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COMPARATIVE ANALYSIS OF VITAMIN C CONTENT IN FRUIT JUICES AVAILABLE IN LOCAL SUPERMARKETS USING THE DCPIP METHOD

Nurul Nadia Zainal Abidin^{1*}, Fadlin Batrisyia Fa'dzli Ikhwan²

¹ Pusat Asasi STEM, Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia
Email: nadia.zainal@umt.edu.my

² Pusat Asasi STEM, Universiti Malaysia Terengganu, Kuala Terengganu, Malaysia
Email: fadlinbatrisyia@gmail.com

* Corresponding Author

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Abstract:

L-ascorbic acid, another name for vitamin C, is an important antioxidant ingredient that is vital to human health. Its presence in various fruit juices serves as a significant indicator of their nutritional value. The study focuses on using titration method with 2,6-Dichlorophenolindophenol solution (DCPIP) to determine the level of vitamin C in various fruit juices. The experiment involved the preparation of standardized 1% of DCPIP solution and titration of the fruit juice samples with the DCPIP solution. Several types of fruit juices bought from local supermarkets, including orange, apple, mango and grape, were tested in this study. Each juice sample was prepared and analysed twice to ensure accuracy and reliability of the results. The titration method used facilitated the quantitative assessment of vitamin C levels in the juices. These results indicated varying concentrations and percentage of vitamin C among the different types of fruit juices tested. Orange juice exhibited the highest concentration of vitamin C, followed by mango, apple, and grape juices, respectively. It was found that vitamin C is present in all juice brands analysed, and in some cases the concentration varies from the value informed on the package label by the manufacturer. This research shows the viability of using the titration method for the determination of vitamin C in a food product, which is relevant to determine vitamin C. This study not only demonstrates the utility of the DCPIP method for assessing Vitamin C levels but also provides insights into the nutritional value of various fruit juices.

Keywords:

Vitamin C, L-ascorbic Acid, DCPIP, Titration, Fruit Juice

Introduction

The importance of Vitamin C in collagen synthesis and its role as an antioxidant for maintaining human health is well established. The recommended daily allowance (RDA) of Vitamin C is 60 mg/day, which is sufficient to prevent scurvy and maintain a steady level of approximately 1500 mg in the body, primarily derived from fruits and vegetables. Both L-ascorbic acid and its oxidized form, dehydroascorbic acid, contribute equally to Vitamin C activity, and their combined presence is used to determine the total Vitamin C content (Brause et al., 2003).

Over 90% of Vitamin C in the human diet is obtained from fruits and vegetables, which are the richest natural sources of ascorbic acid. Humans and other primates cannot produce this essential water-soluble nutrient, Vitamin C (ascorbic acid), due to genetic mutations affecting the enzyme L-gulonolactone oxidase (GLO). This enzyme is crucial for the final step of Vitamin C synthesis (Feszterová et al., 2019).

Vitamin C is a crucial nutrient involved in various essential bodily functions, including the hydroxylation of proline and lysine during collagen synthesis and the antioxidant defense mechanisms that bolster immune responses. High Vitamin C intake has also been associated with lower blood pressure and a reduced risk of cardiovascular diseases. Given the importance of this water-soluble vitamin, the guidelines from the European Food Safety Authority (EFSA) recommended average daily intake is 91 mg, with 110 mg for men and 78 mg for women.

Fruits and vegetables are the primary natural sources of Vitamin C, but processed foods, such as 100% fruit juice, can also contribute to meeting these nutritional needs. Juices have become a significant source of Vitamin C in modern diets, particularly with today's fast-paced lifestyles favoring convenient options. However, the Vitamin C content in juices can be affected by manufacturing processes and storage conditions.

Conversely, ascorbic acid (AA), the reduced form of vitamin C, is commonly used in food products as a stabilizer and antioxidant to extend the shelf life of processed foods and beverages. Consequently, it is important to carefully control the quantity of ascorbic acid added during the production of juices and other beverages within the food industry (López-Pastor et al., 2020).

In the era of rapid technological advancements, a specialized machine has been developed to measure the Vitamin C content in food products. However, this equipment is relatively costly and its use requires significant research and time investment. Among the various methods for measuring Vitamin C in fruit juices, titration is a straightforward and cost-effective approach. This method allows for the rapid and economical determination of Vitamin C levels in fruit juices.

This project also aims to compare Vitamin C content across different fruit juices, which is crucial for human health. By assessing the Vitamin C levels in several popular fruit juices, consumers will be better informed about their choices and the nutritional benefits of the products they select.

The objectives of the study are:

- i. to determine vitamin C in different type of fruit juices by using titration method
- ii. to evaluate which fruit juice varieties are the highest in vitamin C content

In today's complex global landscape, dietitians and nutritionists across the country are placing a strong emphasis on fundamental food technology. This focus is crucial to address various issues that can adversely affect people (Spinola et al., 2014). Examples of such issues include inconsistent food coloring and the presence of inappropriate ingredients in dishes.

This study has made a valuable contribution to society by enhancing general knowledge on Vitamin C content. It demonstrates that the accurate measurement of Vitamin C in fruit juice can be achieved without relying on the latest technological devices. Titration remains the simplest and most effective method for determining Vitamin C levels.

By meeting the necessary conditions, we can also identify which fruit juices contain high levels of Vitamin C. This information empowers us to make informed choices as consumers. For example, infants aged 0 to 5 months require 25 mg of Vitamin C daily, while children aged 6 to 12 years need 30 mg. Adequate Vitamin C in their diet supports healthy growth and development (Riley et al., 2019).

L-ascorbic acid is typically abundant in a well-balanced diet; however, the body's need for this vitamin increases in situations such as burns, exposure to high temperatures, or following significant injury or major surgery. Individuals at higher risk of deficiency include smokers, women using estrogen-containing contraceptives, and those living in cities with high levels of carbon monoxide from traffic. Contrary to some beliefs, research indicates that consuming large amounts of ascorbic acid may help prevent or alleviate the symptoms of the common cold (El-Ishaq et al., 2015).

Literature Review

This chapter highlights previous studies on the quantity of vitamin C is present in fruit juices.

Introduction of Vitamin C

Albert Van SzentGyorgyi, who discovered that vitamin C may prevent and cure scurvy, first isolated the tiny carbohydrate molecule known as ascorbic acid, often known as vitamin C, in the 1920s. Scurvy is a degenerative, potentially fatal ailment that affects people who are deprived of fruits and vegetables for long periods of time. Kazimierz Funk had compiled a list of dietary components known as vitamins ten years prior, which inadequacies lead to serious illnesses in people. Funk designated a factor that is currently undetermined but known to prevent scurvy with the letter "C" in his list. Ascorbic acid is later chemically identified as C by SzentGyorgyi and Haworth, who gave it that name because it is one of the most widely used medications in history.

Apart from its deficiency causing scurvy in humans, vitamin C is also vitally important to other species. Neither animals nor plants can live without vitamin c and it is therefore suprising that some animals (some fishes and birds, and a few mammals, including guinea pigs and human) have lost the capability to produce it over the course of evolution (El-Ishaq et al.,2015).

L-ascorbic Acid

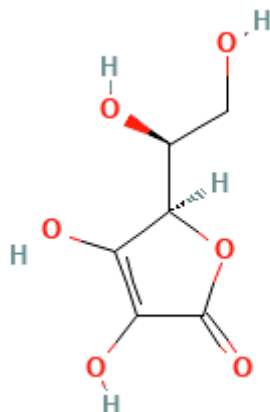


Figure 1: Structure of L-ascorbic Acid

Source: National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 54670067, Ascorbic Acid. Retrieved November 27, 2024 from <https://pubchem.ncbi.nlm.nih.gov/compound/Ascorbic-Acid>.

Figure 1 shows the general structure of L-ascorbic acid. From the structure of Vitamin C that the functional groups present in it are -OH group or other name is hydroxyl group, -C=O (carbonyl group) and -O- group is ether group.

The activity of various enzymes involved in biochemical reactions within the human body, such as metallo-enzymes of the oxygenases category (monooxygenases, intra- and intermolecular dioxygenases), which contain iron and copper ions, is significantly influenced by vitamin C, also known as ascorbic acid(Cioroi,2022).

Vitamins are chemical compounds that are necessary for maintaining, promoting, and producing normal physiological processes. Since a vitamin is an organic substance, it contains carbon. Usually, a very small amount is required for the body to function at its best. When your body doesn't acquire enough vitamins, a lack, or a shortfall of a particular vitamin, a deficiency syndrome develops. Humans must get vitamins from external sources such as food, where they are one of their natural components and are present in trace amounts. This is because humans cannot produce the majority of vitamins. Because vitamin C cannot be synthesized by humans, unlike most animals, it is a vital nutritional component.

Vitamin C or ascorbic acid (AA) is a water-soluble vitamin that possesses diverse functions in the body. However, due to the lack of L-gulonolactone oxidase in the final step of the vitamin C synthesis, vitamin C cannot be synthesized in humans and some species. Daily intake of vitamin C is therefore needed because vitamin C cannot be stored in the human body. Vitamin C plays an important role in many physiological processes such as immune response, iron absorption, collagen synthesis, and oxidative stress reduction (Boonpangrak et al.,2015).

Ascorbic acid, another name for vitamin C, is a necessary nutrient that is abundant in fruits and vegetables and is recognized for strengthening the immune system. It is a water-soluble vitamin and potent antioxidant that aids in connective tissue maintenance, including that of the skin, blood vessels, and bones.

Additionally, vitamin C is recognized to be essential for a variety of biological activities, including the absorption of inorganic iron, inhibition of nitrosamine synthesis, collagen synthesis, reduction of plasma cholesterol levels, and immune system stimulation.

An organic substance called ascorbic acid is made up of carbon, hydrogen, and oxygen. Synthetic ascorbic acid is produced from the sugar dextrose and is a white solid. It is utilized as a food preservative and in vitamin supplements (Mansor S. Bofars.,2022).

Fruit Juices

Fruit juices are consumed all over the world because of the advantages to health, the delicious taste, and the distinctive flavor and scent. Fruit juices in their natural state are a good supply of vitamins, minerals, soluble and insoluble fibers, as well as a significant source of vital bioactive chemicals that are crucial for human health. Fruit juices are foods with varied consequences for body balance because of their potential nutritional and biological value. Fruit juice use as a regular diet component to prevent or treat cardiovascular problems is growing in popularity.

Fruit juice is a fermentable but unfermented liquid or juice that is designed for direct consumption. It is produced mechanically by extracting the edible component of healthy, mature fruit that is still fresh and in good condition. As a natural liquid, fruit juice can also be made by crushing or squeezing fresh fruits or reconstituted concentrates (Devolli et al.,2022). Fruits have always been an essential part of the human diet. Fruits and their juices exhibit an extremely complex makeup with about 500 different components. They are mostly composed of water (between 80% and 90%), as well as a variety of fruit byproducts such carbohydrates, organic acids, amino acids, and peptides, as well as minerals, vitamins, fragrance compounds, vibrant anthocyanins, and carotenoids. Every fruit has unique qualities, according to analysis of the fruits and their juice.

The emergence of chemicals and biological materials as well as the use of new technological processes for the manufacturing of fruit juice became common in recent years as a result of advancements in food technology.

There are many different fruit juice products available on the market today, including both straight juices and reconstituted from concentrates. There are variations in rough material, composition, quality, nutrient content, packaging, and brand name (IFU et al.,2017).

Materials and Methods

This section outlines the procedures and materials used in the experiment. The materials include the specific chemicals, equipment, and sample types, while the methods detail the step-by-step process followed to achieve the experiment's objectives. This section ensures that the experiment can be replicated, providing clarity on how the data was collected and analyzed.

Preparation of 1.0% of DCPIP Solution

To prepare the DCPIP solution, first, carefully measure 1 gram of DCPIP powder using a laboratory balance. Ensure that the balance is calibrated and the powder is accurately measured. Next, measure 100 cm of distilled water using a graduated cylinder, ensuring the meniscus is at the 100 cm mark for accuracy.

Add the measured 1 gram of DCPIP powder into a clean beaker, and simultaneously pour the 100 cm of distilled water into the same beaker. This allows the DCPIP powder to begin dissolving as soon as it is added. Using a glass rod, gently stir the mixture to facilitate the dissolution of the powder in the water. Stir the solution continuously until the DCPIP powder is fully dissolved, resulting in a clear, blue DCPIP solution.

Once the solution is fully mixed and dissolved, it is ready for use in the titration process.

Apparatus

The apparatus used in this procedure includes a magnetic bar, a hot plate stirrer, a glass rod, a 250 cm³ beaker, a plastic tray, and a graduated cylinder. The instrument used for measuring the DCPIP powder is a laboratory balance.

Titration of Fruit Juices with DCPIP Solution

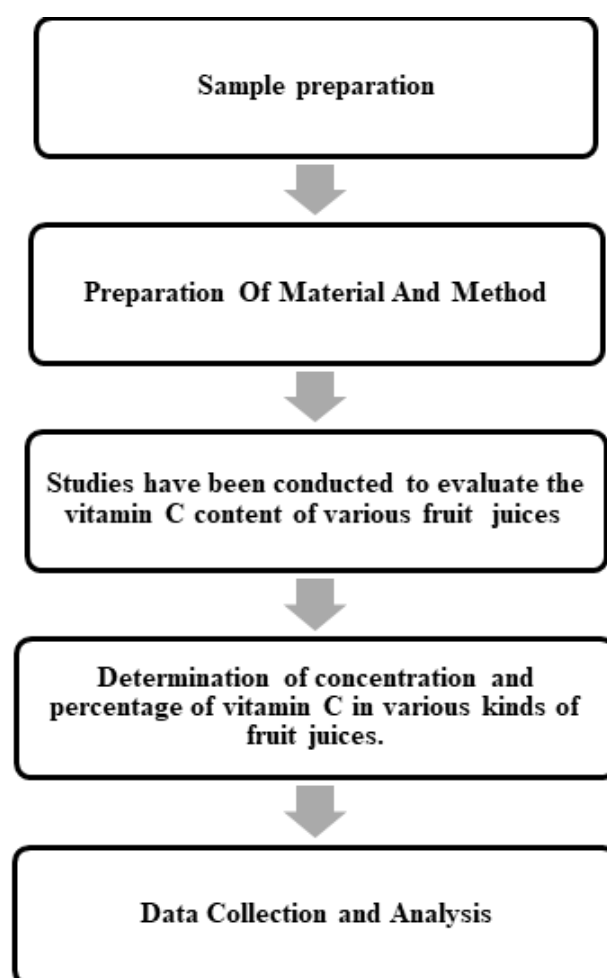


Figure 2: Flow Chart of Methodology

A 0.5 cm syringe is used to carefully measure 0.5 cm³ of a 1.0% DCPIP solution, which is then placed into a clean test tube to start the analysis. Next, a syringe is used to precisely measure 1 cm of an ascorbic acid solution that contains 1 g/100 cm³. Then, with continuous swirling to

guarantee adequate mixing, the ascorbic acid-containing fruit juice is titrated drop by drop into the test tube holding the DCPIP solution.

As the titration progresses, the fruit juice is added slowly and with great care, drop by drop, until the blue color of the DCPIP solution disappears and turns colorless, signaling that the vitamin C has fully reacted with the DCPIP. The exact volume of the 1 g/100 cm³ ascorbic acid solution used to achieve this endpoint is recorded.

To ensure accuracy and reliability of the results, the entire process is repeated for a total of three titrations, with the volume of ascorbic acid used being measured and recorded for each trial. Finally, the data collected from the three titrations is used to construct a standard curve, which will be used to calculate the concentration of vitamin C in unknown samples based on the volume of ascorbic acid consumed.

Determination of The Concentration of Vitamin C

To calculate the concentration of vitamin C (ascorbic acid) in the fruit juice sample using the DCPIP method, you can use the following equation based on the volume of ascorbic acid solution required for the titration:

$$C_{\text{ascorbic acid}} = \frac{V_{\text{ascorbic acid}} (\text{cm}^3) \times C_{\text{ascorbic acid standard}} \left(\frac{\text{mg}}{\text{cm}^3}\right)}{V_{\text{fruit juice added}} (\text{cm}^3)}$$

The unit for the concentration of vitamin C is mg/cm³.

Results and Discussion

This section contains all the results from the characterization. The results are separated into three parts; the volume of fruit juice added to determine vitamin C concentration, the percentage of vitamin C content in fruit juices and the concentration of vitamin C using DCPIP solution to observed colour changing.

The Volume of Fruit Juice

The volume of fruit juice added to determine the vitamin C concentration is a crucial factor in the titration process. Typically, a known volume of DCPIP solution is measured and used in the titration to react with a standard ascorbic acid solution and the fruit juice. This volume is carefully recorded to ensure the accuracy of the final calculation.

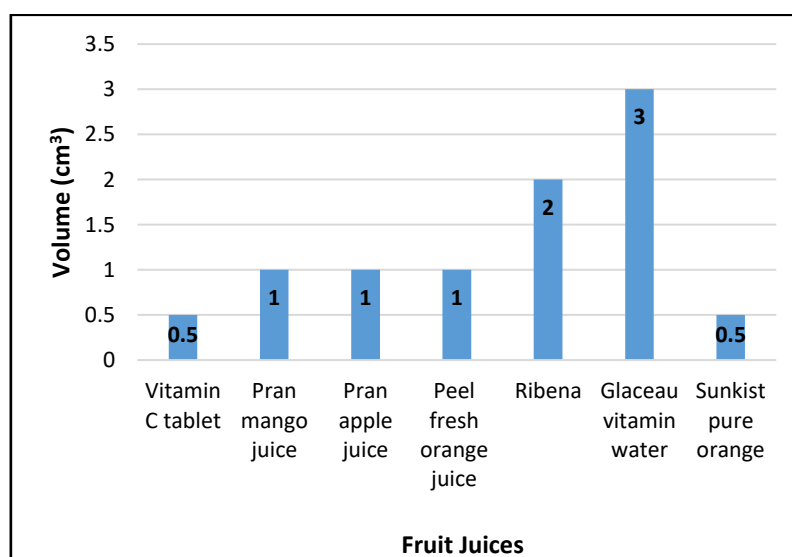
Once the fruit juice is introduced into the test tube, the titration is performed by slowly adding the ascorbic acid solution drop by drop until the DCPIP solution decolorizes, signalling the endpoint. The volume of ascorbic acid solution used to achieve this endpoint is then recorded and applied in the calculation to determine the vitamin C concentration in the juice.

Table 1: Volume of Fruit Juice Required For Titration with DCPIP Solution

Fruit juices	Titration 1 Volume (cm ³)	Titration 2 Volume (cm ³)	Titration 3 Volume (cm ³)	Mean volume (cm ³)
Vitamin C Tablet	0.5	0.5	0.5	0.5
Pran Mango Juice	1	1	1	1
Pran Apple Juice	1	1	1	1
Peel Fresh Orange Juice	1	1	1	1
Ribena	2	2	2	2
Glaceau Vitamin Water	3	3	3	3
Sunkist Pure Orange	0.5	0.5	0.5	0.5

To ensure the accuracy and reliability of the results, the titration process is performed three times, with the volume of ascorbic acid used recorded for each trial. The data from all three titrations is then compiled to create a standard curve, which will be used to calculate the vitamin C concentration in unknown samples based on the volume of ascorbic acid consumed.

In the titration process, a Vitamin C tablet can serve as a reference or standard for calibration purposes. The purpose of using a Vitamin C tablet is to provide a known and reliable source of ascorbic acid, which allows for the determination of the concentration of Vitamin C in the fruit juices.

**Figure 3: Mean Volume Of Fruit Juice Required For Titration With DCPIP Solution**

The amount of Vitamin C is assessed by the volume of fruit juice required to decolorize the DCPIP solution from dark blue to colorless. Vitamin C tablet is being used a reference standard. It serves as a benchmark to calibrate the measurements and compare the Vitamin C content in different fruit juices. As illustrated in Figure 3, the graph depicts the volume of fruit juices

necessary to complete the titration. It shows that Glaceau Vitamin Water required the highest volume (3 cm) for the titration, while Sunkist Pure Orange Juice required the lowest volume (0.5 cm). In summary, a higher volume of fruit juice needed for titration indicates a lower Vitamin C content in the juice.

Table 2: Concentration Of Vitamin C In Various Fruit Juices

Fruit juices	Concentration of vitamin C (mg/cm ³)
Vitamin C Tablet	20.0
Sunkist Pure Orange	20.0
Pran Mango Juice	10.0
Pran Apple Juice	10.0
Peel Fresh Orange Juice	10.0
Ribena	5.0
Glaceau Vitamin Water	3.3

Table 2 presents a comparison of the Vitamin C concentrations across various fruit juices. The graph highlights that Sunkist Pure Orange Juice has the highest Vitamin C concentration, measuring at 20 mg/cm³, indicating it is the richest in this nutrient among the samples tested. In contrast, Glaceau Vitamin Water shows the lowest Vitamin C concentration at just 3.3 mg/cm³, suggesting a significantly lower vitamin C content compared to the other juices.

Additionally, the graph reveals that Pran Mango Juice, Pran Apple Juice, and Peel Fresh Orange Juice each contain a Vitamin C concentration of 10 mg/cm³, placing them at a moderate level within the range of tested juices. This distribution of Vitamin C concentrations across the different brands provides a clear overview of how various fruit juices compare in terms of their nutrient content, with orange-based juices generally exhibiting higher concentrations of Vitamin C.

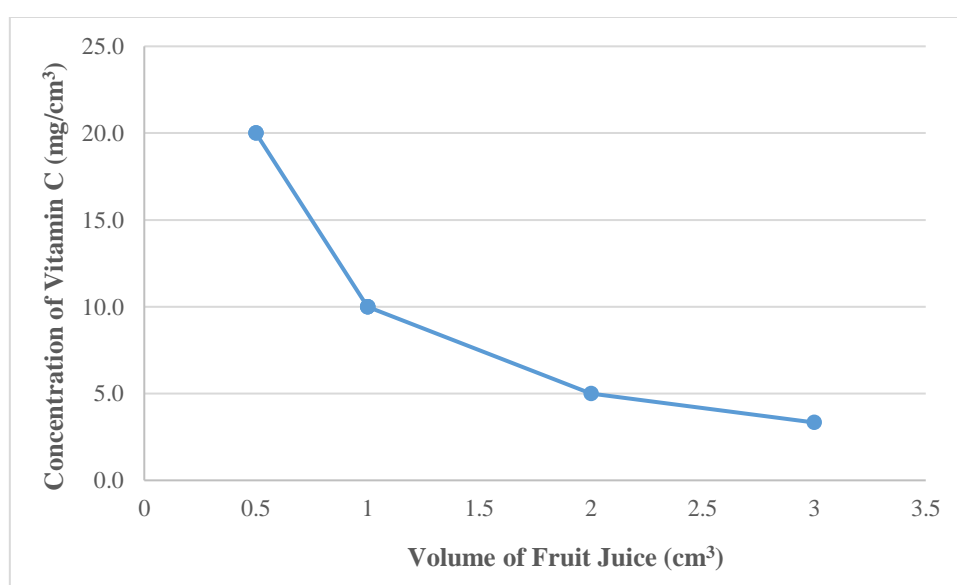


Figure 4: Relationship Between Volume of Fruit Juice Added and Vitamin C Concentration

The graph in Figure 4 illustrates the relationship between the volume of fruit juice added to a 1% DCPIP solution and the concentration of vitamin C in the juice. The trend shown in the graph highlights an inverse relationship between the two variables: volume of fruit juice (x-axis) and concentration of vitamin c (y-axis).

At low volumes of fruit juice, the vitamin C concentration is high. For example, at 0.5 cm³ of juice, the concentration of vitamin C is around 20 mg/cm³. As the volume of fruit juice increases, the concentration of vitamin C decreases progressively. This indicates that as more juice is required to decolorize the DCPIP, the vitamin C concentration in the juice is lower.

The decolorization of DCPIP depends on the amount of vitamin C in the juice. A smaller volume of juice needed to decolorize the solution means the juice has a high concentration of vitamin C. Conversely, if more juice is required to achieve the same effect, the juice has a lower vitamin C concentration. Adding a larger volume of fruit juice implies that the juice contains less vitamin C per unit volume, as more juice is required to reduce the same amount of DCPIP.

Conclusion

Vitamin C Tablet As Standard

The Vitamin C tablet serves as an essential reference and standard in this experiment due to its consistent and known concentration of vitamin C. Acting as a benchmark, the tablet allows for accurate comparisons with the vitamin C content of the tested fruit juices and beverages. By using a substance with a precise and stable concentration of ascorbic acid, the experiment ensures reliability in measuring the vitamin C levels of other samples.

As a control, the Vitamin C tablet provides a clear point of reference for determining how effective other sources are in delivering vitamin C. It represents a pure, potent source of the nutrient, showcasing the ideal scenario for decolorizing the DCPIP solution with minimal volume. This makes it particularly valuable for assessing the relative strength of natural and processed products, such as fruit juices or fortified drinks.

Findings From the Experiment

Sunkist Pure Orange Juice have the highest concentration of vitamin C (20.0 mg/cm³) and require only 0.5 cm³ to decolorize the DCPIP solution. This highlights that it is highly potent sources of vitamin C and also ideal choice for meeting dietary vitamin C requirements efficiently due to high concentration.

Pran Mango Juice, Pran Apple Juice, and Peel Fresh Orange Juice are moderate sources of vitamin C, sharing similar characteristics in terms of their vitamin C content. These juices demonstrate a balanced vitamin C concentration, making them suitable for individuals who may prioritize taste, variety, or availability. Although their vitamin C content is noticeably lower compared to the Vitamin C tablet and Sunkist Pure Orange Juice, they still provide a reasonable amount of the nutrient, making them practical choices for casual consumption.

Ribena, on the other hand, contains a significantly lower concentration of vitamin C. Its reduced potency suggests that it is not primarily consumed as a rich source of this nutrient but more for its flavor and appeal. This product may cater to individuals who value taste over nutritional benefits, making it less ideal for those who need a higher intake of vitamin C.

Glaceau Vitamin Water has the lowest vitamin C concentration among the tested samples. With minimal potency, it appears to be marketed more as a hydration option rather than a nutritional supplement. Its lower vitamin C content reflects a focus on refreshing consumers rather than providing a meaningful contribution to their daily vitamin C requirements.

Overall, the data showcases a direct relationship between the volume required to decolorize the DCPIP solution and the vitamin C concentration of the samples. Products like the Vitamin C tablet and Sunkist Pure Orange Juice emerge as the most efficient sources of vitamin C, offering high levels of the nutrient in small amounts. Meanwhile, juices like Pran Mango, Pran Apple, and Peel Fresh Orange Juice serve as moderate options, while Ribena and Glaceau Vitamin Water are less effective in delivering significant quantities of vitamin C. This emphasizes the importance of selecting beverages based on individual dietary goals, whether for taste, hydration, or nutritional value.

Fruit Juice As the Source of Vitamin C

Choosing fruit juice as a primary source of vitamin C can be both practical and enjoyable, but it is important to make informed decisions about the type and quality of juice. Freshly squeezed juices or minimally processed options, such as pure orange juice, are among the best choices as they retain high levels of natural vitamin C. Fortified juices can also serve as excellent alternatives for those who prefer convenience or need additional supplementation.

However, not all fruit juices are equally effective in delivering vitamin C. Commercial juices that are heavily processed or diluted, such as Ribena or Glaceau Vitamin Water, tend to have significantly lower vitamin C content. These products are often chosen for their taste or hydrating properties, but they may not be ideal for meeting daily vitamin C requirements. Additionally, factors like storage and processing can diminish vitamin C levels, making freshly packed or refrigerated juices a better choice.

While fruit juice can provide a convenient source of vitamin C, it is important to consume it in moderation. High-vitamin-C juices, like orange juice, can easily meet daily nutritional needs with relatively small servings. However, over-reliance on fruit juice may lead to excessive sugar consumption, which can have negative health effects over time. Where possible, incorporating whole fruits into the diet is recommended, as they offer additional benefits like dietary fiber, which is typically lost during the juicing process.

In summary, fruit juices can be an effective and enjoyable way to supplement vitamin C intake when chosen carefully. By selecting fresh or fortified juices, monitoring portion sizes, and balancing their consumption with whole fruits, fruit juices can support a healthy and well-rounded diet.

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