EVALUATION OF NUTRITIONAL PROPERTIES AND SENSORY ACCEPTANCE OF COMMERCIALLY AVAILABLE ORANGE JUICES IN KOTA BHARU, KELANTAN

by

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Dissertation submitted in partial fulfilment

of the requirements for the degree

of Bachelor in Nutrition with Honours

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CERTIFICATE

This is to certify the dissertation entitled “Evaluation of Nutritional Properties and Sensory Acceptance of Commercially Available Orange Juices in Kota Bharu, Kelantan” is the record of research work done by Nor Wahidah binti Johari during period record of October 2024 until January 2025 under my supervision. I have read this dissertation and that in my opinion, it confirms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation to be submitted in partial fulfilment for the degree of Bachelor in Nutrition with Honours.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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Nor Wahidah binti Johari

Date: 5 April 2025

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LIST OF SYMBOLS

p	p-value
>	greater than
<	lower than
mL	millilitre
µL	microlitre
g	gram
mg	milligram
rpm	revolution per minute
min	minute
Ca	calcium
Mg	magnesium
K	potassium
%	percentage

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrometry
AIJN	European Fruit Juice Association
WHO	World Health Organization
ANOVA	One-way Analysis of Variance
HPLC	High-Performance Liquid Chromatography
dd water	deionised distilled water
SSB	sugar-sweetened beverages

PENILAIAN KHASIAT NUTRISI DAN PENERIMAAN SENSORI JUS OREN KOMERSIAL DI KOTA BHARU, KELANTAN

ABSTRAK

Jus oren terkenal dengan khasiat pemakanannya dan merupakan pilihan jus buah yang popular di seluruh dunia. Walau bagaimanapun, maklumat mengenai kandungan nutrisi dan penerimaan sensori jus oren komersial dalam konteks Malaysia adalah terhad. Kajian ini bertujuan untuk menilai kandungan gula, vitamin C, kalsium, magnesium, kalium dan penerimaan sensori lima jenama jus oren yang berbeza (sampel A, B, C, D dan E) yang terdapat di Kota Bharu. Lima sampel jus oren telah dianalisis untuk sifat pemakanannya menggunakan kaedah enzimatik untuk gula, kaedah titrasi indophenol untuk vitamin C dan spektrofotometri penyerapan atom (AAS) untuk mineral. Hasil penilaian nutrisi yang diperoleh menunjukkan julat nilai berikut untuk jumlah gula (7.94 – 11.00 mg/100 mL), glukosa (2.24 – 5.93 mg/100 mL), fruktosa (2.35 – 3.67 g/100 mL), sukrosa (1.86 – 6.40 g/100 mL), vitamin C (0.16 – 1.41 mg/100 mL), kalsium (0.30 – 0.68 mg/100 mL), magnesium (1.10 – 2.20 mg/100 mL) dan kalium (11.65 – 19.70 mg/100 mL). Kandungan gula, vitamin C dan mineral berbeza-beza di antara kelima-lima sampel. Sampel A mempunyai kandungan magnesium (2.20 mg/100 mL) dan kalium (19.70 mg/100 mL) tertinggi manakala Sampel C mempunyai kandungan gula tertinggi (11.00 mg/100 mL). Seterusnya, sampel D mempunyai kandungan kalsium (0.68 mg/100 mL) tertinggi manakala sampel E mempunyai kandungan vitamin C (1.41 mg/100 mL). Bagi penerimaan sensori pula, lima sampel telah dinilai melalui penilaian sensori, di mana sampel dinilai menggunakan skala hedonik 7 titik. Sampel B mempunyai penerimaan keseluruhan tertinggi (6.18) manakala sampel E mempunyai penerimaan terendah (3.12). Secara umumnya, jus oren komersial merupakan sumber gula semula jadi, vitamin dan mineral yang baik dan harus ditambah dalam diet untuk manfaat kesihatannya.

EVALUATION OF NUTRITIONAL PROPERTIES AND SENSORY ACCEPTANCE OF COMMERCIALLY AVAILABLE ORANGE JUICES IN KOTA BHARU, KELANTAN

ABSTRACT

Orange juice is well-known for its nutritional benefits and has been a popular fruit juice choice worldwide. However, information on the nutritional content and sensory acceptance of commercial orange juices in the Malaysian context is limited. This study aimed to evaluate the sugar, vitamin C, calcium, magnesium, potassium and sensory acceptance of five different brands of orange juice (samples A, B, C, D and E) available in Kota Bharu. Five orange juice samples were analysed for their nutritional properties using enzymatic method for sugars, indophenol titration method for vitamin C and atomic absorption spectrophotometry (AAS) for minerals. Results of nutritional properties obtained showed the following range of values for total sugars (7.94 – 11.00 mg/100 mL), glucose (2.24 – 5.93 mg/100 mL), fructose (2.35 – 3.67 g/100 mL), sucrose (1.86 – 6.40 g/100 mL), vitamin C (0.16 – 1.41 mg/100 mL), calcium (0.30 – 0.68 mg/100 mL), magnesium (1.10 – 2.20 mg/100 mL) and potassium (11.65 – 19.70 mg/100 mL). The sugars, vitamin C and mineral contents varied among the different orange juice samples. Sample A had the highest magnesium (2.20 mg/100 mL) and potassium (19.70 mg/100 mL) content while sample C had the highest total sugar content (11.00 mg/100 mL). Next, sample D had the highest calcium content (0.68 mg/100 mL) while sample E had the highest vitamin C (1.41 mg/100 mL). For sensory acceptance, five samples were subjected to sensory evaluation, in which the samples were rated using a 7-point hedonic scale. Sample B had the most overall acceptability (6.18) while sample E had the least (3.12). In general, commercial orange juice is a fair source of natural sugars, vitamins and minerals and should be supplemented in the diet for its health benefits.

CHAPTER 1: INTRODUCTION

1.1 Study Background

For ages, fruits have been one of the major sources of nutrients and nourishment for humans. Besides the rich nutrients, humans consume various fruits to enjoy a variety of pleasant and unique tastes.

In the context of this study, the citrus fruit; the orange is the fruit of focus. Historically, citrus fruits have been long acknowledged for their palatability and nutritional value (Gupta et al., 2023). In particular, oranges possess a flavour profile that enjoys widespread global popularity. Asia Pacific Food Industry (2017) reported that orange juice continues to be the most popular taste worldwide, accounting for 46% of 100% juice sales regardless of increasing demand for the health-conscious alternative of mixed vegetables and mixed fruit juices.

In addition, research increasingly supports the health benefits associated with whole orange consumption as oranges and orange-derived products are well-recognized sources of essential vitamins, minerals, and dietary fibre, all of which play a crucial role in optimal growth, development, and overall well-being (Gupta et al., 2023).

Since the local population might have distinct food preferences, it can be valuable for the consumers, the locals and the general public to understand their acceptance alongside the nutritional quality of commercially available orange juices marketed around them. This might facilitate them in gaining the optimum benefits over the consumption of commercial orange juices.

Thus, this study aims to evaluate the nutritional properties and sensory acceptance of commercially available orange juices in the local context (Kota Bharu, Kelantan). The research will evaluate the sugar content, vitamin C content and other key minerals like calcium, magnesium, and potassium in the selected orange juice brands. The sensory acceptance will be evaluated by panellists to assess likeness on attributes such as colour, aroma, sweetness, sourness, and overall acceptability.

1.2 Problem Statement

Micronutrient deficiency or “hidden hunger” is a recent nutritional concern. A study by Passarelli et al. (2024) indicated that over half of the world's population does not consume enough essential nutrients like calcium, iron, vitamins C and E. A previous study by Rowe & Carr (2020) also indicated an increasing prevalence of vitamin C deficiency, particularly among low-income populations and in low-middle-income countries.

This issue is also mirrored in Malaysia, where studies by Murugesan (2022) and Ja’afar et al., (2024) highlight significant deficiencies. Murugesan (2022) found that a considerable number of Malaysian children do not meet the recommended daily intake of crucial nutrients like vitamin D and calcium while Ja’afar et al., (2024) highlighted the high prevalence of nutrient deficiencies such as magnesium, manganese, and various vitamins among older adults.

These micronutrient deficiencies require attention for intervention as they may cause serious consequences if left unaddressed. For instance, it can impair the cognitive and physical development of children and impair their growth as well as increase infectious disease susceptibility in general (Ritchie & Roser, 2017).

Compounding to micronutrient deficiency, another nutritional concern in Malaysia is poor dietary habits such as low fruit and vegetable intake and high consumption of sugar-sweetened beverages (SSBs). Data from the National Health and Morbidity Survey (NHMS) 2022: Adolescent Health Survey reveals a declining trend in fruit and vegetable consumption compared to previous years (Institute for Public Health, 2022). Furthermore, caffeinated drinks and sweetened fruit beverages were the most commonly consumed beverages often laden with sugar and condensed milk (Mohd Fahmi Teng et al., 2019).

One of the many ways to intervene with the abovementioned issues is promoting the consumption of healthier beverages such as 100% fruit juices, particularly citrus juices. Citrus fruits are rich sources of essential vitamins, minerals, and bioactive compounds, beneficial for health maintenance (Berk, 2016a). Moreover, fruit juices can be a more palatable and convenient option for some individuals, such as children and the elderly, compared to whole fruits (Khaw et al., 2016)

On a global scale, numerous studies have investigated commercial orange juices. For example, in Nigeria, Ndife et al. (2013) compared the nutritional and sensory quality of various brands, while Bolarinwa et al. (2021) investigated the safety and composition of commercial orange juices versus soft drinks. Similarly, Chanson-Rolle et al. (2016) in France compared the nutritional content of commercially produced and homemade orange juices. These studies highlight the wealth of existing information and valuable findings regarding commercial orange juices worldwide.

However, research on the nutritional content and sensory acceptance of commercial orange juices among urban Malaysian consumers remains limited. While there is existing literature analysing the nutrient content or consumer acceptance of other fruit juices like pineapple (Faridah et al., 2011), kuini (Rusli et al., 2020), and lycopene-rich drinks (Rusli et al., 2021), a limited body of research exists on commercial orange juice in Malaysia. Notably, only one study by Seow et al. (2015) specifically analyses the nutrient components of commercially available orange juice in the country for potential fraud. However, the study focused on the analysis of total soluble solids content, total titratable acidity, sugar composition and amino acid profiles.

This research gap underscores the need for the present study, which aims to investigate the nutritional content (sugars, vitamin C and minerals) and sensory acceptance of commercial orange juices among Malaysian consumers specifically in the local area of Kota Bharu, Kelantan. This in hope will provide valuable nutritional information to the population thus improving their beverage choices.

1.3 Study Rationale

Due to the lack of scientific data regarding commercial orange juices, there is potentially a gap between the actual nutritional composition with the perceived health benefits. Therefore, by evaluating the nutritional and sensory properties of commercially available orange juices in Kota Bharu, beneficial outcomes may be gained.

Firstly, the findings of this study can provide local consumers with information and awareness regarding sensory attributes, sugar, vitamin C and minerals content of the juices. Thus, this can facilitate them in making informed decisions which may promote better dietary habits to suit their nutritional needs. For instance, the information gained from this study hoped to influence consumers to make better beverage purchases.

Next, this study can add to the scientific knowledge on orange juices that already exist by providing data specific to the local context of Kota Bharu, Kelantan. The results can be used as a basis for future studies on the nutritional properties and sensory acceptance of orange juices in other regions in Malaysia, thus, expanding the knowledge of this well-known beverage.

1.4 Research Questions

1. What is the sugar content of commercially available orange juices in Kota Bharu, Kelantan?
2. What is the vitamin C content of commercially available orange juices in Kota Bharu, Kelantan?
3. What is the mineral content of commercially available orange juices in Kota Bharu, Kelantan?
4. What are the consumers' sensory acceptance of commercially available orange juices in Kota Bharu, Kelantan?

1.5 Objectives

1.5.1 General Objectives

To compare the nutritional properties and sensory acceptance of commercially available orange juices in Kota Bharu, Kelantan.

1.5.2 Specific Objectives

1. To determine the sugar content of commercially available orange juices in Kota Bharu, Kelantan.
2. To assess the vitamin C content of commercially available orange juices in Kota Bharu, Kelantan.
3. To determine the mineral content (calcium, magnesium and potassium) of commercially available orange juices in Kota Bharu, Kelantan.
4. To evaluate the consumers' sensory acceptance of commercially available orange juices in Kota Bharu, Kelantan.

CHAPTER 2: LITERATURE REVIEW

2.1 Orange Fruits and Orange Juices

Fruits and vegetables are famously incorporated into beverages due to the rich presence of vitamins, minerals, and antioxidants. This processed product can contribute to the fulfilment of daily dietary requirements besides being a convenient form of consumption (Giri et al., 2023).

Oranges (Citrus sinensis) are a famous example of citrus fruits that are utilized for production. Oranges are widely consumed mainly for their rich vitamin C content which fulfil individual's daily needs as well as for their tangy taste (Agbaje et al., 2020). Pera, Pineapple, Valencia, and Hamlin are some of the main varieties of oranges cultivated for orange juice production (Chanson-Rolle et al., 2016). Moreover, these citrus fruits hold a significant position in global fruit production, with a substantial portion of around 30% processed into juice (Gupta et al., 2023). This makes the citrus processing industry the second largest globally after the grape and wine production industry (Gupta et al., 2023). In addition, orange juice also topped the chart as the most popular fruit juice worldwide, especially in Europe (Chanson-Rolle et al., 2016).

2.2 Classification of Orange Juices Based on Composition

The term ‘fruit juice’ has a few definitions, related terms, and classifications. (Rajauria & Tiwari, 2018) defined ‘fruit juice’ as an “extract, or an extractable fluid content of cells or tissues made by mechanically squeezing or pressing out the natural liquid contained in ripe fruits without using any heat or solvent”. Chanson-Rolle et al. (2016) also adopt a similar definition which is “juice obtained by mechanical extraction (squeezing) of fruits harvested at maturity, followed by pasteurization”. In the context of orange juice, the European Fruit Juice Association (AIJN) defined 100% orange juice as juice made by crushing or squeezing the fruit without the addition of any other substances. The composition of the juice outlined by AIJN is (90-100%) orange juice water, vitamin C, natural sugars, other vitamins, minerals, and phytonutrients. Thus, in general, it can be understood that fruit juices are pure extracts which are mechanically processed from ripened fruits without added substances.

Next, the related terms to ‘fruit juice’ are ‘fruit juice from concentrate’ and ‘fruit nectar’. It is important to note that these terms cannot be simply used interchangeably due to their differing definition. Firstly, ‘fruit juice from concentrate’ refers to juice derived from concentrated fruit juice that has been reconstituted by the addition of water (Mihalev et al., 2018). It is compulsory for a juice containing a blend of fruit juice and fruit juice from concentrate to be clearly labelled as "partially from concentrate" close to the product name (Mihalev et al., 2018).

Then, ‘fruit nectar’ refers to fruit juice or fruit puree with added substances such as water, sugar, or artificial sweetener (Chanson-Rolle et al., 2016). However, for a beverage to be labelled as fruit nectar, it must contain a minimum of 25-50% fruit content depending on the fruit type.

2.3 Classification of Orange Juices Based on Preservation Method

Transforming easily perishable fruits into fruit juices is one of the traditional and natural preservation methods. However, juices in their raw, fresh and unprocessed form are not greatly marketed due to the challenge of changing taste over time and storage (Gupta et al., 2023). Therefore, modern production and preservation methods were innovated to tackle quality, safety, and nutritional challenges regarding fruit juices.

As a result, different types of fruit juices which are classified based on preservation methods are available in the market. Firstly, ‘freshly squeezed juice’ represents the most unprocessed form, offering the maximum nutritional content but with the shortest shelf life. Next, ‘chilled juice’ requires refrigeration throughout the supply chain as it is marketed as a chilled product. On the other hand, ‘pasteurized juice’, only required ambient storage as it is commercially sterilized. Finally, ‘concentrated juice’ possesses the longest shelf life and is the most flexible in terms of storage (Mihalev et al., 2018).

2.4 Nutrient Composition of Orange Juice & Its Health Impact

2.4.1 Sugars

In an umbrella review by (Huang et al., 2023), dietary sugars can be classified into five main classifications which are monosaccharides, disaccharides, polyols, free sugars and added sugars. Monosaccharides include glucose, fructose and galactose; disaccharides include sucrose, lactose and maltose while polyols include sorbitol and xylitol. Next, free sugars comprise any sugars added by the consumer or manufacturer as well as naturally found sugars in fruit or vegetable juices, honey and syrups. Finally, added sugars refer to sugars added to food or beverage products during processing or meal preparation.

In orange juice samples; sucrose, glucose, and fructose are the major sugars discovered. According to Seow et al. (2015), the ratio of fructose, glucose and sucrose should be either 1:1:1 or 1:1:2 if the samples claimed to be 100% juice and they contributed to the sweetness and sensory acceptance of the juice. These free sugars although naturally found in orange juices have raised debate and concerns over their effect on health. However, there were mixed findings regarding its impact on health especially in terms of weight gain, diabetes and dental caries.

In terms of weight gain, Nguyen et al. (2024), reported a positive association between 100% fruit juice intake and body weight, particularly in children. A slight increase (0.03) in body mass index (BMI) was linked with each additional daily serving of fruit juice (237 ml). Rapid ingestion of liquid calories as compared to solid calories as well as rapid absorption of fructose in the liver due to reduced dietary fibre in juices compared to whole fruits were potentially attributed to the association. However, Hägele et al., (2018) reported consumption pattern could prevent positive energy balance thus reducing the risk of weight gain when consuming 100% orange juice. Hägele et al. (2018) reported that consuming orange juice with main meals rather than consuming it in between meals or as snacks could mediate the negative impact on weight gain.

Next, 100% orange juices were associated with an increased risk of diabetes. This association was linked to the ability of fructose to induce diabetes as well as atherosclerosis through the formation of toxic advanced glycation end products (AGEs) (Serpen, 2012). However, it is important to note that natural fructose in orange juices typically does not exhibit similar detrimental effects as added sugar in sugar-sweetened beverages (SSBs). According to Ruxton & Myers (2021), fruit juices possess a nutritional profile more alike to whole fruit than SSBs. Furthermore, Monteiro-Alfredo et al. (2021) highlighted that the presence of fibre, phenolic compounds and antioxidants acted as protective factors in preventing glycation and reducing oxidative stress. In addition, these components impact the satiety mechanism which lowers food intake and better control blood glucose. Thus, orange juices exhibited different health impact than SSBs although both largely composed with sugars.

Finally, sugars in 100% orange juice were also associated with dental health issues. Liska et al., (2019) outlined that sugar content and acidic pH of 100% fruit juice might contribute to tooth erosion and dental caries. However, existing evidences regarding this association showed mixed findings thus remain inconclusive. In contrast, Berk (2016a) stated that the acidic pH could be beneficial to dental health as it can reduce the activity of dental plaque bacteria. Furthermore, Berk (2016a) disagreed with the association of orange juices and enamel erosion because most studies which exposed human or bovine enamel to beverages were done under experimental conditions that differ significantly from the natural environment of the mouth. This conditions typically disregarded factors such as presence of other foods or water and normal consumption process and normal saliva flow.

2.4.2 Vitamin C

Vitamin C is proven to exhibit immune-boosting effects through increased production of antibodies. This can prevent viruses' entry and help the body fight against infection (Giri et al., 2023) In addition, vitamin C in orange juice can facilitate the absorption of iron which can also prevent anaemia (Gupta et al., 2023). According to Yuan & Baduge, (2018), consuming 2 servings of commercial 100% orange juice can fulfilled the daily recommended vitamin C intake for healthy adults. Thus, 100% orange juice is proven to be a good dietary source of vitamin C.

In a comparative study by Chanson-Rolle et al. (2016), it was reported that the vitamin C content was lower in commercial orange juice when compared with homemade ones. However, the observed differences were within expected ranges considering potential vitamin loss due to pasteurization and storage temperature. Thus, the differences were regarded as minor. In fact, the findings showed that commercial orange juice retained approximately 85% of the vitamin C content measured in homemade juice, suggesting a satisfactory preservation of vitamin C despite processing.

2.4.3 Minerals

Potassium, phosphorus, calcium, and magnesium are some of the most abundant minerals in 100% orange juice per 100 ml (European Fruit Juice Association (AIJN), n.d.-b) For example, potassium facilitates optimum muscle function and maintenance of blood pressure. Ruxton & Myers (2021) proposed that potassium alongside other nutrients such as folate, vitamin C and polyphenols resulted in this positive vascular effect. Furthermore, higher dietary potassium intake has also been linked to a decreased risk of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke.

The consumption of a single serving (150ml) of orange juice, can provide approximately 227 mg of potassium, fulfilling 10% of the daily recommended intake thus reducing the abovementioned health risks.

CHAPTER 3: MATERIALS & METHODOLOGY

3.1 Sample Collection

Five (5) different brands of orange juice with a “100% orange juice” label were purchased from MYDIN Kubang Kerian Hypermarket and AEON Mall Kota Bharu in Kelantan. The orange juices were then labelled as A, B, C, D and E and were chilled in the refrigerator during the period of analytical investigation. The details of each juice are summarised in Table 3.1 below.

Table 3.1: Details of five different brands of commercial orange juice

Sample	Product	Origin country	Ingredients list on label	Claims
A		Malaysia	Water, orange juice concentrate (16%)	• No sugar added • No preservatives • With vitamin C
B		Thailand	100% mandarin orange juice with orange pulp from mandarin orange juice concentrate	• No sugar added • No preservatives • No artificial colour
C		Singapore	Freshly squeezed orange juice (100%)	• No sugar added • No preservatives • No additives • Never from concentrate
D		Cyprus	Orange juice made with concentrate orange juice, vitamins A, C, E	• Rich in vitamins A,C,E
E		Turkey	Water, orange juice concentrate	• No sugar added

3.2 Sugar Analysis (Enzymatic Method)

The enzymatic method using commercially available enzymatic kits was chosen due to its practicality. For instance, the kits were supplemented with detailed instructions and information such as the principle of the analysis, step-by-step procedures and calculations. (Nielsen, 2017). Furthermore, other practical advantages of utilising an enzymatic kit include ready-to-use liquid reagents, highly stable reagents even after opening and a standardised pipetting scheme across all tests (R-Biopharm, n.d.).

The detailed procedures for the sugar analysis were adopted from the GTN106 Food Analysis Laboratory Manual (2024) and Enzytec Kit Usage Instruction and as below:

3.2.1 Carrez Clarification

Carrez clarification is a crucial preliminary step for samples containing colour and protein. This process is essential for producing a clear solution, a prerequisite for enzymatic methods which involve spectrophotometer reading. Carrez clarification effectively precipitates proteins, breaks down emulsions, and absorbs some of the colour present in the sample (BeMiller, 2017)

Reagent Preparation:

- Carrez I solution: 3.6 g potassium hexacyanoferrate (II) was dissolved and diluted in a 100 mL volumetric flask with distilled water.
- Carrez II solution: 7.2 g zinc sulfate was dissolved and diluted in a 100 mL volumetric flask with distilled water.

Carrez Clarification Procedure:

1. About 10 mL of juice samples were transferred from their original packaging into sterile sealed containers prior to analysis.
2. Then, 1 mL of the juice sample was transferred into a 100 mL volumetric flask containing 60 mL of distilled water.
3. Subsequently, 5ml of Carrez I solution and 5ml of Carrez II solution were added.
4. The solutions were adjusted to a pH of 7.5 – 8.5 with 0.1M sodium hydroxide, with mixing occurring after each addition.
5. Finally, the volumetric flasks were filled to the mark with distilled water, mixed thoroughly, and filtered (if pulps were present).

3.2.2 Centrifugation

Centrifugation was done to further clarify the cloudy solution thus ensuring clear solutions were obtained. Centrifugation separated the sample solution based on density. As a result, two layers of clear solution and precipitate were formed.

Centrifugation Procedure:

1. 2.0 mL solutions were transferred to centrifuge tubes.
2. The solutions were centrifuged for 5 minutes at the speed of 3000 rpm using an Eppendorf MiniSpin Mini Centrifuge machine.

3.2.3 Dilution

The solutions were further diluted before enzymatic treatment and absorbance reading. 0.1 mL of the centrifuged solution was diluted with 0.9 mL of distilled water in another tube. This process was crucial to ensure solutions were suitable for spectrophotometer reading.

3.2.4 Enzymatic Treatment & Absorbance Reading

Enzytec Liquid Sucrose/D-Glucose/D-Fructose and Enzytec D-Glucose/D-Fructose kit were purchased from R-Biopharm Company, Darmstadt, Germany. Biochrom WPA Biowave II UV/Visible Spectrophotometer was utilised for absorbance reading. The absorbances were measured at 340 nm and plastic cuvettes were used to minimize absorbance interference and ensure accurate readings at this specific wavelength (Hamid et al., 2018). The results obtained were used in the calculation formula for the concentration of total sugar, glucose, fructose and sucrose.

Total Sugars (Sucrose D-Glucose/D-Fructose) Enzymatic Test Procedure:

1. Firstly, 100 µL of distilled water and 2000 µL of reagent 1 (reagent blank) were pipetted into the cuvette. Next, 100 µL of sample solution and 2000 µL of reagent 1 were pipetted into another cuvette.
2. Then, the solutions were mixed using cuvette mixer and were incubated for 15 minutes.
3. After 15 minutes, absorbance (A_1) were measured.
4. Next, 500 µL of reagent 2 were pipetted into the reagent blank cuvette and sample cuvette each.
5. Then, the solutions were mixed using cuvette mixer and were incubated for 15 minutes.
6. After 15 minutes, absorbance (A_2) were recorded.

D-Glucose/D-Fructose Enzymatic Test Procedure:

1. Firstly, 100 µL of distilled water and 2000 µL of reagent 1 (Reagent Blank) were pipetted into the cuvette. Next, 100 µL of sample solution and 2000 µL of reagent 1 were pipetted into another cuvette.
2. Then, the solutions were mixed using cuvette mixer and were incubated for 3 minutes.
3. After 3 minutes, absorbance (A_1) were measured.
4. Next, 500 µL of reagent 2 were pipetted into the reagent blank cuvette and sample cuvette each.
5. Then, the solutions were mixed using cuvette mixer and were incubated for 15 minutes.
6. After 15 minutes, absorbance (A_2) were recorded.
7. Finally, 500 µL of reagent 3 were pipetted into the reagent blank cuvette and sample cuvette each.
8. Then, the solutions were mixed using cuvette mixers and were incubated for 10 minutes.
9. After 10 minutes, absorbance (A_3) was recorded.

3.2.5 Calculation of Sugar Concentration

The general formula to calculate sugar concentration is as below:

$$concentration\ of\ sugar, c\ (g/l\ ) = \frac{\boldsymbol{V}\ \times MW}{\varepsilon\ \ \times d\ \times v\ \times\ 1000} \times\ \boldsymbol{\Delta A}$$

where:
V = final volume
v = sample volume (0.1 ml)
MW = molecular weight of glucose (180.16 g/mol)
d = light path (1 cm)
ε = extinction coefficient of NADPH at 340 nm (6.3 x $mmol^{-1}$ x cm^{-1})

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{blank}$:

where:
A_1= absorbance 1
A_2 = absorbance 2
df = dilution factor of optical densities, because of reagent volumes:
(sample volume + R1)/ (sample volume + R1 + R2) = 0.808

Depending on the enzymatic kit used, the values of V (final volume) and ΔA differed.

The specific formulas to calculate different types of sugars are as below:

Calculation for Enzytec Total Sugars (Sucrose/ D-Glucose/D-Fructose)

$$concentration\ of\ total\ sugars\ , c\ (g/L\ ) = \frac{\mathbf{2.600}\ \times 180.16}{6.3\ \times 1.0\ \times 0.1 \times 1000} \times\ \Delta A$$
$$c\ = \mathbf{0.744}\ \times\ \Delta A$$

Calculation for Enzytec D-Glucose:

$$concentration\ of\ glucose\ , c\ (g/L)\ = \frac{\mathbf{2.600}\ \times 180.16}{6.3\ \times 1.0\ \times 0.1 \times 1000} \times\ \Delta A$$
$$c\ = \mathbf{0.744}\ \times\ \Delta A$$

Calculation for Enzytec D-Fructose

$$concentration\ of\ fructose\ , c\ (g/L\ ) = \frac{\mathbf{3.100}\ \times 180.16}{6.3\ \times 1.0\ \times 0.1 \times 1000} \times\ \Delta A$$
$$c\ = \mathbf{0.887}\ \times\ \Delta A$$

Calculation for Sucrose

$$c_{sucrose}(g/L) = (c_{total\ sugars}) - \ (c_{glucose} + c_{fructose})$$

3.3 Vitamin C Analysis (Indophenol Titration Method)

2,6 dichloroindophenol (DCP) titrimetric method (AOAC Method 967.21) was chosen as it is an established method for the analysis of vitamin C in juices (Nielsen, 2017). Furthermore, this method used inexpensive chemicals and simple equipment which was very suitable for undergraduate research (Najwa & Azrina, 2017).

3.3.1 Reagent Preparation :

- **Ascorbic acid standard solution:**

  50 mg of ascorbic acid was accurately weighed on an analytical balance and recorded. Then, it was transferred to a 50 mL volumetric flask and immediately diluted to volume with metaphosphoric acid-acetic acid solution prior to use.

- **Metaphosphoric acid-acetic acid solution:**

  100 mL of deionised distilled (dd) water and 20 mL of acetic acid were added to a 250 mL beaker. 7.5 g of metaphosphoric acid was then added and stirred to dissolve. The mixture was diluted to 250 mL with distilled water, filtered through fluted filter paper into a bottle and stored under refrigeration until use.

- **Indophenol dye solution:**

  In a 150 mL beaker, 50 mL of dd water was added. 42 mg of sodium bicarbonate and 50 mg of 2,6-dichloroindophenol sodium salt were added sequentially and stirred to dissolve. The mixture was then diluted to 200 mL with dd water and filtered through fluted filter paper into an amber bottle. The bottle was stored under refrigeration until use.

3.3.2 Standardisation of indophenol dye

1. Three 50 mL Erlenmeyer flasks labelled S1, S2, and S3 were filled with metaphosphoric acid-acetic acid solution (5 mL), followed by ascorbic acid standard solution (2 mL).
2. The indophenol dye solution was titrated into each flask until a persistent rose-pink colour was achieved.
3. The volumes of dye used were calculated (initial burette reading subtracted with final burette reading).
4. This process was repeated for two additional standard samples (S2, S3).

3.3.3 Titration of blank

1. Three 50 mL Erlenmeyer flasks labelled B1, B2, and B3 were filled with the metaphosphoric acid-acetic acid solution (7 mL) and distilled water with an equivalent volume to the average dye volume used for the standards.
2. These blanks were also titrated with the dye solution in the same way as standardisation steps.

3.3. 4 Titration of indophenol dye with orange juice samples

1. For juice sample analysis, three 50 mL Erlenmeyer were filled with the metaphosphoric acid-acetic acid solution (5 mL) and orange juice sample (2 mL)
2. Each samples were titrated with the dye solution until the same rose-pink endpoint was reached.
3. The volumes of dye used for each titration were determined by recording the initial and final burette readings.

3.3.5 Calculation of vitamin C content

The vitamin C content of the juice samples was determined using the volume of dye used during titration (titrant volume) and the titre of the dye solution. The formula for calculating the ascorbic acid content in mg/mL of juice is as below:

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

where:

X = average millilitres of titrant used for the sample titration
B = average millilitres of titrant used for the blank titration
F = titre of the dye solution (mg ascorbic acid/mL dye)

$$F = \frac{mg\ ascorbic\ acid\ weighed}{50\ mL} \times 2\ mL$$

E = volume of juice sample used in the titration (=2 ml)
V = volume of the initial assay solution added before titration (=7 ml)
Y = volume of the juice aliquot titrated (=2 ml)

3.4 Minerals Analysis: Atomic Absorption Spectrophotometry (AAS)

The methodology to determine the major mineral contents (calcium, magnesium and potassium) was adopted from a study by Ajai et al. (2014) which analysed trace metals and essential minerals in selected fruit juices in Nigeria. This was due to the similarity in sample type and minerals analysed.

3.4.1 Digestion of orange juice samples

Digestion was part of the sample preparation procedure. This was to ensure organic material present in the sample broke down thus liberating the minerals (Bolarinwa et al., 2021).

Digestion Procedures:

1. 10 mL of concentrated nitric acid (HNO_3) was added to a beaker containing 5 mL of orange juice samples.
2. The solution was heated for 1 hour in a fume hood.
3. After it was cooled down, the solution was filtered into a 50 mL volumetric flask using Whatman No. 1 filter paper to remove any undigested particles.
4. Finally, the volumetric flasks were filled to the mark with distilled water.
5. The solution was transferred into a polyethene container and chilled in the refrigerator prior to analysis.

3.4.2 AAS Analysis

Flame Atomic Absorption Spectrophotometer (PerkinElmer PinAAcle 900 Series) was used to analyse the concentration of calcium (Ca), magnesium (Mg) and potassium (K) presented in the digested orange juice samples. Before the samples were analysed, standard calibration was prepared.

Standard Calibration

Standard calibration for each mineral (Ca, Mg, K) were prepared with five different concentrations by serial dilution from 1000mg/L standard stock solution stated in Table 3.2 below:

Table 3.2: Concentration of standard solution for each mineral

Mineral	Concentration of Standard Solution (mg/L)
Ca	0.3125, 0.625, 1.25, 2.5, 5.0
Mg	0.3125, 0.625, 1.25, 2.5, 5.0
K	0.125, 0.25, 0.5, 1.0, 2.0

Determination of mineral concentration

After standard calibrations were conducted, the concentration of each mineral was determined. The AAS analysis produced three readings and expressed the concentration of the minerals in mg/L. Mean and standard deviation were also generated.