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Influence of storage temperature and time on the physicochemical properties of fruit juice: a leading concern for human health

By

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DEDICATION

- I dedicate this achievement to my Father and my wonderful mother. They have been by my side since the very beginning with endless love and support. So much so that my dream became their dream.
- To my dear brothers and sisters.
- To my supportive close friends and colleagues.
- To our Assistant professor Dr. Shaida Anwar kakil

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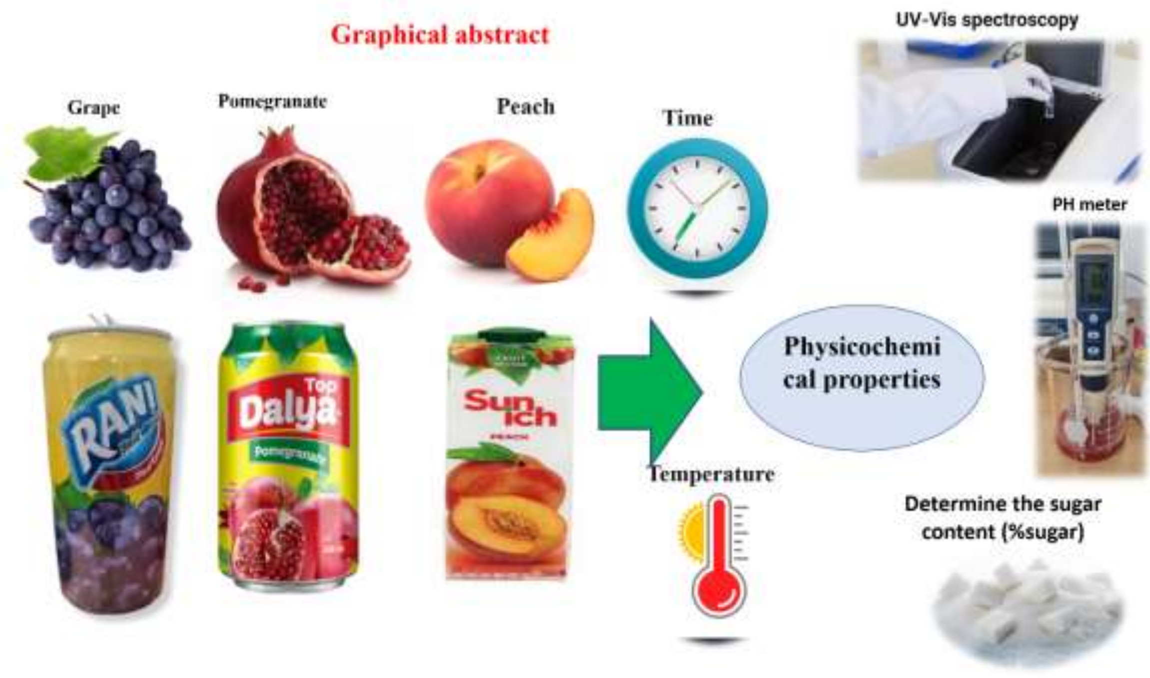
Graphical Abstract



Abstract

This study investigates the significant impact of storage temperature and duration on the physicochemical attributes of both fruit juice and fresh fruit juice, highlighting their critical role in addressing human health concerns. Employing a multifaceted approach, UV-Visible spectroscopy, and pH meter analysis, a comprehensive examination was conducted to discern molecular changes, compositional shifts, and acidity variations induced by diverse storage conditions. UV-visible spectroscopy elucidated changes in chromophore composition and absorbance patterns, offering valuable information on antioxidant content and overall quality fluctuations. Additionally, pH meter analysis captured fluctuations in acidity levels, reflecting the influence of storage conditions on juice freshness and stability. These findings underscore the susceptibility of fruit juice and fresh fruit juice to deterioration under varying storage conditions, emphasizing the urgency of implementing proper storage practices to mitigate potential health risks. Integration of spectroscopic techniques and pH measurements enhances our understanding of the underlying mechanisms governing these transformations, facilitating the development of targeted preservation strategies. Addressing these concerns is paramount for safeguarding public health and ensuring the nutritional integrity of fruit-based products in the market.

List of Contents

Chapter Five
Conclusions and Future Work

Chapter one
Introduction

1.1 Background of the study

The sweetness is a taste sensation that is difficult to measure scientifically; therefore, the sugar content is measured as an alternative (Harker *et al.,* 2002)Practical methods currently used for measuring the sugar contents in a fluid include Brix gauge probing and chemical analysis. Brix gauge probing is traditional, accurate and effective, but can only be operated manually (Lynnworth, 1989) which is unacceptable in automated food processing.

. Low-power ultrasound and infrared which are emitted from equipment isolated from the target material are a well-studied candidate in food processing, as they do not introduce extraneous contamination nor alter the properties of the substance (Kuo, Sheng and Ting, 2008a).It is cheap and has been used by the authors for various inspection of food products. It has been well understood that the propagation of ultrasound in a fluid is affected by the density, compressibility, temperature and composition of the fluid (Harker *et al.,* 2002). Thus, ultrasonics has been utilised for more than half a century as an effective tool in measurements of suspensions in milk ((Hueter, Morgan and Cohen, 1953) and density of fluid (The Brix index of fluid is directly linked with the concentration of the dissolved sugar (Boulton et al., 1996, McClements, 1995). Hence, we may expect a correlation between the Brix index of fluid and the behaviour of the ultrasound travelling in the fluid. A similar correlation can also be expected on viscosity since a fluid with more sugar becomes more sticky and (Pryor and Roscoe, 1954)hence has a higher viscosity (Greenwood, Adamson and Bond, 2006) , Both pulse-echo (PE) and transmission-through (TT) ultrasonic techniques are employed in this study to investigate how the sugar content in orange juice affects the nature of ultrasound propagation. Ultrasound for measuring sugar content . Accurate density measurement combined with ultrasonic velocity measurement is a widely used and indispensable method for determining the adiabatic compressibility of fluids (Nölting, 1995)and a wide range of thermodynamic parameters (Ewing, 1993)The velocity of sound in aqueous sugar solution appears to correlate with the temperature, sugar concentration, and solution density (McClements, 1995) Different sugar compositions exhibit different characteristics in acoustic velocity. Using different ranges of electromagnetic radiation provides information about the different properties of samples.(Nicolai *et al.,* 2007).The spectra in this range contain bands coming from overtones and combination tones of basic vibrations of groups having a hydrogen atom, mainly C–H, O–H and N–H chemical bonds. Thus, the NIR spectra enables study of organic major and minor food constituents, containing these structures. The NIR spectra may be measured directly for samples in different forms without the need for dilution or other preparation.(Nielsen, 2010).The bands in the UV-VIS spectra correspond to different chromophores presented in foods such as carbonyl groups, nitro groups, double and triple bonds, conjugated double bonds, etc. The absorption in VIS region, responsible for food color, is due to the presence of pigments with conjugated double bonds systems.

The use of these spectroscopic techniques for quality assessment is based on the development of multivariate calibration models. This technique can also be useful for testing juice. Several studies have shown the possibility of using NIR spectroscopy for apple juice analysis, particularly to predict sugar content in apple juice (Temma, Hanamatsu and Shinoki, 2002a), detect adulteration (León, Kelly and Downey, 2005a)وdetect deterioration of apple juice during storage and heating Zhu *et al.*, 2011) and determine quality parameters of commercial apple juice łodarska, Khmelinskii and Sikorska, 2018). Only few papers reported application of UV or UV-VIS spectroscopy for determination quality parameters of apple juice (Chang *et al.,* 2016a)(Iqbal *et al.,* 2020a) A rapid and low-cost screening method proposed to detect adulterations of pomegranate juices. Potential application for detecting addition of filler juices and water to pomegranate juice. In these last years, interest in functional foods is increasing in almost all in dustrialised countries ((Bech-Larsen and Scholderer, 2007), (Siro *et al.,* 2008). In particular, fruit juices – and, among them, pomegranate (PG) juices – are considered a good source of phenolic compounds with strong antioxidant activity

((Watson and Preedy, 2009), whose consumption could improve cardiovascular health and inhibit the proliferation of many cancers ((Braga *et al.,* 2005), Malik *et al.*, 2005),(Noda *et al.*, 2002), *t al.*, 2014b)(Vidal *et al.,* 2003). PG contains a significantly high level of powerful antioxidants ellagitannins such as ellagic acid, punicalagin and punicalin ((Lansky and Newman, 2007), (Visioli and Hagen, 2007)), as well as anthocyanins Several studies have even shown that authentic PG contain much more antioxidant compounds than other common fruit juices and beverages ((Gil *et al.,* 2000). UV–VIS spectroscopy was used recording for each sample the whole UV–VIS spectrum. In this way, each sample analysed was described by a vector of absorbances, which can be considered a fingerprint of that sample. Since many studies have established that PG have superior antioxidant activity compared to other fruit juices, the antioxidant activity of all the samples was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method an easy method commonly implemented for measuring the antioxidant activity of fruit and vegetable juices or extracts (Gil *et al.,* 2000), .

Fruit juice as a primary part in the beverage industry is growing fast with the development of food processing techniques. Many different kinds of fruits are processed into juices but orange juice still takes an important part in the beverage industry sold around the world. Both pure juice and beverage with different concentrations of orange juice are demanded in the market. Due to the loss of some nutrients in the process of fresh orange juice, cheaper juices, sugars, acids, colorants, water and other additives are often added into the pure juice to adjust the flavor and color in that the problem of adulteration of beverages consequently appears. Fruit quality is essential for human beings due to its excellent tests like acidness, sweetness and bitterness. These tests make feeling in the mouth of a human being due to its structures. The quality of juices has a great role in balancing factors such as acidness, sweetness, and bitterness, which are the most important factors for peoples. Particularly the corrosive and sugar substance in natural products and their proportion are exceptionally vital components for the quality of assessment by buyers (Gebishu *et al.*, 2022). Quality of natural products such that mango and orange juice were investigated by a spectroscopic technique such as UV/Visible and Fluorescence in Japan, Denmark, and China. Still now, testing natural product juices was not investigated in Ethiopia. The main objective of this study is to investigate the quality of mango and orange fruit juice by UV/Vis and Fluorescence spectroscopy.(Gebishu *et al.*, 2022). World Health Organization, low fruit and vegetable consumption is estimated to cause some 2.7 million deaths each year and was among the top 10 risk factors contributing to mortality (Reducingrisks, 2002). Thus, current evidence supports a significant association between fruit and vegetable intake (recommending the consumption at least five servings each day) and health (Who and Consultation, 2003), (Tucker *et al.,* 2005), (Liu, 2004) This protective action has been attributed mainly to their bioactive compounds, which have antioxidant properties. In this sense, vitamin C is the most important water-soluble antioxidant nutrient that contributes to antioxidant defense against oxidative stress. This fact is related with its ability to function as a reducing agent and as an oxygen scavenger (Kitts, 1997). Traditionally, it has been pasteurized by heat treatment to prolong its shelf life.

Some investigations have showed that haze formation was mostly associated with the presence of protein and polyphenol in juice (Van Buren and Way, 1978a), (Siebert, 1999), (Beveridge and Wrolstad, 1997), (Calderon, Van Buren and Robinson, 1968). In fruit juices, one of the main problems posed is its unstable colour after bottling. Both enzymatic and nonenzymatic browning have been implicated in the darkening that occurs in fruit juices during processing and storage, (Toribio and Lozano, 1986), (Toribio and Lozano, 1984), (Nicolas *et al.,* 1994),(Beveridge, Harrison and Weintraub, 1997) , with the nonenzymatic (Maillard) type predominating during storage, (Cornwell and Wrolstad, 1981). With enzymatic browning, there is initial enzymatic oxidation of phenols into slightly coloured quinones which undergo subsequent reactions to form coloured pigments. (Tajchakavit, Boye and Couture, 2001)showed that temperature may play an important role in the reactions leading to increased browning in apple juice during storage. Nutritional quality of food during storage has become increasingly important problem. The loss of some nutrients such as ascorbic acid (vitamin C) might be a critical factor for the shelf life of some products as citrus juice concentrates (Laing, Schlueter and Labuza, 1978) since vitamin C content of citrus juices undergoes destruction during storage,(Johnson, Braddock and Chen, 1995) , (Lee and Nagy, 1988), (Solomon, Svanberg and Sahlström, 1995). Ascorbic acid (AA) is an important component of our nutrition and used as additive in many foods because of its antioxidant capacity. It is necessary to describe ascorbic acid degradation and investigate kinetics of AA loss in stored citrus juices. Fruit juice is a popular choice of beverage, and the fruit juice market is one that has grown substantially over recent years Fruit juice is a drink consisting of 100% pure fruit juice, which typically contains no preservatives or other added ingredients [British Soft Drinks Association (BSDA 2009)]. However, they may undergo high-pressure treatment and/or modifiedatmosphere packaging to increase their shelf life. From concentrate In the case of juices ‘from concentrate’, the juice is extracted from the fruit in exactly the same way as described previously. However, the extracted fruit juice is then concentrated (by evaporating the water naturally present in the juice) (BSDA 2009). This concentrate is often frozen before being transported to its destination, where water is added back to reconstitute the juice to 100% fruit juice (or alternatively, it can be used as an ingredient in a cordial/squash drink). The type of heat treatment the juice is subjected to affects the shelf life of the product: Long-life products These products are pasteurized by exposing them to a temperature of approximately 90°C for a short time period (10–20 seconds). They are then packaged into sterilized containers where the air is removed, so the products remain sterile.Short-life/chilled products A lower temperature (70–75°C) is used in the pasteurization of chilled products, which are again heat-treated for approximately 10–20 seconds.(Caswell, 2009). Anthocyanins are responsible for the brilliant red color and its different hues in many fruits and berries. Attractive color is one of the main sensory characteristics of fruit and berry products, and this important quality parameter strongly affects consumer behavior. Unfortunately, the color of red juices is unstable and easily susceptible to degradation leading to a dull, weak, and brownish juice color. The color stability of anthocyanins is influenced by pH, storage temperature, presence of enzymes, light, structure and concentration of the anthocyanins, and the presence of other berry compounds such as other flavonoids and phenolics , (Markakis, 1982) This technique can also be useful for testing juice. Several studies have shown the possibility of using NIR spectroscopy for apple juice analysis, particularly to predict sugar content in apple juice(Temma, Hanamatsu and Shinoki, 2002b) , detect adulteration (León, Kelly and Downey, 2005b), differentiate between the juices based on heat treatment and apple varieties ,(Reid *et al.*, 2005) detect deterioration of apple juice during storage and heating (Zhu *et al.*, 2011)and determine quality parameters of commercial apple juice (Włodarska *et al.*, 2021). Only few papers reported application of UV or UV-VIS spectroscopy for determination quality parameters of apple juice(Chang *et al.*, 2016b) , (Iqbal *et al.*, 2020b) In routine analysis of fruit juices, usually only selected parameters are controlled, including soluble solids content (SSC) and titratable acidity (TA), related to the sensory attributes and stability of juice. The ratio of soluble solids content to titratable acidity (SSC/TA) is also determined as an index of sensory acceptability of the fruit taste (Abu-Khalaf and Bennedsen, 2004b) Important constituents of apple juice are phenolic compounds. They

affect organoleptic properties of juice: their color, flavor, and astringency (Robbins, 2003). Phenolics due to their antioxidant properties account for the health benefits to humans related to consumption of apple juice (Kalinowska *et al.*, 2014). Beta-carotene, known as provitamin A, is the most common carotenoid in fruits with the highest vitamin A activity (Stinco *et al.*, 2019). Beta-carotene as a natural fat-soluble compound in many flowers and fruits and a strong antioxidant and scavenger of singlet oxygen, beta-carotene contributes to the majority of yellow,
red and orange colorations (Marx, Schieber and Carle, 2000). Because of the features mentioned above that could confer them with a preventive role in cardiovascular disease and cancer, measuring the amount of beta-carotene in commercial fruit juices (e.g., carrot, mango, orange, and apple) and quality control of this products is very important(Sakaew *et al.*, 2018) . Beta-
carotene is sensitive to degradation and especially to oxidation due to its substantial number of double bonds. The beta-carotene lose is mainly observed during fruit processing, i.e., juice or puree production. In fact, the loss of tissue integrity, temperature increase and exposure to oxygen and light during thermal treatment drastically increase the rates of destruction reactions (Pénicaud *et al.*, 2011) The methods proposed to determine beta-carotene include HPLC (Brabcová *et al.*, 2013) ultra-performance liquid chromatography (Delpino-Rius *et al.*, 2014) liquid chromatography-mass spectrometry fluorometry near-infrared spectroscopy and thin layer chromatography-densitometry UV-Vis spectroscopy. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes. In this method, determining the analyte concentration in the unknown sample is very simple and fast. Measuring the analytes in the complex samples is, however, difficult and sample preparation, extraction and pre-concentration steps are required before measurement. Although the amount of Beta-carotene in commercial fruit juices may be higher than the detection limit of a UV/Vis spectroscopy method, however, the complexity of the matrix in these samples does not allow direct measurement of it. Methods of extraction that are used before the final spectrophotometric determination of analytes therefore include solvent extraction and partitioning (Zheng *et al.*, 2015)dispersive liquid-liquid microextraction (Sereshti, Ahmadvand and Asgari, 2014)and solid phase extraction (Ma and Lin, 2004) Mango (Mangifera indica L) is one of the most important and widely cultivated fruits of the tropical and subtropical world (Akhter *et al.*, 2012)It is also known as the king of the tropical fruits (Akram *et al.*, 2021). It is an excellent

source of fiber, vitamins A, C, and B complex, iron, and phosphorus (Akhter *et al.,* 2012). Many studies have been conducted on physicochemical and antioxidant properties of roselle extract (Tsai and Huang, 2004). However, few studies have been conducted on roselle-fruit juice blends, and practically none on the effects of storage time and temperature on roselle-fruit juices. The aim of the present study was to investigate the influence of storage time and temperature on the physicochemical and antioxidant properties of roselle-fruit blends stored in plastic bottles.

1.2 Research Objectives

- This study investigates the influence of storage temperature and time on the physicochemical properties of both commercially available and fresh fruit juices(grapes, peach, and pomegranate, emphasizing their significance for human health.
- Storage temperature and duration represent critical variables that can exert a substantial impact on the physicochemical characteristics of fruit juices, irrespective of their commercial or freshly prepared origin.
- UV-visible spectroscopy was employed to analyze key parameters such as absorbance, color, and chemical composition
- The study discusses the implications of these changes for human health, highlighting concerns related to potential alterations in the nutritional content of fruit juices. UV-Visible spectroscopy and Ph meter emerge as a valuable tool for monitoring and understanding these changes, offering insights that can guide both producers and consumers in making informed decisions.
- In addition to our spectroscopic differentiation between natural and commercial juices, an important aspect is the examination of sugar content in juices and its potential correlation with diabetes. This aspect warrants further investigation as it holds significance in understanding the health implications associated with different types of juices.

1.3 Thesis Organization

The thesis is organized in the following way:

Chapter 1: Introduction and some background information on fruit juice

Chapter 2: Physiochemical properties of Fruit juice, Factor affecting fruit juice quality, Importance of storage conditions , Some main compound of juice and advantages and disadvantages.

Chapter 3: Materials and Methods, Description of fruit juice sample , Experimental setup for UV-Visible and FTIR spectroscopic analysis , pH Measurement of Fruit Juice , Procedure for determination the % (w/v)sugar and Mendeley and Origin8.5.

Chapter 4: Result and Discussion**,** Comparison of Ingredients in Marketed and Fresh Juice, UV/visible spectroscopic investigation of both commercially available and fresh fruit juices (grapes, peach, and pomegranate)quality in case of Erbil City , PH Measurement Juice Commercial and Fresh Juice , Determination the % (w/v)sugar (Commercial -Juice and Fresh juice).

Chapter 5: Conclusions and Future Work and references

Chapter two
Physiochemical properties of Fruit juice

2-1 Introduction

The physicochemical characteristics of juices considered in quality assessment include pH, titratable acidity (TA), total soluble solids (°Brix), dry matter contents, ash content, crude protein, ascorbic acid, total sugar, reducing sugar and °Brix (sugar)/acid ratio.(Nonga, Simforian and Ndabikunze, 2014)

One of the primary physiochemical properties of fruit juice is its pH level, which measures the acidity or alkalinity of the juice. The pH of fruit juice can vary significantly depending on the type of fruit used and its ripeness. in fruit juice analysis, both absorbance and transmission measurements are essential for characterizing the optical properties of the juice and identifying key components contributing to its color, flavor, and nutritional content. By analyzing the UV-visible spectra of fruit juice samples, researchers can gain valuable insights into their chemical composition, quality, and authenticity, supporting various applications in food science, quality control, and product development within the fruit juice industry.

In this Chapter, we aim to delve deeper into the physiochemical properties of fruit juice, focusing on a specific range of fruits and their corresponding juices. Our objective is to analyze and compare various parameters such as pH, sugar content, organic acid composition, color attributes, and other relevant factors across different fruit juices.

2.2 Factor affecting fruit juice quality

1. The exposure of juice to sunlight can have a significant impact on the vitamins present, potentially resulting in alterations to their composition and affecting the overall nutritional quality of the juice. (El-Ishaq and Obirinakem, 2015)
2. Effect of Sunlight Exposure on Anthocyanin and Non-Anthocyanin Phenolic Levels in Pomegranate Juices. (Di Stefano *et al.*, 2020)
3. reducing ascorbic acidity and antioxidant activity. (Mathew *et al.*, 2018a)
4. The pigments responsible for the color of peach juice may be sensitive to sunlight. Extended exposure can lead to color changes, potentially causing a shift in the appearance of the juice. (Minas, Tanou and Molassiotis, 2018)
5. Peach juice contains antioxidants that contribute to its health benefits. Sunlight exposure may affect the stability of these antioxidants, potentially diminishing the juice's ability to combat oxidative stress. (Wang et al., 2020
6. Storage Condition
7. The method used to extract juice from oranges can influence the sensory and nutritional characteristics of the final product. Common extraction methods include mechanical squeezing, reaming, and pressing. Gentle extraction methods help minimize damage to the juice and preserve flavor compounds.(Akyıldız, Dundar Kirit and Ağçam, 2023)
8. Packaging juice and techniques, such as glass bottles, plastic containers, or Tetra Pak cartons, play a significant role in maintaining the quality of orange juice. Packaging should provide adequate protection against light, oxygen, and moisture to preserve flavor and nutritional integrity.(Youssef, 2013) .

2.3 Importance of storage conditions

Moreover, sugar content for juice had minimum and maximum decrease at refrigeration and ambient temperatures, respectively. Storage at ambient temperature resulted in growth of microbes which was observed after 15 days for juices without preservatives and 75 days for juices with preservatives, with no E. coli growth.(Aluko, Kassim and Makule, 2023).

2.4 The main Compound of juice:

2.4.1 Citric acid

(2-HYDROXY-1,2,3-PROPANETRICAR- BOXYLIC ACID) is a weak tricarboxylic acid that is naturally concentrated in citrus fruits. At physiologic blood pH, and to a lesser extent in urine, it exists mainly as the trivalent anion. Citric acid is frequently used as a food additive to provide acidity and sour taste to foods and beverages Citrate retards stone formation by inhibiting the calcium oxalate nucleation process and the growth of both calcium oxalate and calcium phosphate stones,(Penniston *et al.,* 2008)

2.4.2 Stabilizer

A preotic inhibitor of pectin methyl esterase (PME), recently discovered in kiwi, was used to stabilize cloud of orange juice concentrate with "Cut-back," 42" Brix. When this colloid is demethylated by pectin methyl esterase PME), naturally present in juice, it causes clarification of the juice because of rapid sedimentation of the solid phase. This is due to formation of free carboxyl groups by PME action on pectin Phytosterol and polyethylene glycol 400 were used as stabilizers at four different concentrations .particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency The formulation stabilized with phytosterol showed higher ES% over 30 days storage period and after pasteurization heating.(Castaldo *et al.,* 1991)

2.4.3 Calcium lactate

Calcium fortification of fruit juice has become increasingly popular in recent years. Commercially available orange, grapefruit, and grape juices as well as lemonade and fruit punch are fortified with various forms of calcium in order to increase their nutritional value Calcium lactate is used by the beverage industry as a source of calcium to fortify fruit juice. The objective of this study was to evaluate the influence of various concentrations of calcium lactate on the fate of pathogenic and spoilage microorganisms in orange juice. The pH of each fortified juice was adjusted to 3.6 or 4.1 . juice can be an effective delivery vehicle for calcium(Yeh, Hoogetoorn and Chen, 2004)

2.4.4 Ascorbic acid

content of commercial fruit juices and its rate of loss with respect to time and temperature of storage were determined. Ascorbic acid content of commercial fruit juices ranged from 2.4 to 43 mg/100 ml of juice.

Fruit juices are a significant source of ascorbic acid for humans and their consumption in the last years increased at very quick rates. .(Kabasakalis, Siopidou and Moshatou, 2000)

2.4.5 Sugar

The sweetness is a taste sensation that is difficult to measure scientifically; therefore, the sugar content is measured as an alternative

Fruit juices have been traditionally concentrated by multi-stage vacuum evaporation, resulting in a loss of fresh juice flavors, color degradation and a “cooked” taste due to the thermal effects. The promising alternative is reverse osmosis concentration. However, it cannot reach concentrations larger than 25–30°Brix with a single-stage RO system due to high osmotic pressure limitation, which is quite below the value of 45–65°Brix for standard products obtained by evaporation.(Kuo, Sheng and Ting, 2008b)

Free sugars overconsumption is associated with an increased prevalence of risk factors for metabolic diseases such as the alteration of the blood lipid levels. Natural fruit juices have a free sugar composition quite similar to that of sugar-sweetened beverages.

Moreover, the current literature strongly suggests that ingestion of sugar-sweetened beverages increases the cardiometabolic risk and risk factors more than is ocaloric amounts of complex carbohydrates , Free sugars are defined as any types of simple sugars (monosaccharides or disaccharides) that have been added to beverages or food products during their transformation or preparation by food industries or by the consumer per se, plus sugars naturally present in fruit juices, fruit juice concentrates, honey, and various syrups While it is mainly accepted that the overconsumption of sugar-sweetened beverages may lead to adverse effects on health The World Health Organization (WHO) recommends reducing the intake of free sugars to less than 10% (and, ideally, less than 5% of total daily energy intake, thus including sugars naturally present in fruit juices in the category of sugars whose consumption should be reduced.(Pepin, Stanhope and Imbeault, 2019) , Sugar concentrations in OJ were measured according to methods published by the International Federation of Fruit Juice Producers (IFU). More precisely, glucose and fructose concentrations were measured according to method IFUMA 55 and sucrose according to method IFUMA56 . (Chanson-Rolle *et al.*, 2016)

2.5 Advantages and Disadvantages

Advantage**;**

1.anti-atherosclerotic

2.anti-hypertensive

3.Antioxidant

4. anti-inflammatory effects in human subjects

5.decreased systolic blood pressure
6.treat osteoarthritis
7. skin cancer chemoprevention (Stowe, 2011)

Disadvantage:
1.Coronary heart disease
2. It causes type 2 diabetes
3. different beverages have erosive potential on teeth depending on the duration of the exposure.
increased surface enamel loss (Stowe, 2011).
4-CVD(cardiovascular disease) .(Mathew *et al.,* 2018b).

Chapter Three
Materials and Methods

3.1 Introduction

The explorations were carried out with the assistance of the taking after gadgets: containers, spatula, column tubes,
inquires, advanced electronic bar adjustments, cuvette used to take the samples for investigation, pipette used to
sort, juice extractors, cone-shaped bottles, as described below

3.2 Description of fruit juice sample

Juice is a drink made from the extraction or pressing of the natural liquid contained in fruit and vegetables. And we have two types of juice Fruit juice and industry juice. as shown in Fig.3.1 and Fig.3.2.

Figure 3.1 illustrates the three types of commercial juice and fresh fruit juice evaluated in the study

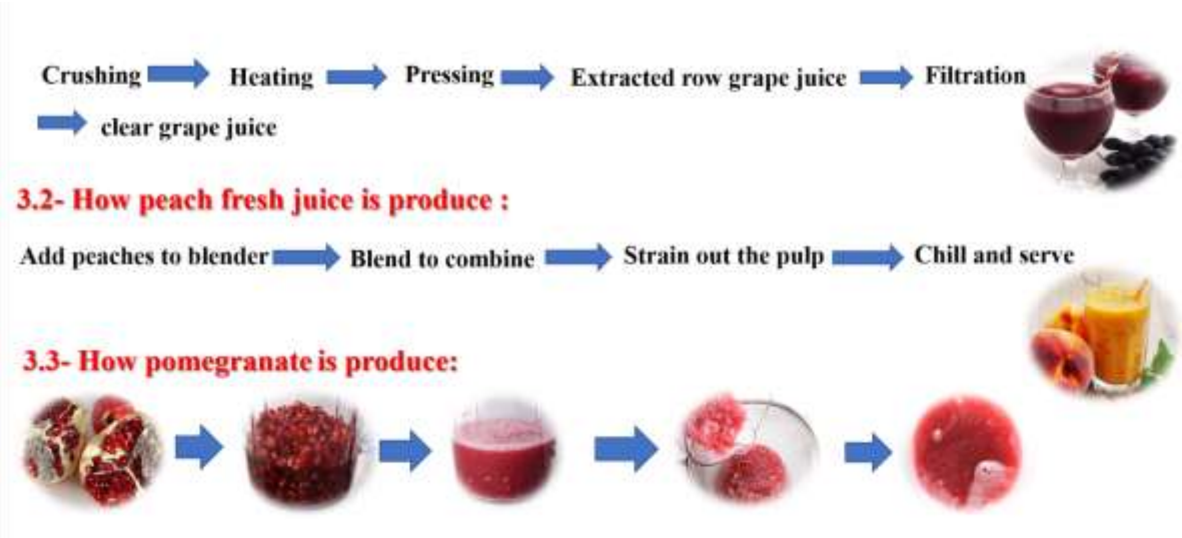


Fig .3.2 Schematic representation of the method for preparing fresh juice

3.3 pH Measurement of Fruit Juice

pH meter: is a measure of hydrogen ion concentration. It is a logarithmic measure of the hydrogen ion concentration of an aqueous solution, and is defined as pH = -log [H +], where log is the base 10 logarithm and [H +] is the hydrogen ion concentration in moles per liter. The procedure at first we calibrated the PH meter To calibrate PH meter we use this 3 powders and the three powders have different

PH that contains (6.86, 9.18 , and 4.00) , and then we found the pH value of each juices .

Fig .3.3 Schematic representation of pH Measurement of Fruit Juice

3.4 Experimental setup for UV-Visible and FTIR spectroscopic analysis

Absorption spectra in the ultraviolet and visible regions were obtained within the range of 190-1100 nm using an Agilent 8453 spectrophotometer with a 1 nm resolution. The cuvettes used were rectangular quartz cells with a 0.1 cm path length.
MilliQ water served as the blank. Prior to analysis, the samples underwent centrifugation at 5000 rpm for 15 minutes.
For each sample of juice, the spectrum was collected in duplicate at room temperature, and the results were subsequently averaged.

UV-Visible Spectroscopy

Cuvettes

3.4 Procedure for determination the % (w/v)sugar

Prepare different solutions containing (0, 5, 10, 15, and 20) % sugar, by weighing (5, 10, 15, and 20) g of sugar and dissolving in a beaker with a small amount of D.W. and transfer (**quantitative transfer**) to a 100 mL volumetric flask and complete to the mark with D.W. and close the volumetric flask with stopper and shake it well. (label the flask) ,Find the mass of an empty, clean, and dry beaker (**W1**). Add a certain volume of liquid (20 mL water), which measured by graduated cylinder (**V**) and place it into the beaker. Find the mass of the liquid and the beaker (**W2**). Calculate the mass of the liquid in the beaker by **W2 – W1**. Find the density of the liquid by ***d =m/v***

3.5 Mendeley and Origin8.5

In This study, you utilized Mendeley for reference management. Mendeley" is a reference management software that allows researchers to organize, annotate, and cite research papers. Also, we used **Origin8.5:** OriginPro is developed by OriginLab Corporation and offers a wide range of tools for data visualization, statistical analysis, curve fitting, and presentation-quality graphing.

Chapter Four
Result and Discussion

4.1 Introduction

In this chapter Presentation of ingredient analysis data for both marketed (commercially available) and fresh fruit juices.

Comparison of ingredient profiles, including sugars, preservatives, additives, and natural components (e.g., vitamins, antioxidants).

Quantitative assessment of ingredient concentrations in each juice type

4.2 Comparison of Ingredients in Marketed and Fresh Juice

4.2.1 Ingredients of marketing (Rani) and fresh grape juice

Table 1: Ingredients of marketing (Rani) and fresh grape juice, This table compares various ingredients commonly found in marketed (Rani) grape juice and fresh grape juice. It indicates whether each ingredient is present in the marketed juice, whereas the fresh juice column highlights the absence of additives and the presence of natural components. Additionally, it mentions the Protein, Fats, Fiber, and Minerals (Iron , Magnesium, Calcium , Potassium, Zinc, Vitamins (B3, B2, B1, B6,C , K) that would be absent in Rani providing insights into potential differences between the two types of juice

4.2.2. Ingredients of marketing and fresh pomegranate (Dalya) juice

Table 2: Ingredients of marketing and fresh pomegranate (Dalya) juice

This table provides a comparison of ingredients commonly found in marketed (Dalya) pomegranate juice and fresh pomegranate juice. Similar to the previous example, it indicates whether each ingredient is present in the marketed juice, while highlighting the absence of additives and the presence of natural components in the fresh juice.

Table 4.1: Ingredients of marketing (Rani) and fresh grape juice

Marketing Grape	Fresh Grapes
Water	Water
Sugar*	Sugar 14.2
Carbohydrate	Carbohydrate 14.8
Sodium	Sodium
Stabilizer ; E415	
Nature identical red grape flavor	
Colors;(E163 ,E150d)	
Acidifier;E330	
	Protein 0.37
	Fats 0.13
	Fiber 0.2
	Minerals (Iron , Magnesium , Calcium , Potassium , Zinc)
	Vitamins (B3, B2, B1, B6 ,C , K)

Table 4.2 : Ingredients of marketing and fresh pomegranate(Dalya) juice

Marketing Pomegranate	Fresh Pomegranate
Water	Water
Sugar	Sugar
Potassium	Potassium
Citric Acid	
Sodium Benzoate	
Sorbate	
Natural identical pomegranate	
	Vitamin C , B5 , K , E
	Magnesium
	Fiber
	Antioxidant

Table 4.3 : Ingredients of marketing and fresh peach juice

Marketing peach juice	Fresh Peach juice
Vitamin C 8	Vitamin (E,C,A,K,B3)
Sugar	Sugar
Potassium	Phosphorus
Carbohydrate	Zinc
Purified Water	Magnesium
Sodium	Choline
Carboxymethyl Cellulose(E466)	Folate
Citric Acid (E330)	Copper, Potassium
Ascorbic Acid(E300)	Protein , Sugar
Stabilizer	Vitamin (E,C,A,K,B3)
	Beta Carotene

This table structure provides a clear comparison of the ingredients between marketed and fresh peach juice. It indicates whether each ingredient is present or not in each type of juice, along with additional information such as nutritional value and potential contaminants

4.3 UV/visible spectroscopic investigation of both commercially available and fresh fruit juices(grapes, peach, and pomegranate)quality in case of Erbil City.

4.3.1 The Influence of Time and Temperature on Absorbance Characteristics in Fresh-Grape Juice

The results of the study demonstrate the sensitivity of fresh grape juice to both time and temperature variations, as evidenced by the changes in absorbance characteristics observed in the UV-visible spectra.

The gradual increase in absorbance values over time suggests ongoing chemical reactions within the grape juice, likely driven by factors such as enzymatic activity, oxidation, and microbial growth. These reactions can lead to the formation of degradation products, which absorb light in the UV-visible range, resulting in higher absorbance values.

The broad peak of fresh grape juice was seen at a wavelength of 455 -500 nm was the absorption peak. The absorbance spectrum of grape juice can provide information about the presence of various compounds such as anthocyanins, flavonoids, and phenolic acids This result depicts that the chemicals found in juices were destroyed at different temperatures and for a longer time

The shift in absorbance peaks at high temperatures, representing the destruction of chemical bonds, aligns with the findings presented in (Kerslake, Longo and Dambergs, 2018)

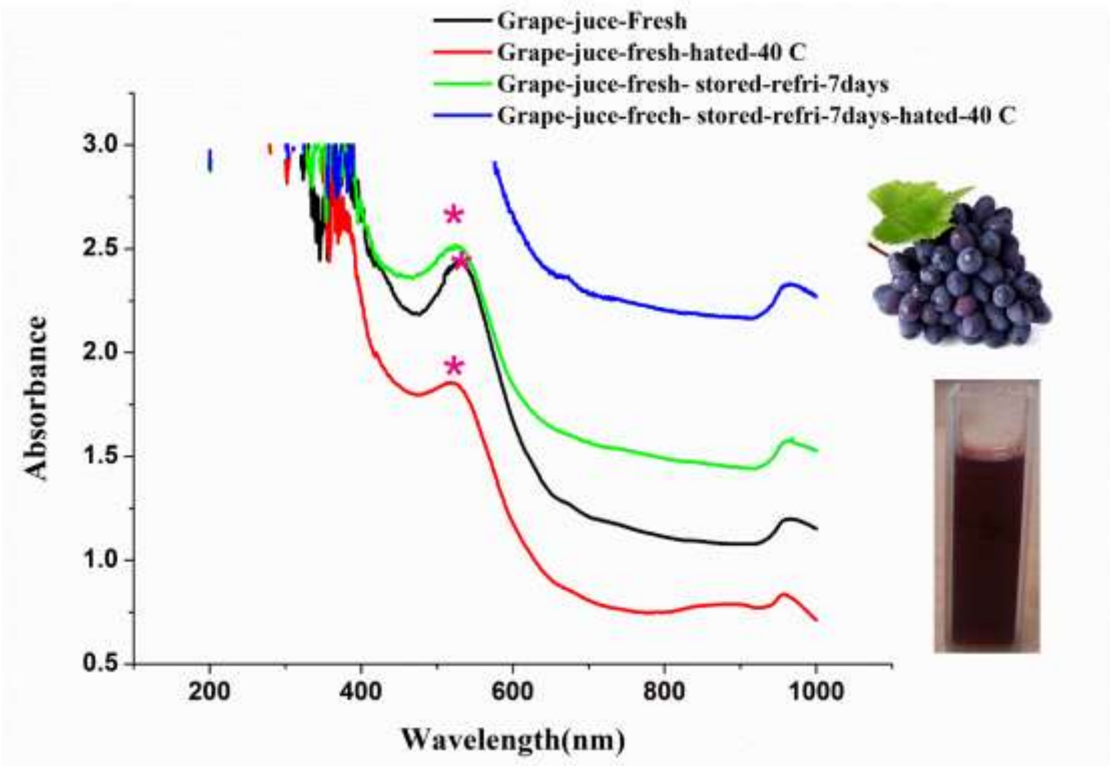


Fig.4.1 UV/Vis absorption spectra of Grape-Fresh juices at different conditions

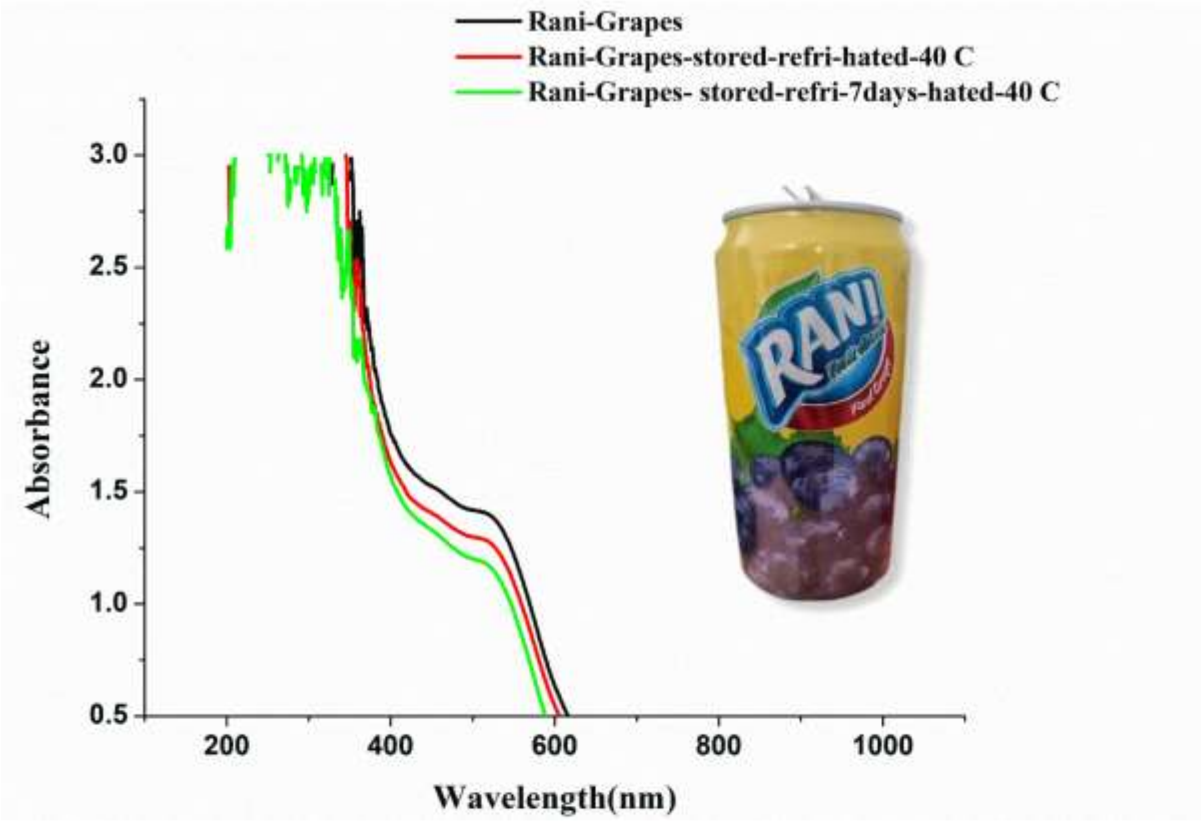


Fig.4.2 UV/Vis absorption spectra of Commercial -Grape Juice at different conditions

4.3.2 The Influence of Time and Temperature on Absorbance Characteristics in Pomegranate Juice.

Studying the influence of temperature on the absorbance characteristics of fresh pomegranate juice using UV-Vis spectroscopy can provide valuable insights into the stability and changes in its chemical composition over time and under different storage conditions Fig.3.3 UV/Vis absorption spectra of pomegranate juice at different conditions

Pomegranate juice has a unique UV-Vis spectrum due to the presence of anthocyanins, which are responsible for the red color of the juice. The spectrum of pomegranate juice shows a maximum absorption peak at around 550 nm. The absorption peak is due to the presence of anthocyanins, which are a type of flavonoid that is responsible for the red color of the juice Our results agree with (Pappalardo, 2022)Higher temperatures may accelerate the degradation of key compounds in the pomegranate juice, leading to more pronounced changes in absorbance characteristics compared to samples stored at lower temperatures

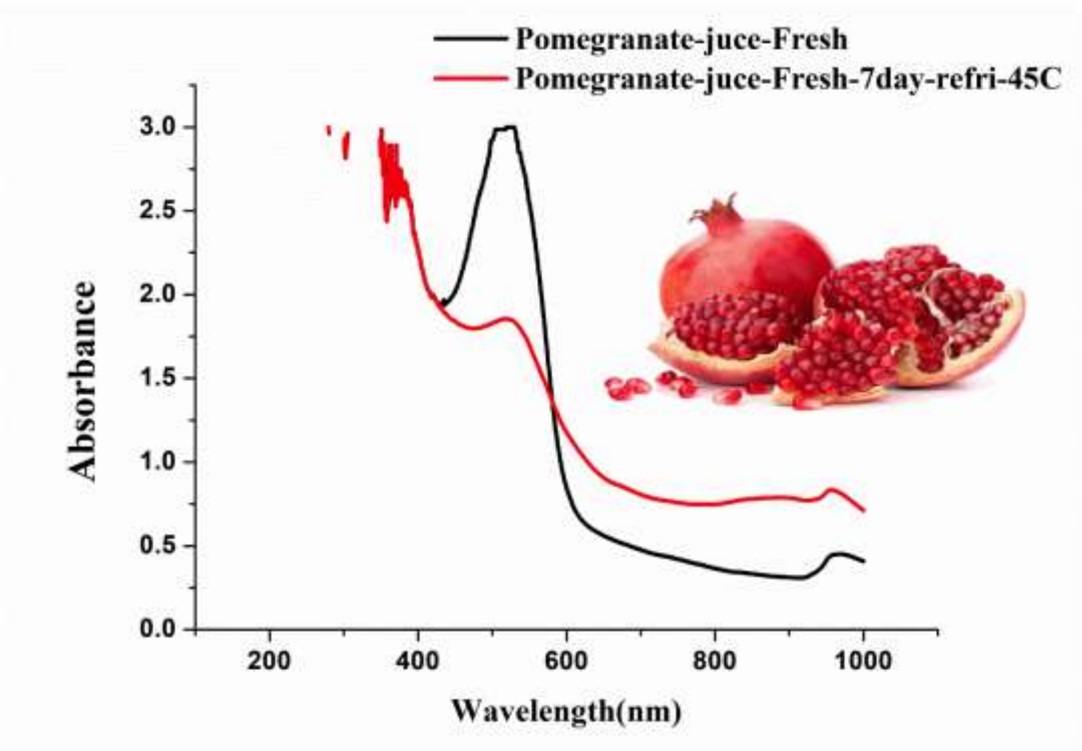


Fig.4.3 UV/Vis absorption spectra of **pomegranate juice** at different conditions

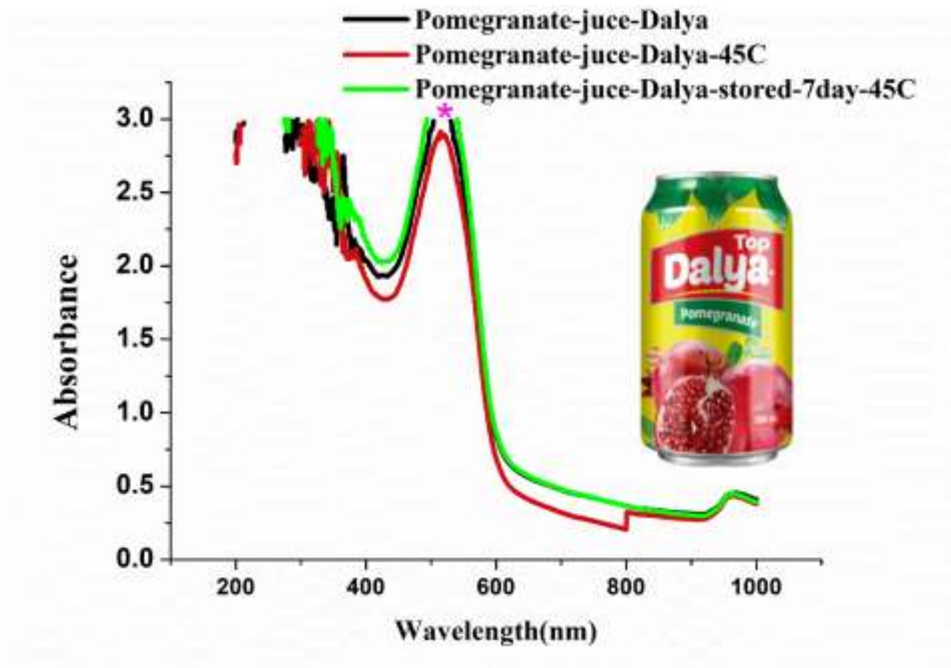


Fig.4.4 UV/Vis absorption spectra of commercial-pomegranate Juice at different conditions

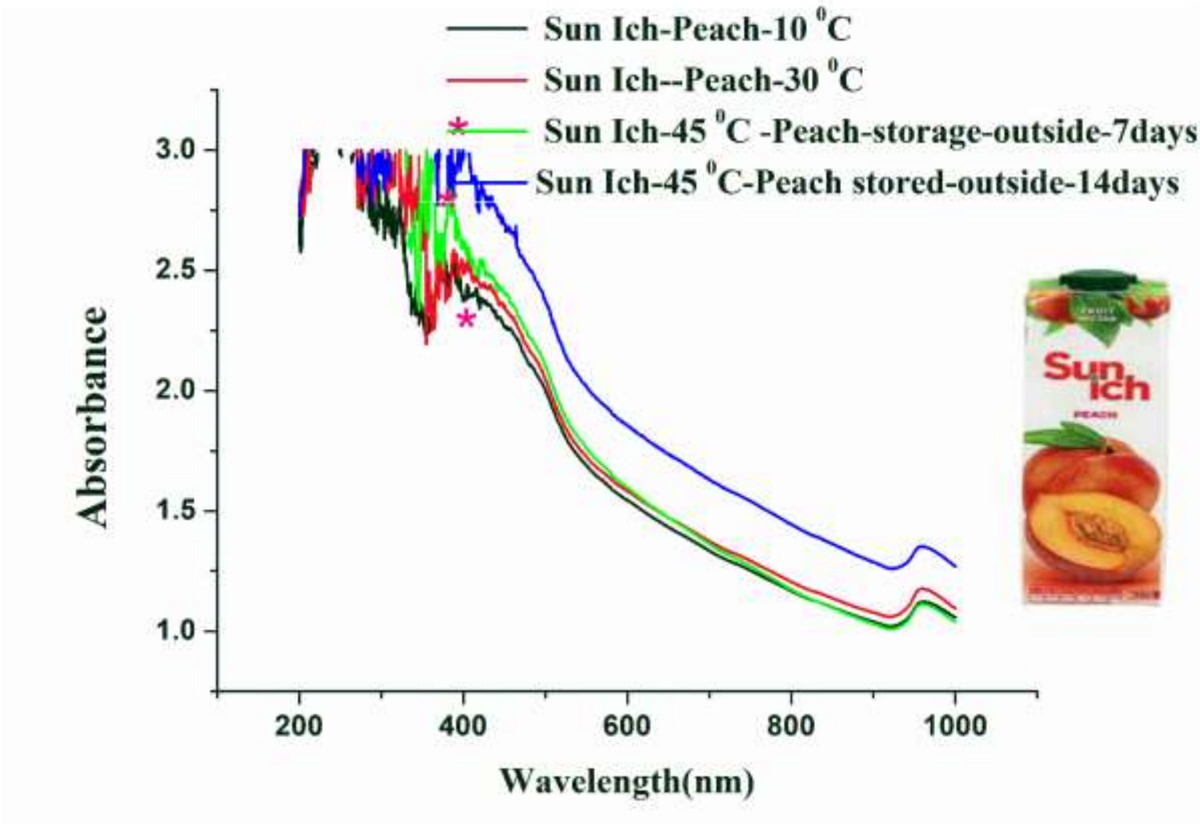


Fig .4.5 UV/Vis absorption spectra of **commercial-Peach Juice** at different conditions

4.3.3 The Influence of Time and Temperature on Absorbance Characteristics in Peach Juice

Based on the provided information, the results of the study indicate that under various time and temperature conditions, the absorbance characteristics of both fresh peach juice and commercial peach juice remain consistent compared to the spectra of fresh juice. This suggests that neither time nor temperature significantly alters the absorbance properties of the juices as measured by UV-visible spectroscopy. This consistency in absorbance spectra implies stability in the chemical composition of the juices over the studied time and temperature ranges, highlighting their potential suitability for various applications without significant degradation.

4.4. PH Measurement Juice Commercial and Fresh Juice

pH is a measure of hydrogen ion concentration. It is a logarithmic measure of the hydrogen ion concentration of an aqueous solution, and is defined as pH = -log [H +], where log is the base 10 logarithm and [H +] is the hydrogen ion concentration in moles per liter.

The pH measurements of grape, peach, and pomegranate juices, both in commercial and fresh forms, were conducted under various time and temperature storage conditions. Generally, there was a decrease in pH values over time for all juice types, indicating an increase in acidity as juices underwent natural degradation processes.

The rate of pH decreases varied among juice types, with some exhibiting faster degradation kinetics than others. Higher temperatures tended to accelerate changes in pH, resulting in more significant decreases in acidity compared to juices stored at lower temperatures.

However, extreme temperatures might have led to unexpected pH fluctuations due to enzymatic or microbial activity.

Table 4.4: PH Measurement for Grap-juice

Grape-Fresh-juice at different conditions	PH
Grape-juice-Fresh	**3.4**
Grape-juice-fresh-hated-40 C	**3.4**
Grape-juice-fresh- stored-refri-7days	**3.2**
Grape-juice-frech- stored-refri-7days-hated-40 C	**3**
Grape- Commercial -juice at different conditions	**PH**
Rani-Grapes	**3.8**
Rani-Grapes-stored-refri-hated-40 C	**3.4**
Rani-Grapes- stored-refri-7days-hated-40 C	**3.2**

Table 4.5: PH Measurement for pomegranate-juice

Pomegranate-Fresh-juice at different conditions	**PH**
Pomegranate-juice-Fresh	**3**
Pomegranate-juce-Fresh-45C	**2.94**
Pomegranate-juce-Fresh-stored-7day-45C	**2**
Pomegranate- Commercial -juice at different conditions	**PH**
Dalya-Pomegranate-juice	**2.45**
Dalya-Pomegranate-juce-45C	**2.12**
Dalya-Pomegranate-7day-45C	**2**

Table 4.6: PH Measurement for Peach-juice

Peach-Fresh-juice at different conditions	**PH**
Peach-juice-Fresh	**3.5**
Peach-juce-Fresh-45C	**3.45**
Peach-juce-Fresh-stored-7day-45C	**3.35**
Peach- Commercial -juice at different conditions	**PH**
Sun-ich-Peach-juice	**4.05**
Sun-Ich-Peach-juce-45C	**3.6**
Sun-Ich-Peach-7day-45C	**3.1**

4.4 Determination the % (w/v)sugar (Commercial -Juice and Fresh juice)

Both commercial and fruit juices can lead to a rapid increase in blood glucose levels due to their high sugar content. However, the extent of this increase may vary depending on factors such as the type of juice, serving size, and individual metabolic response.

Commercial fruit juices, especially those with added sugars or high-fructose grape, orange syrup..., tend to have a more pronounced effect on blood glucose levels compared to freshly squeezed or homemade juices, which may contain less added sugar and higher fiber content.

(Yagi *et al.*, 2020) They investigated lemon juice consumption have a beneficial effect on postprandial blood glucose levels when consumed with rice or other high-carbohydrate foods. However, the overall dietary context, including the type and quantity of foods consumed, plays a significant role in blood glucose regulation and should be considered for optimal health outcomes. As shown Fig.4.6

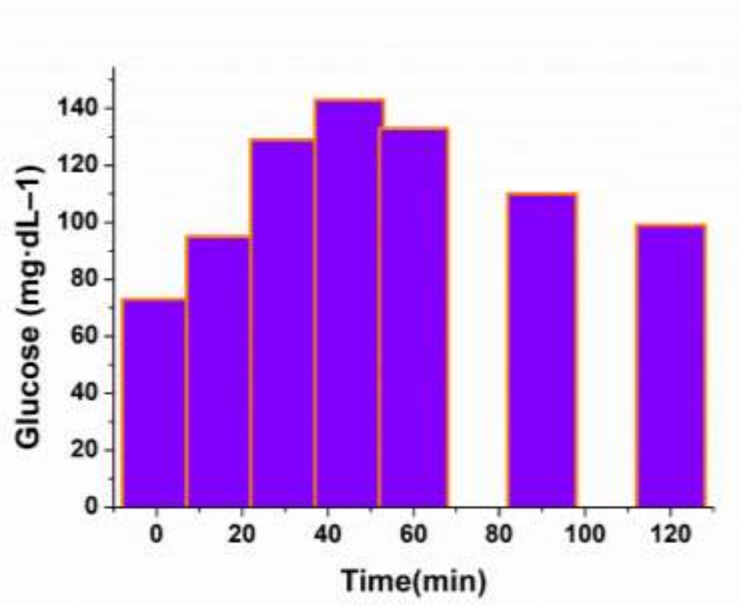


Fig .4.6 The amount of maximum blood glucose level changes after taking test food and lemon juice

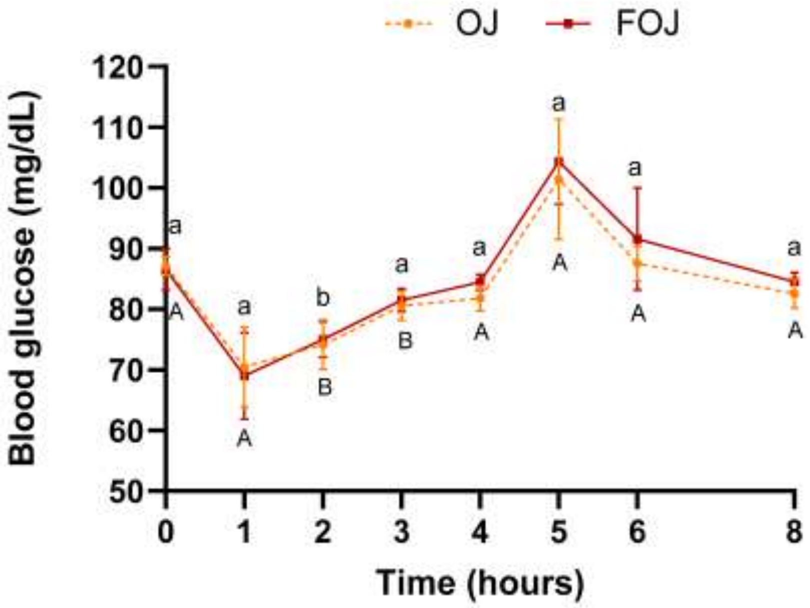


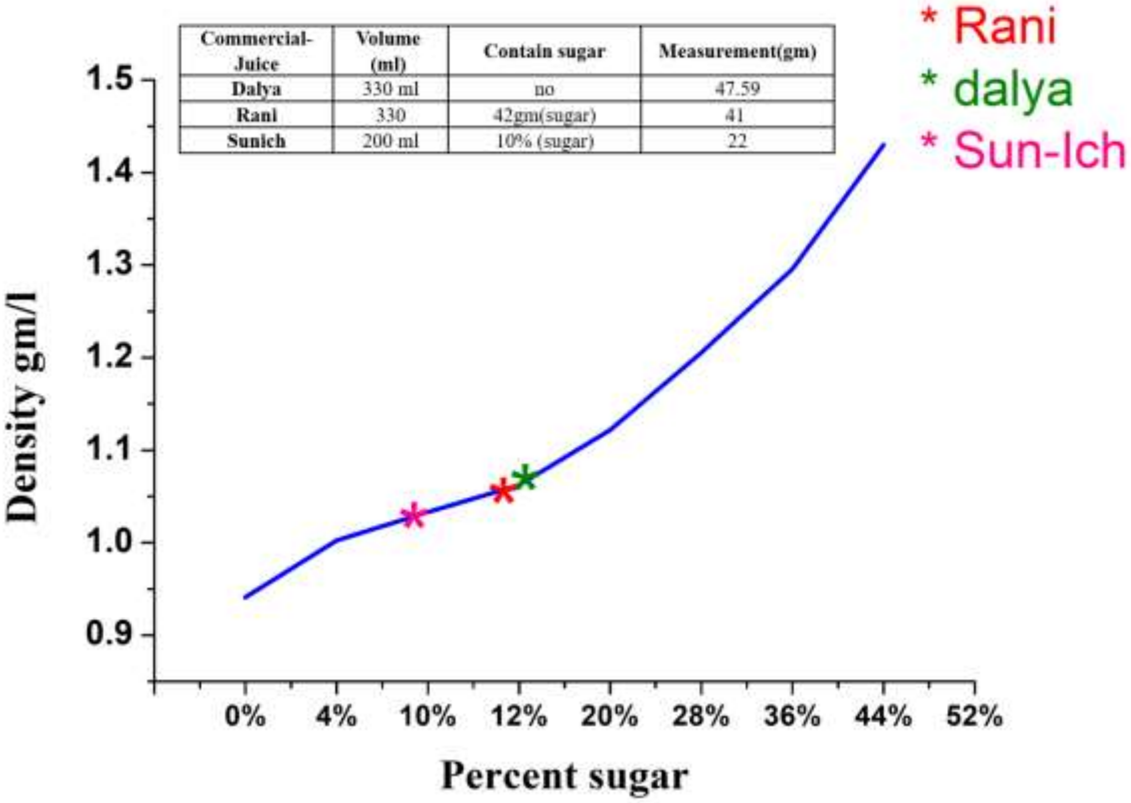
Fig.4.6 Glucose levels throughout the entire experiment (0–8 h) after orange juice (OJ) or fermented orange juice (FOJ) consumption.

And (Escudero-López *et al.,* 2022)They described a postprandial effect of fermented orange juice through the attenuation of plasma glucose and triglyceride levels compared with orange juice, probably due to its suitable profile of highly bioavailable (poly)phenols. As show fig. 4.7

The percentage of sugar (% w/v) in commercial juice can vary widely depending on factors such as fruit type, processing methods, and added sweeteners.

High sugar intake from commercial juice has been linked to various health issues, including obesity, type 2 diabetes, and dental decay.

Consumers may unknowingly consume excessive amounts of sugar if the sugar content of commercial juice is not clearly labeled. Fig.4.7 shows Measure the density of unknown Commercial juice the lack of labeling on some commercial juice products regarding sugar content underscores the need for stronger regulatory oversight, consumer education, and advocacy efforts to promote transparency and empower consumers to make healthier dietary choices.



Commercial-Juice	Volume (ml)	Contain sugar	Measurement(gm)
Dalya	330 ml	no	47.59
Rani	330	42gm(sugar)	41
Sunich	200 ml	10% (sugar)	22

Fig.4.7 Measure the density of unknown Commercial juice

Chapter Five
Conclusions and Future Work
Conclusion

- The investigation into the influence of storage temperature and time on the physicochemical properties of fruit juice, utilizing UV-visible spectroscopy and pH measurements, reveals compelling evidence of the importance of careful storage practices for maintaining the quality and safety of this beverage.
- UV-visible spectroscopy elucidated changes in color and potential alterations in the concentration of compounds responsible for the sensory attributes of fruit juice.
- The absorption peak observed at 580 nm in grape juice corresponds to anthocyanins, flavonoids, and phenolic acids. When subjected to a temperature of 45°C, the peak undergoes a noticeable shift towards lower wavelengths, indicating changes in the composition or structure of these compounds. Notably, after seven days of storage in a refrigerator, the peak at 580 nm disappears entirely. In commercial Grape juice, the peak does not appear at all conditions.
- This disappearance suggests alterations in the concentration or stability of the aforementioned compounds, potentially influenced by temperature variations during storage.
- The distinctive UV-Vis spectrum of pomegranate juice, characterized by a prominent peak at 560 nm, is attributed to anthocyanins, the compounds responsible for the juice's vibrant red color. Notably, this peak vanishes after seven days of storing the juice at room temperature.
- weak absorption peak of peach juice consistently appears at 400 nm under various conditions. This observation suggests the presence of specific compounds contributing to the overall optical characteristics of peach juice.
- pH meter analysis played a pivotal role in monitoring acidity levels in fruit juice. Fluctuations in pH values were indicative of potential alterations in taste, microbial stability, and enzymatic activity. Maintaining optimal pH levels through proper storage conditions is essential for ensuring the desirable flavor and safety of the juice. grape juice has a moderate to high acid content, and frequent consumption may result in acid reflux and dental erosion.

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