.2.10 Total Phenolics Content

The Folin-Ciocalteu technique as reported by Kupina et al. (2018), was used to determine the total phenolic content. Approximately 3 mL of 20% Sodium carbonate are added together with 1 mL of 1 N Folin-Ciocalteu's phenol reagent and 1 mL of sample juice solution. The solution was incubated in the dark for 2 hours. A spectrophotometer (DLAB Scientific SP-V1000, Beijing, China) was used to measure the absorbance at 765 nm in comparison to a blank. In order to calculate the total phenolic content, a standard curve was built using various gallic acid concentrations (40, 80, 120, 160, and 120 mg/mL). The outcomes were represented as mg of gallic acid equivalent (GAE) per g of samples (mg GAE/g).

3.2.11 Total Antioxidant Capacity

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the method of Tan et al. (2022). Approximately 2 mL of newly made 30 mg/L DPPH solution in methanol was combined with 0.25 mL of sample extract solution. After the mixture was vigorously shaken and mixture was incubated at room temperature for 5 minutes. A spectrophotometer (DLAB Scientific SP-V1000, Beijing, China) was used to quantify the reduction in absorbance at 517 nm with pure methanol serving as the blank. All sample extracts were analyzed in triplicate. The antiradical activity percentage of the extract on DPPH radicals in comparison to the control was used to express the results. The following formula was used to determine the radical scavenging activity (RSA) percentage:

Percentage of radical scavenging activity (RSA) = $(1 - A_1/A_0) \times 100\%$

Where: A_1 = absorbance of testing sample solution

A_0 = absorbance of control/extraction solvent

3.2.12 Microbiological Analysis

In order to perform microbiological analysis, the total plate counts (TPC) were measured using the plate count agar (PCA) (Chia et al., 2012). For the microbiological examination of nectars, fruit juice samples (1 mL) were obtained immediately after the storage conditions. Using sterile 0.1% peptone water, 1 mL of the homogenized sample was serially diluted 10 times (10^{-1} to 10^{-5}). 1 mL of the appropriate dilutions was pour-plated onto approximately 15 mL of the appropriate plate count agar (PCA) media. The PCA media was incubated at 37°C for 24 hours after the colony-forming units were counted. All fruit juice sample extracts were analyzed in triplicate. Colony-forming units (CFU) were used to represent the results in terms of juice volume. The given fruit juice sample's microorganism count can be determined using the following formula:

CFU/mL = (total number of colonies obtained × total dilution factor) / volume of inoculum transferred

3.2.13 Statistical Analysis

The paired samples t-test was used in statistical analysis to examine the impact of storage temperature on orange juice quality. By contrasting the characteristics of the stored juice with the control (freshly cold-pressed orange fruit juice), the impact of storage temperature was evaluated. At a significance level of 0.05, the difference in means was found. All statistical analyses were performed using SPSS version 26, with a significance level of 0.05. The outcomes were presented as mean ± standard deviation.

CHAPTER 4

RESULTS

4.1 Effect of Storage Temperature on The Physicochemical Properties of The Cold-Pressed Orange Juices

Table 4.1 depicts how the total soluble solid in orange juice is affected by storage temperature. The total soluble solid in orange juices kept at room temperature (24°C) and at refrigerator cold (4°C) varied from 11.8 to 8.0 °Brix. The total soluble solid of orange juice did not change significantly ($p > 0.05$) while stored at refrigerator cold (4°C) compared to the control (freshly cold-pressed orange juice) before storage. However, after 12 days of storage at room temperature (24°C), a significant reduction ($p < 0.05$) in the total soluble solid was seen between the control and the stored sample.

Likewise, Table 4.1 displays how the pH of orange juice changed while it was being stored. For orange juices kept at room temperature (24°C) and refrigerator cold (4°C), the pH varied from 3.42 to 3.61. The pH of orange juice was not significantly impacted by storage at refrigerator temperature (4°C). However, after 12 days of storage at room temperature (24°C), the pH was significantly lower ($p < 0.05$) than the control.

Similarly, the color changes of orange juice kept at various storage temperatures are displayed in Table 4.1. Orange juices kept at room temperature (24°C) and refrigerator at 4°C varied in L* values from 52.02 to 53.21. The L* values increased on average in most cases. After 12 days, there was a significant difference ($p < 0.05$) in L* values between the control and orange juice held at room temperature (24°C) and refrigerator cold (4°C). The a* values, on the other hand, varied from 2.46 to 3.76 for orange juices kept at room temperature (24°C) and room temperature (4°C). After 12 days, there was no significant difference in a* values between the control and all of the stored samples. During storage, orange juice's b* values often increased. The b* values for orange juices kept at room temperature (24°C) and refrigerator cold (4°C) varied from 16.09 to 16.81. After 12 days, there were significant differences ($p < 0.05$) between the control and orange juice held at refrigerator cold (4°C), and room temperature (24°C).

Correspondingly, Table 4.1 illustrates how orange juice's browning index was affected by storage temperature. For orange juices kept at room temperature (24°C) and refrigerator cold (4°C), the browning index fell between 6.40 to 8.16. After 12 days of storage, there was no significant difference between the control and refrigerator cold (4°C) and room temperature (24°C) in terms of the browning index.

The viscosity of orange juice was affected by storage temperature, as seen in Table 4.1. The viscosity of orange juice held at room temperature (24°C) and refrigerator cold (4°C) varied from 19.60 mP.a.s to 16.00 mP.a.s Throughout storage, orange juice's viscosity often decreased. Significant viscosity variations ($p < 0.05$) between the control and stored samples were found after 12 days of storage in refrigerator cold (4°C) and room temperature (24°C). After 12 days of storage, there were significant variations ($p < 0.05$) in viscosity among the orange juice sample held at room temperature (24°C) and refrigerator cold (4°C) when comparing the various storage temperatures.

Table 4.1 demonstrates how orange juice's titratable acidity changed as it was being stored. The titratable acidity for orange juice held at room temperature (24°C) and refrigerator cold (4°C) varied between 3.07g to 3.46g citric acid/100mL. Although the titratable acidity increased generally during the course of storage, no significant variations were found between the control and the fruit juice held at room temperature (4°C). Nevertheless after 12 days of storage at room temperature (24°C), titratable acidity was found to have significantly increased ($p < 0.05$) in comparison to the control.

Table 4.1: Physicochemical properties of cold-pressed orange juices stored for 12 days at 4°C and 24°C

Physicochemical parameters	Freshly cold-pressed orange juice	Storage temperature of cold-pressed orange juices	
		Refrigerator cold (4°C)	**Room temperature (24°C)**
Total soluble solid (°Brix)	11.90 ± 0.20^{a}	11.77 ± 0.06^{a}	8.10 ± 0.10^{b}
pH	3.64 ± 0.02^{a}	3.56 ± 0.06^{a}	3.49 ± 0.07^{b}
Color			
L*	48.22 ± 0.14^{a}	52.64 ± 0.49^{b}	52.57 ± 0.48^{b}
a*	3.83 ± 0.18^{a}	3.39 ± 0.48^{a}	2.97 ± 0.69^{a}
b*	13.93 ± 0.41^{a}	16.77 ± 0.04^{b}	16.54 ± 0.39^{b}
Browning index	8.43 ± 0.32^{a}	7.70 ± 0.70^{a}	7.10 ± 0.92^{a}
Viscosity (mP.a.s)	33.20 ± 0.00^{a}	19.47 ± 0.23^{b}	16.67 ± 0.61^{c}
Titratable acidity (g citric acid/100ml)	2.91 ± 0.11^{a}	3.23 ± 0.20^{a}	5.25 ± 0.15^{b}

Data are presented as mean ± standard deviation of samples (n = 3).

Different letters ($^{a,\ b\ \&\ c}$) in the same row indicate significant differences ($p < 0.05$) between samples.

4.2 Effect of Storage Temperature on The Nutritional Contents of The Cold-Pressed Orange Juices

The ascorbic acid content of orange juice held at various storage temperatures is displayed in Table 4.2. The quantity of ascorbic acid in orange juice that was stored at room temperature (24°C) and in refrigerator cold (4°C) varies between 5.37 mg/100 mL to 21.48 mg/100 ml. Throughout storage, ascorbic acid content often decreased. After 12 days of storage at both room temperature (24°C) and refrigerator cold (4°C), significant changes ($p < 0.05$) in ascorbic acid content between the control and stored sample were found. After 12 days of storage, there were significant changes ($p < 0.05$) in the ascorbic acid content between orange juice samples maintained at room temperature (24°C) and refrigerator cold (4°C).

Table 4.2 exhibits how the total phenolic content of orange juice is affected by storage temperature. The total phenolic content of orange juice stored at room temperature (24°C) and refrigerator cold (4°C) varied between 61.24 mg GAE/100mL to 49.33 mg GAE/100ml. After 12 days of storage at room temperature (24°C) and refrigerator cold (4°C), there was a significant decrease ($p < 0.05$) in the total phenolic content between the control and stored samples. After 12 days of storage, there were significant differences ($p < 0.05$) in the total phenolic content between orange juice samples kept at room temperature (24°C) and refrigerator cold (4°C), according to a comparison of the various storage temperatures.

Table 4.2 exhibits how orange juice's total antioxidant capacity changed as it was being stored. The radical scavenging activity for orange juices kept at room temperature (24°C) and refrigerator cold (4°C) varied from 49.11% to 58.61%. After 12 days, the total antioxidant capacity between the control sample and the stored sample significantly decreased ($p < 0.05$) at both room temperature (24°C) and refrigerator cold (4°C) storage. After 12 days of storage, a sample of orange juice held at room temperature (24°C) and refrigerator cold (4°C) showed statistically significant differences ($p < 0.05$) in total antioxidant capacity.

Table 4.2: Nutritional properties of cold-pressed orange juices stored for 12 days at 4°C and 24°C

Nutritional parameters	Freshly cold-pressed orange juice	Storage temperature of cold-pressed orange juices	
		Refrigerator cold (4°C)	**Room temperature (24°C)**
Ascorbic acid (mg/100ml)	30.87 ± 0.67^{a}	20.81 ± 0.67^{b}	6.04 ± 0.67^{c}
Total phenolic content (mg GAE/100ml)	73.41 ± 0.46^{a}	60.44 ± 0.99^{b}	51.08 ± 1.53^{c}
Total antioxidant capacity (%RSA)	63.25 ± 1.64^{a}	57.96 ± 0.76^{b}	50.74 ± 1.71^{c}

Data are presented as mean ± standard deviation of samples (n = 3).

Different letters ($^{a,\ b\ \&\ c}$) in the same row indicate significant differences ($p < 0.05$) between samples.

4.3 Effect of Storage Temperature on The Microbiological Loads of The Cold-Pressed Orange Juices

Table 4.3 represents how storage temperatures affect the development of microbial loads. The microbiological loads for orange juices kept at room temperature (24°C) and refrigerator cold (4°C) ranged from 2.1×10^5 CFU/mL to 4.9×10^6 CFU/ml. After 12 days of storage in refrigerator cold (4°C), there were no significant variations in the microbial loads between the control and stored samples. However, refrigerator cold (4°C) storage caused a slow but not significant rise in microbial loads. However, after 12 days of storage at room temperature (24°C), there were significant differences ($p < 0.05$) between the control and the stored sample in terms of the increase of microbiological loads.

Table 4.3: Microbiological loads of cold-pressed orange juices stored for 12 days at 4°C and 24°C

Microbiological parameters	**Freshly cold-pressed orange juice**	**Storage temperature of cold-pressed orange juices**	
		Refrigerator cold (4°C)	**Room temperature (24°C)**
Total plate counts (CFU/ml)	$2.14 \pm 0.37 \times 10^{5\ a}$	$2.88 \pm 0.83 \times 10^{5\ a}$	$3.29 \pm 1.15 \times 10^{6\ b}$

Data are presented as mean ± standard deviation of samples (n = 3).

Different letters ($^{a,\ b\ \&\ c}$) in the same row indicate significant differences ($p < 0.05$) between samples.

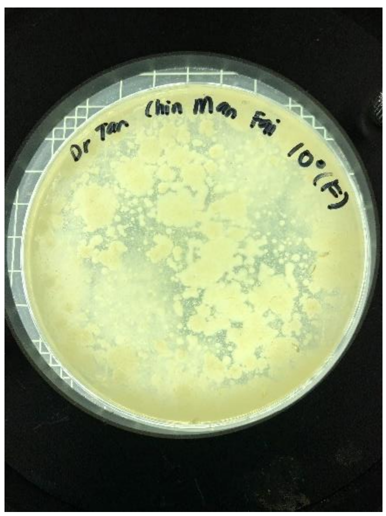

Figure 4.3.1a: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^0)

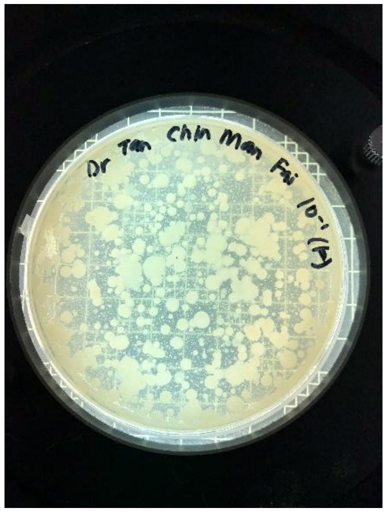

Figure 4.3.1b: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^{-1})

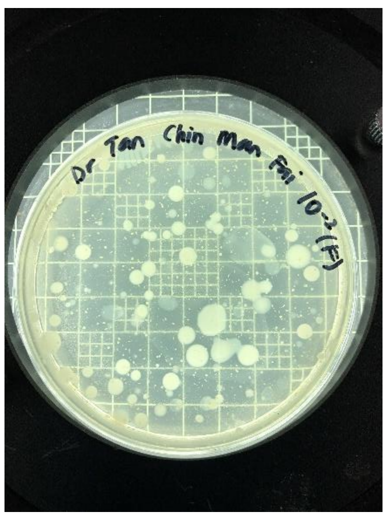

Figure 4.3.1c: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^{-2})

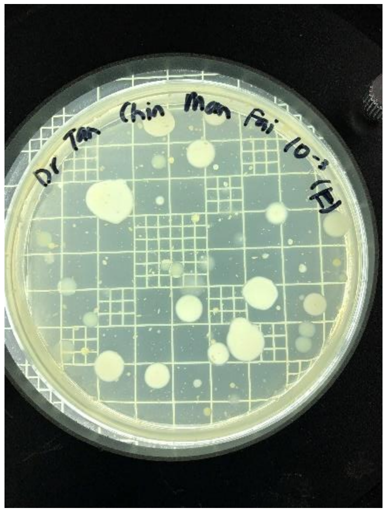

Figure 4.3.1d: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^{-3})

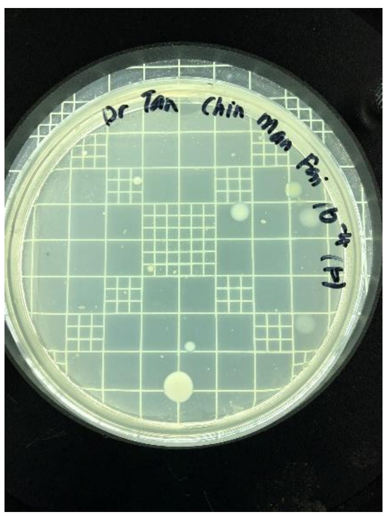

Figure 4.3.1e: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^{-4})

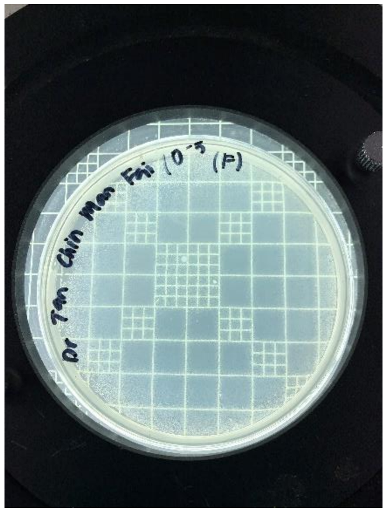

Figure 4.3.1f: Freshly Cold-Pressed Orange Juice (Dilution factor: 10^{-5})

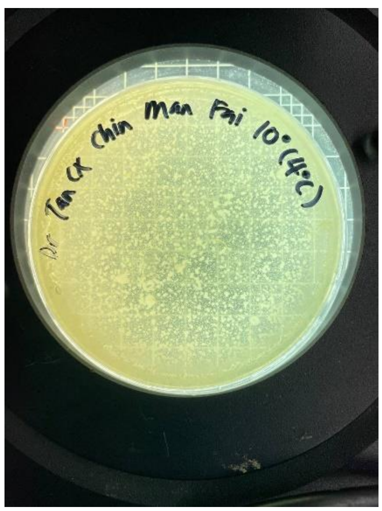

Figure 4.3.2a: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{0})

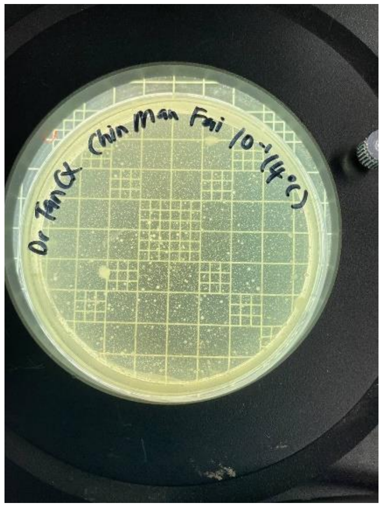

Figure 4.3.2b: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-1})

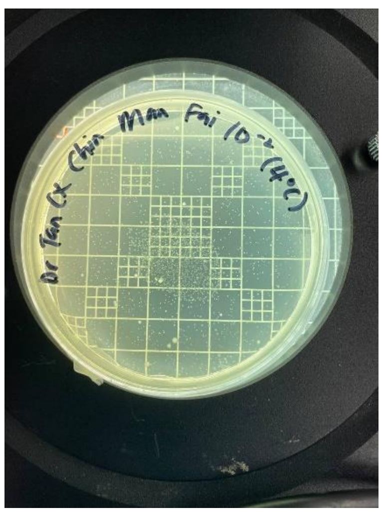

Figure 4.3.2c: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-2})

Figure 4.3.2d: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-3})

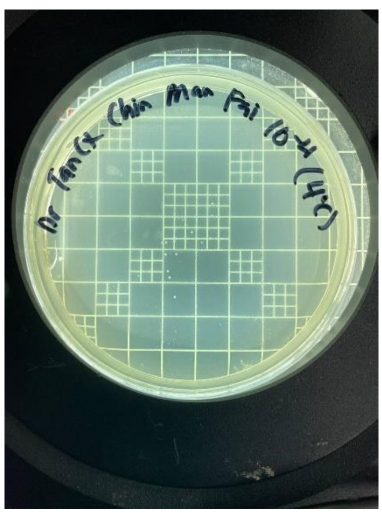

Figure 4.3.2e: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-4})

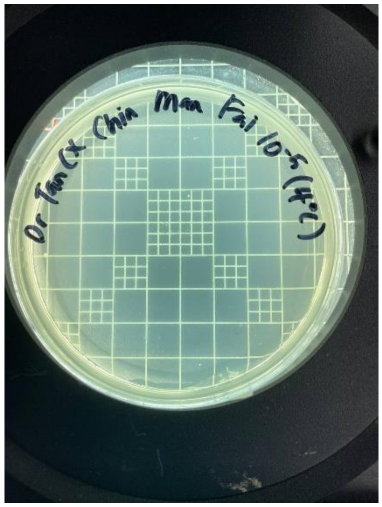

Figure 4.3.2f: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-5})

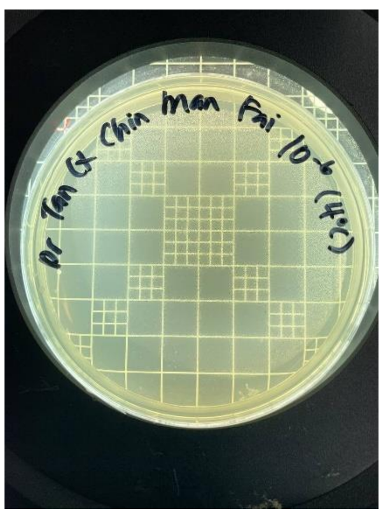

Figure 4.3.2g: Storage temperature (4°C) of Cold-Pressed Orange Juice (Dilution factor: 10^{-6})

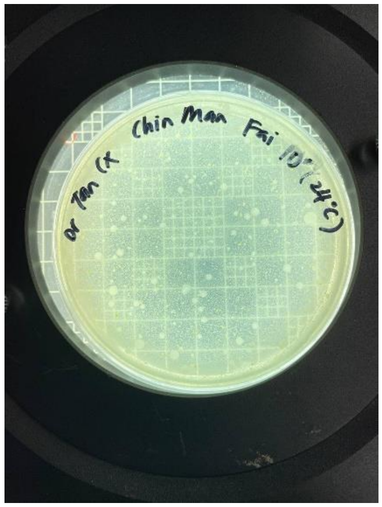

Figure 4.3.3a: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{0})

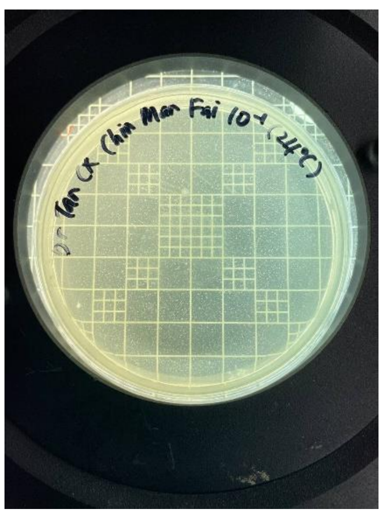

Figure 4.3.3b: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-1})

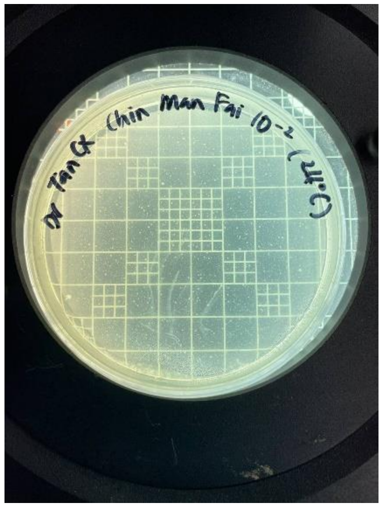

Figure 4.3.3c: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-2})

Figure 4.3.3d: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-3})

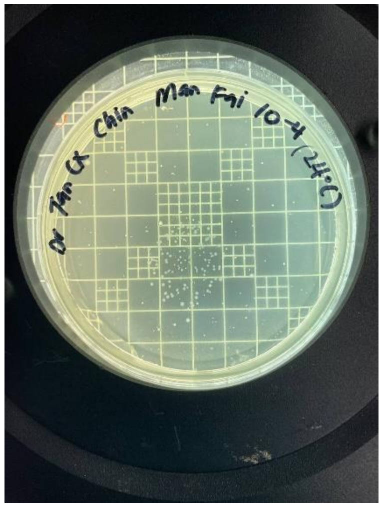

Figure 4.3.3e: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-4})

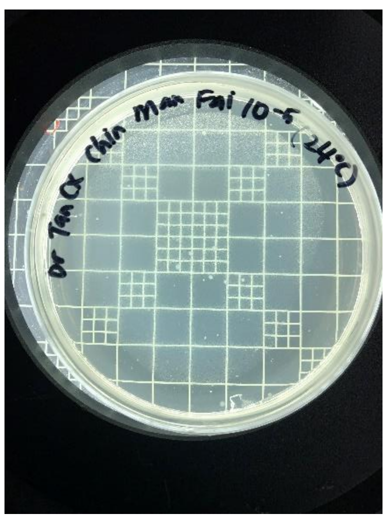

Figure 4.3.3f: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-5})

Figure 4.3.3g: Storage temperature of (24°C) Cold-Pressed Orange Juice (Dilution factor: 10^{-6})

CHAPTER 5

DISCUSSION

5.1 Effect of Storage Temperature on The Physicochemical Properties of The Cold-Pressed Orange Juices

5.1.1 Total Soluble Solids

Total soluble solid was utilized to assess the degree of sweetness of fruit juice (Choo et al., 2023). Cold-pressed orange juice kept in the refrigerator cold (4°C) after 12 days of storage had no change in total soluble solids compared to the control (Table 4.1). This is same to the findings of previous studies (Jerry and Bright, 2019; Anaya-Esparza et al., 2017). The retention of the total soluble solid may be caused by the decreased hydrolysis of sucrose and ingestion of reduced sugars by the minimal bacteria load in orange juice. Orange juices retain their total soluble solids throughout storage to maintain their quality such as pleasant taste experience (Bhardwaj and Pandey, 2011). Therefore, orange juice preserves its total soluble solids content when stored at a low temperature (4°C).

Nevertheless, after 12 days of storage, cold-pressed orange juice kept at room temperature (24°C) had less total soluble solid than the control (Table 4.1). Fruit juice’s sweetness ultimately decreased as the amount of soluble solids decreased. The increased respiration rate that takes place during storage, particularly at a higher temperature (24°C), can be blamed for the alterations in the total soluble solid of orange juice (Mishra and Kar, 2014). Fruit juice microorganisms thrive as a result of sucrose hydrolysis and the use of reducing sugar by microbes, which reduces the total soluble solid (Mishra and Kar, 2014; Jerry and Bright, 2019). This study, therefore, demonstrated that fruit sugar content may be influenced by storage temperature, which would ultimately result in a decrease in the total soluble solid of the juice.

5.1.2 pH

The pH scale was used to determine the amount of hydrogen ions present in fruit juice (Tan et al., 2023) and to represent the flavor, fragrance, and taste of fruit (Suriati et al., 2020). In comparison to the control, the pH of cold-pressed orange juice kept in the refrigerator cold (4°C) remained unaltered after 12 days of storage (Table 4.1). Other fruit juices kept at a low temperature (4°C) had a similar effect. The pH of thermo-sonicated soursop nectar did not significantly alter throughout 45 days of storage at 4°C (Anaya-Esparza et al., 2017). Additionally, thermally pasteurized pineapple juice did not significantly change in pH when stored in a refrigerator (Chia et al., 2012). Low temperatures (4°C) often prevent or slow the development and multiplication of microorganisms but frequently do not kill germs. According to Erkmen and Bozoglu (2016), the pH of the orange juice stayed stable during the storage temperature due to decreased microbial metabolic activity that led to a reduction in the conversion of the juice’s sugars to organic acids.

Nonetheless, compared to the control after 12 days of storage, cold-pressed orange juice held at room temperature (24°C) had a lower pH (Table 4.1). The pH drop is consistent with Touati et al. (2016) observed in grape, orange, and pear nectars that have undergone thermal processing. Orange juice stored at room temperature (24°C) showed a significant pH reduction, indicating that mesophilic bacteria grew more rapidly under ideal circumstances (Kaddumukasa et al., 2017). The acidic environment promotes the growth of harmful microbes that can withstand acid (Kaddumukasa et al., 2017). By responding to resources like sugars in the orange juice and producing organic acids, these pathogenic bacteria are able to live and trigger the production of spores and toxins, raising the pH of the orange juice (Kaddumukasa et al., 2017). Low pH encourages the growth of bacteria that can withstand acid, which can deplete nutrients and perhaps lead to the creation of spores and toxins. Therefore, reducing storage stability due to high microbial load and buildup of metabolic by-products that degrade and contaminate the juices (Kaddumukasa et al., 2017).

5.1.3 Color

Fruit juice's color is employed by the customers to evaluate the overall quality of the product and is connected to its organoleptic quality (Choo et al., 2023). Cold-pressed orange juice held in all storage conditions increased in L* and b* values after 12 days, while the a* values remained stable in comparison to the control (Table 4.1). The findings of the current study conflict with Jerry and Bright (2019), who found that at 4°C pasteurized soursop juice showed a decrease in L* value and b* value between the control (freshly pasteurized soursop juice) and the stored samples after 12 weeks, but not at 25°C, where it showed an increase in L* value and b* value.

The noticeable rise in the L* (whiteness) and b* (yellowness) values, which may be caused by the oxidative degradation of carotenoids, cis or trans alterations, and modifications to epoxide rings over the storage period, were primarily blamed for the visible change in the color of orange juice. According to Wibowo et al. (2015), carotenoids are the natural pigments that give orange juice its orange hue. Singlet oxygen molecules and peroxyl free radicals, which act as oxidants, are effectively scavenged by carotenoids. The influence of storage on the quality of orange juice has been strongly demonstrated by the correlation between the change in color of the juice and a considerable decline in its overall antioxidant capacity (Khaksar et al., 2019).

5.1.4 Browning Index

The browning index evaluates the level of purity of a juice's brown hue. When processing or storing juice, it is crucial to monitor this parameter to determine if enzymatic or non-enzymatic browning events take place. Cold-pressed orange juice held in all storage circumstances for a period of 12 days did not alter the browning index compared to the control (Table 4.1). The findings of the current study differ from those of Mohamad Salin et al. (2022), who found that after 9 days of storage, the watermelon juice showed a greater browning response at 25°C and 4°C than the control (freshly extracted watermelon juice).

In the current study, cold-pressed orange juice's browning index stayed constant compared to the control sample. This could be because orange fruit juice contains a lot of ascorbic acids, which might convert colored quinone to colorless phenol and stop the browning reaction from occurring (Landi et al., 2013).

5.1.5 Viscosity

Viscosity refers to the ability of a fluid to resist deformation when it is exposed to shear stress (Wong and Wong, 2013b). The viscosity of fruit juices is significantly influenced by varietal traits and the ripeness of the fruits at the time of processing (Aguiló-Aguayo, Soliva-Fortuny, and Martín-Belloso, 2010). Cold-pressed orange juice kept at room temperature (24°C) and refrigerator cold (4°C) for 12 days had less viscosity than the control (Table 4.1). After 12 days, there were significant decreases in viscosity between juice samples held at 4°C and 24°C when comparing the various storage temperatures. Similar observations were made by these researchers (Bhukya et al., 2021, Wang et al., 2021). The activity of pectinolytic enzymes including polygalacturonases (PG) and pectin methylesterase (PME) can affect viscosity variations. According to Aguiló-Aguayo, Soliva-Fortuny and Martín-Belloso (2010), pectin methylesterase partly hydrolyzes the methyl ester moiety of pectin, and polygalacturonases then cleave the polygalacturonic acid that results, reducing the viscosity of orange juice. The pectin's ongoing breakdown and the creation of polygalacturonic acid will result in a drop in the viscosity of the orange juice. Thus, this study showed that storage temperature may affect the sugar viscosity of fruit juices, which would ultimately cause the fruit juices' viscosity to decrease.

5.1.6 Titratable Acidity

The fruit juice's total acid content is measured by titratable acidity. According to Tan et al. (2023), titratable acidity is frequently used for determining the acidity of fruit juice. Cold-pressed orange juice kept in the refrigerator cold (4°C) maintained the same titratable acidity after 12 days of storage as compared to the control (Table 4.1). Other fruit juices kept at a low temperature (4°C) had a similar effect. The titratable acidity of pasteurized soursop juice did not significantly alter throughout the course of 12 weeks of storage at 4°C (Jerry and Bright, 2019). At low temperatures (4°C), microbiological growth and proliferation are limited. The inhibition of the conversion of the orange juice's sugars to organic acids through fermentation may have been the reason for the retention of titratable acidity (Kaddumukasa et al., 2017). This would have prevented the titratable acidity from changing during the course of the storage temperature.

On the other hand, cold-pressed orange juice kept at room temperature (24°C) for 12 days had higher titratable acidity than the control (Table 4.1). Anaya-Esparza et al. (2017) in unpasteurized and thermo-sonicated soursop nectars and Jerry & Bright (2019) in pasteurized soursop juice both noted an increase in titratable acidity. The buildup of organic acids derived from sugars produced through microbial organism's metabolic activities like fermentation, which resulted in an increase in titratable acidity, may have been the cause of the changes in titratable acidity of orange juice during storage at a higher temperature (24°C) (Kaddumukasa et al., 2017). As a result, this study demonstrated that storage temperature may affect the amount of fruit organic acids, ultimately raising the titratable acidity in juice.

5.2 Effect of Storage Temperature on The Nutritional Properties of The Cold-Pressed Orange Juices

5.2.1 Ascorbic Acid Content

Ascorbic acid is a crucial vitamin that has antioxidant properties and offers defense against free radicals. According to Jerry and Bright (2019), ascorbic acid is the primary element impacting the nutritional quality of the majority of fruit juices. Cold-pressed orange juice kept at room temperature (24°C) and refrigerator cold (4°C) after 12 days of storage loses ascorbic acid concentration compared to the control (Table 4.2). Significant drops in ascorbic acid concentration were seen between juice samples held at 4°C and 24°C after 12 days when comparing the various storage temperatures. These findings agreed with those published by Ibrahim (2016) in pineapple, watermelon, and pawpaw juice, Touati et al. (2016) in grape, orange, and pear nectars that had undergone thermal processing, and Jerry and Bright (2019) in pasteurized soursop juice.

According to Chia et al. (2012), ascorbic acid decrease during storage might be employed as a measure of quality and shelf-life indicator for items like citrus juice. Oxygen from the air causes ascorbic acid to degrade while being stored. Additionally, the presence of oxygen, exposure to light and heat, and the action of certain enzymes like ascorbate oxidase and peroxidase can initiate the oxidative process, leading to the loss of ascorbic acid. Ascorbic acid decomposition during storage is influenced by storage temperature and processing methods (Mgaya-Kilima et al., 2014). In general, low temperatures can slow down the rate of ascorbic acid deterioration, while juice samples kept at room temperature may have experienced significant [high] losses due to residual oxygen oxidation, followed by decomposition that may have been sped up by the storage temperature (Ibrahim, 2016). This demonstrates that ascorbic acid in orange juice degrades depending on the temperature.

5.2.2 Total Phenolics Content

Fruits contain a variety of phenolic compounds, which are secondary metabolites. Fruit juice's antioxidant action is mostly derived from phenolic compounds. Compared to the control, cold-pressed orange juice kept at room temperature (24°C) and refrigerator cold (4°C) after 12 days of storage has reduced total phenolic content (Table 4.2). Comparing the juice samples held at 4°C and 24°C over the course of 12 days, significant drops in total phenolic content were found. It was found in several studies that the storage temperature may have an impact on the rate at which phenolic compounds degrade (Kim et al., 2018; Khaksar et al., 2019; Lin et al., 2020; Mohamad Salin et al., 2022).

Due to their unstable structure, phenolic compounds are sensitive and may deteriorate under a variety of environmental circumstances, including light, pH, oxygen, temperature, and ions (Ali et al., 2018). Juice quality gradually declines as a result of the altered phenolic structure's diminished antioxidant capacity, which also adds to nutrient loss (Ali et al., 2018). According to the study, storage temperature, especially elevated storage temperature, had an impact on and ultimately enhanced phenolic compound breakdown. This confirms the conclusion of a recent study that total phenolic content is temperature-dependent (Mohamad Salin et al., 2022). Due to these factors, controlling the storage temperature is an effective strategy to prevent orange juice's phenolic compounds from degrading.

5.2.3 Total Antioxidant Capacity

According to Fallik and Ili (2021), an antioxidant is a substrate that delays prevent, or removes oxidative damage. Using a DPPH radical scavenging method, the antioxidant potency of orange juice obtained from a cold-pressed extractor was assessed. A methanol solution of the stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) has a rich violet hue. Over time, the color of the orange juice changes from deep violet to yellow as a result of the antioxidant substances in the juice scavenging the free radicals. By measuring the absorbance at 517 nm, the color shift may be assessed (Tan et al., 2022). Cold-pressed orange juice kept at room temperature (24°C) and refrigerator cold (4°C) after 12 days of storage loses antioxidant content overall compared to the control (Table 4.2). The total antioxidant capacity of the juice samples stored at 4°C and 24°C showed significant reductions after 12 days of storage. According to recent research, storage temperature has an impact on antioxidant capacity (Kim et al., 2018; Khaksar et al., 2019; Lin et al., 2020; Mohamad Salin et al., 2022).

According to the most recent findings, orange juice’s antioxidant content degraded quickly during storage. The storage temperature and duration exposures had a significant impact on the effectiveness of antioxidant activities (Mohamad Salin et al., 2022). A lower storage temperature may be able to counteract the declining DPPH scavenging activity since the effect of storage time on DPPH scavenging capacity was more pronounced at higher storage temperatures (Lin et al., 2020). Additionally, higher storage temperatures accelerated the breakdown of phenolic compounds and ascorbic acid, impairing their ability to scavenge free radicals (Zori et al., 2017; Zhang et al., 2021). According to the research’s findings, orange juice’s antioxidant scavenging capabilities degraded during the course of 12 days of storage and were influenced by storage temperature, which is consistent with the findings of an earlier study (Mohamad Salin et al., 2022). In order to prevent the degradation of phenolic compounds and ascorbic acid, which is connected to antioxidant capacity, orange juice should be stored at a low temperature.

5.3 Effect of Storage Temperature on The Microbiological Properties of The Cold-Pressed Orange Juices

Microbiological evaluation determines if fruit juice is acceptable based on the absence, presence, or quantity of microorganisms in a predetermined amount of sample, which is a component of food safety management. Cold-pressed orange juice kept in the refrigerator cold (4°C) for 12 days had the same microbial load as the control (Table 4.3). Other fruit juices kept at a low temperature (4°C) also showed a similar effect. The microbial load of unpasteurized soursop nectar did not significantly alter following 45 days of storage at 4°C (Anaya-Esparza et al., 2017). Additionally, no significant changes in microbial load were seen throughout the refrigeration storage of thermally pasteurized pineapple juice (Jerry and Bright, 2019).

Temperature, pH, food availability, and water activity are the variables that affect the development of microorganisms including bacteria and fungi (Pham, 2014). The "danger zone" for food safety is generally understood to be the range of temperatures between 5°C to 60°C when microbes grow most quickly (Singh et al., 2019). It can significantly affect the metabolic functions of bacteria at lower temperatures. At lower temperatures, enzyme activity, which is in charge of catalyzing chemical processes within the cell, is frequently diminished. This is because lower temperatures can make enzymes less active or inactive entirely (Daniel et al., 2008). Enzymes normally have a certain ideal temperature range at which they work most efficiently. Likewise, bacteria's ability to absorb nutrients may be hindered at lower temperatures. For development and reproduction, many bacteria need certain nutrients, and in order to use them, these nutrients must be delivered across the cell membrane. Lower temperatures, however, may cause the cell membrane to stiffen and become less flexible, which may decrease the microorganism's ability to absorb nutrients (Morita and Moyer, 2001). On top of that, DNA replication and protein synthesis both slow down at low temperatures, which can limit the pace at which microbes proliferate. As a result, the lag phase, during which microorganisms are adjusting to their surroundings and getting ready to develop, may end up being longer (D'Amico et al., 2006). Therefore, most bacteria that could be present in the juice develop more slowly due to the refrigerator's low temperature. As the bacteria take longer to develop to levels that might cause spoilage or foodborne disease, this slower growth rate may result in the juice having a longer shelf life.

Despite this, after 12 days of storage, cold-pressed orange juice held at room temperature (24°C) had a higher microbiological load than the control (Table 4.3). These researchers noted similar findings (Jerry and Bright, 2019). On fruits and vegetables, microorganisms like bacteria, yeast, and mold are naturally present. These bacteria may be introduced to the juice during the cold-pressing process of oranges. Freshly extracted orange juice has a relatively low initial microbial load, but if it is kept in an environment that encourages the growth of microorganisms, the load might eventually rise (Osman Erkmen and Faruk Bozoglu, 2016). One of the key elements influencing the development of microbes in food is temperature. Microorganisms can only grow and replicate within a restricted temperature range. This range is between 5°C to 60°C for the majority of bacteria. Because it permits bacteria to proliferate quickly and might result in food spoiling and probable foodborne disease, this temperature range is referred to as the "danger zone" for food safety (Singh et al., 2019). The danger zone for microbial development is reached when cold-pressed orange juice is kept at room temperature (24°C), creating ideal conditions for microbe growth. Thus, as a result of microorganisms growing more quickly due to the higher temperature, the microbial load of the juice gradually increases.

5.4 Significance of Results

The physicochemical features of cold-pressed orange juice may be impacted by storage temperature, which is a key finding of this study on how storage temperature affects the juice's nutritional, microbiological, and physical characteristics. The strength of this study is that it allows for a comparison of the changes in these qualities throughout the course of 12-day storage at two distinct settings (room temperature and refrigerator cold). It provides insight into the degree of juice degradation under various storage settings that may be gained from the rate of change in these qualities. Furthermore, the temperature at which cold-pressed orange juice is stored might have an impact on its nutritional quality. Analyzing the variations in these variables during the course of storage at various temperatures for a 12-day period will reveal the magnitude of this effect. The influence of the temperature of storage on the orange juice's nutritional content may be evaluated with the use of this information. Moreover, the storage of cold-pressed orange juice might make it vulnerable to microbial deterioration. The microbial load during storage at various temperatures for a period of 12 days can be utilized to assess the intensity of the influence of storage temperature on the juice's microbiological quality. This data may be used to assess how well different storage temperatures inhibit microbial development and maintain the juice's safety. By analyzing the changes in these characteristics during the course of storage at various temperatures for a set period of time, the overall strength of the research may be ascertained. This information may be used to determine the juice's ideal storage temperature in order to preserve its quality and safety.

5.5 Limitations of Study

Apart from that, the lack of practical applicability in real-world settings is the potential limitation of the study on how orange juice qualities are affected by storage temperature. The results may not entirely apply to situations in the real world when juices are held in less controlled circumstances (such as during shipping or in a home refrigerator) if the research was carried out under well-controlled conditions in the laboratory. This may restrict the findings' applicability to other contexts and make it challenging to draw reliable conclusions about how storage temperature affects juice qualities in real-world situations. In addition, different methods of analysis can produce various findings, which could have an impact on how cold-pressed orange juice's physicochemical, nutritional, and microbiological attributes changed during the course of storage. When determining the juice's ascorbic acid content, total phenolic content, and overall antioxidant capacity, for instance, various methodologies might produce varying findings and more than one antioxidant capacity assays should be used to compare the results. Therefore, it is crucial to measure the intended qualities of the juice using consistent, reliable, and standardized analytical procedures such as ultra-performance liquid chromatography (UPLC) should be used to analyze the vitamin C content. Not only that, but there is also a chance that the original microbial population of the cold-pressed orange juice will differ, which may have an impact on how quickly bacteria multiply when they are stored. Microbial development has the ability to affect the sensory qualities of the juice, and potentially endanger consumer health. Therefore, to reduce the juice's original microbial load, adequate sanitation and handling procedures should be followed. Microbial studies should also be carried out to track microbial development throughout storage. Additionally, microbiological studies should also employ suitable detection techniques, such as selective media and PCR-based approaches, to precisely ascertain the presence and amount of specific food-borne bacteria. The study also left out sensory evaluation, which is crucial for assessing whether the juice is generally acceptable, such as taste and scent analyses. Evaluation of the senses is essential since it establishes customer preferences and acceptability. As a result, sensory analysis is required to ascertain the general quality of the juice and how storage temperature affects it. Overall, there are several constraints that should be considered even if the proposed study might offer useful information on how storage temperature affects the physicochemical, nutritional, and microbiological attributes of cold-pressed orange juice.

5.6 Recommendation For Future Studies

Future study on the impact of storage temperature on orange juice attributes has made several key recommendations, including the need for constant monitoring of storage conditions, particularly temperature, to guarantee that the juice is kept at the right temperature. Temperature loggers, which take frequent temperature readings, or other monitoring tools can be used to do this. These gadgets offer real-time information on the storage conditions, making it simpler to spot any temperature variations that can compromise the juice quality. For instance, if the temperature exceeds the advised range, this might speed up microbial development and affect the juice's quality. In order to avoid any possible risks, storage conditions must be regularly monitored. Another crucial issue is that routine microbiological testing is required to keep track of microbial development in the juice during storage. One of the main reasons juice spoils and changes in texture, flavor, and color is bacterial contamination. Having harmful bacteria around can potentially result in foodborne diseases. In order to avoid any potential risks, routine microbiological analysis can aid in the early detection of any microbial growth. The analysis entails taking a sample of the juice and utilizing several techniques, such as coliform count, to check for microbial contamination. Notably, a nutritional analysis of orange juice is required to keep track of any modifications to its nutritional composition that may occur during storage. One of the elements that affect the juice's quality and shelf life is nutrient degradation. Ascorbic acid and phenolic molecules are two nutrients whose concentration might change over time because of their sensitivity to oxidation and heat. The juice's shelf life and the ideal storage conditions for preserving its nutritious value may thus be determined with the use of routine nutritional analysis. In the examination, the juice is examined for its nutritional content using a variety of techniques, including spectrophotometry or high-performance liquid chromatography (HPLC). The outcomes can be used to determine any nutrient deterioration and the ideal storage circumstances for preserving the juice's nutritious integrity. The research on how juice's physicochemical, nutritional, and microbiological attributes are affected by storage temperature, therefore, emphasizes the significance of monitoring, microbial analysis, and nutritional analysis. By keeping an eye on these elements, juice's quality and shelf life may be improved, cutting down on food waste and raising customer satisfaction.

CHAPTER 6

CONCLUSION

In this study, cold-pressed orange juice is exposed to storage temperatures (4°C & 24°C) for 12 days, which causes physicochemical, nutritional, and microbiological attributes to degrade, reducing the juice's quality, nutritional value, and shelf life. All storage temperatures resulted in a decline in the quality of cold-pressed orange juice. The deterioration trend, however, varies depending on the storage temperatures and circumstances. The findings indicated that refrigerator cold (4°C) storage for 12 days is the ideal temperature for cold-pressed orange juice. Fresh cold-pressed orange juice intake has been proven to be fairly nutritious and healthy. Cold storage in a refrigerator, however, may be employed to preserve the majority of the juice's physicochemical, nutritional, and microbiological attributes while delaying the deterioration of the juice. The results of the current work offer a practical method for storing cold-pressed orange juice, but more research is needed to determine the kinetic shelf-life of orange juice under various circumstances (freeze-dried and refrigerator freeze) and to determine the best ways to do so while preserving the majority of its quality and nutritional value.

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APPENDICES

Appendix A

FM-IAD-005 Form

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

UTAR UNIVERSITI TUNKU ABDUL RAHMAN

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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	Chin Man Fai
ID Number(s)	20ADB03024
Programme / Course	Bachelor of Science (Hons) Dietetics
Title of Final Year Project	Effect of storage temperature on the physicochemical, nutritional and microbiological properties of cold-pressed orange juice

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 10 **%** **Similarity by source** Internet Sources: 6 % Publications: 6 % Student Papers : 3 %	
Number of individual sources listed of more than 3% similarity: 0	

Parameters of originality required and limits approved by UTAR are as follows:

(i) Overall similarity index is 20% and below, and

(ii) Matching of individual sources listed must be less than 3% each, and

(iii) Matching texts in continuous block must not exceed 8 words

Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

TanCX

Signature of Supervisor

Name: Tan Chin Xuan

Date: 28/4/2023

Signature of Co-Supervisor

Name: ______________________

Date: ______________________

Appendix B

Summary page of the Turnitin Originality Report

Document Viewer

Turnitin Originality Report

Processed on: 28-Apr-2023 17:09 +08
ID: 2077981590
Word Count: 14679
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CHIN MAN FAI_FYP THESIS By Chin Man Fai

Similarity Index	Similarity by Source	
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Lucía Plaza, Concepción Sánchez-Moreno, Pedro Elez-Martínez, Begoña de Ancos, Olga Martín-Belloso, M. Pilar Cano. "Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization", European Food Research and Technology, 2006

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