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HADIAH

COMPARISON OF SPECTROPHOTOMETRIC METHOD WITH TITRIMETRIC METHOD IN DETERMINATION OF ASCORBIC ACID

LOW MUN YEW

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE (CHEMISTRY)

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DECLARATION

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ABSTRACT

Ascorbic acid content in five commercial fruit juices, apple juice, guava juice, kiwi juice, mango juice and orange juice with fortified ascorbic acid (vitamin C) of 15 mg/100 mL of fruit juice as labelled was analysed using spectrophotometric method and titrimetric method. In the spectrophotometric method, average content of ascorbic acid obtained from each fruit juice ranged from 14.07 to 14.27 mg/100 mL. The range of average ascorbic content obtained by the titrimetric method was 13.1 to 15.9 mg/100 mL. Although both methods had produced satisfactory results, spectrophotometric method had showed a relatively higher consistency and accuracy when compared with the titrimetric method. The spectrophotometric method is also a very simple and direct method for ascorbic acid determination.

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ABSTRAK

Kandungan asid askorbik dalam lima jus buah-buahan komersil, iaitu jus epal, jus jambu batu, jus kiwi, jus mangga dan jus oren yang telah ditambahkan asid askorbik (vitamin C) dengan kepekatan 15 mg/ 100 mL jus seperti yang dilabelkan telah dianalisa dengan kaedah spektrofotometri dan kaedah titratan. Untuk kaedah spektrofotometri, purata kandungan asid askorbik yang diperolehi untuk setiap jus berada dalam julat 14.07 to 14.27 mg/100 mL. Julat kandungan asid askorbik yang diperolehi dengan kaedah titratan adalah 13.1 to 15.9 mg/100 mL. Walaupun kedua-dua kaedah telah memberikan keputusan yang memuaskan, kaedah spektcofotometri telah menunjukkan data yang lebih konsisten dan tepat berbanding dengan kaedah titratan. Kaedah spektrofotometri juga merupakan kaedah yang lebih mudah untuk penentuan kandungan asid askorbik.

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CONTENTS

UMS
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UMS UNIVERSITI MALAYSIA SABAH

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURES

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF SYMBOLS

cm	centimetre
mg	milligram
g	gram
ml	millilitre
L	litre
^{o}C	degree Celsius
AA	ascorbic acid
DHAA	dehydroascorbic acid
UV-Visible	Ultraviolet-Visible

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CHAPTER 1

INTRODUCTION

1.1 Vitamins

Vitamins comprise a diverse group of organic compounds that are nutritionally essential micronutrients. Vitamins are minor but essential constituents of food. They are required for the normal growth, maintenance and functioning of the human body. A deficiency of vitamins can result in hypovitaminosis and if more severe, in avitaminosis. Both can occur not only as consequences of insufficient supply of vitamins food intake but can be caused by disturbance in resorption, by stress and by disease (Belitz & Grosch, 1999).

Vitamins are a chemically and functionally inhomogeneous group of biomolecules. Vitamins are usually divided into two general classes; the fat soluble vitamins and water soluble vitamins. Owing their insolubility in water, vitamins such as A, D, E and K_1 can be accumulated in fat tissues and excessive intake cause hypervitaminoses. The water soluble vitamins, vitamin B_1, vitamin B2, vitamin B6, vitamin B12, nicotinamide, pantothenic acid, biotin, folic acid and C generally can not be stored and intake exceeding actual need is excreted in the urine (Hasnian, 1992).

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The natural source of the essential compounds which have vitamin activity of a man is the metabolism of microorganisms and plants. Sporadic vitamins can be synthesized by man because all the steps of the biosynthetic pathway have been conserved but only to an adequate extent for example niacin from the amino acid tryphophan or vitamin D_3 from its precursor cholecalciferol. Vitamins functions in vivo in several ways, including:

a. as coenzymes or their precursors (niacin, thiamin, riboflavin, biotin, pantothenic acid, vitamin B_6, vitamin B_{12} and folate.
b. as components of the antioxidative defense system (ascorbic acid, certain carotenoids and vitamin E)
c. as factors involved in genetic regulation (vitamin A, D and potentially several others)
d. in specialized functions such as vitamin A in vision, ascorbate in various hydroxylation reactions and vitamin K in carboxylation reaction (Fennema, 1996).

1.2 Need and Use of Vitamins

Vitamins and related biofactors belong to those few chemicals with a positive appeal to most people. Indeed that human need daily intake of vitamins, which should normally be provided via a balanced and varied diet. Principally, the staple food of man, including cereals, rice, potatoes, vegetables, fruits, milk, fish, meat and eggs form the basic source of vitamins and biofactors. Adequate nutrition should thus supply this daily vitamin; however, the need increases with physical exercise, pregnancy, lactation, active growth, convalescence, drug abuse, stress, pollution etc. However, current food habits or

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preferences or food processing and preservation methods do not always provide a sufficient natural daily vitamin supply, even for a healthy human being; this is all the more true for stressed or sick individuals. Pathological situations (intestinal malabsorption; stressed intestinal flora; liver/gall diseases; drug; antibiotic or hormone treatment; enzyme deficiencies; etc) can also lead to vitamin shortages despite a sufficient intake. Malnourishment in many countries also requires direct medical attention, combined with diet and vitamin adjustment. Although modern society is seldom confronted with the notorious avitaminoses of the past, these still occur frequently in overpopulated and poverty- and famine-struck regions in many parts of the world (Kirschmann & Kirschmann, 1996).

Apart from their *in vivo* nutritional-physiological roles as growth factors and/or coenzymes for man, animals, plants and microorganisms, vitamins compound are increasingly being introduced as food/feed additives, as medical-therapeutic agents, as health aids and as cosmetic and technical aids. Indeed, today many processed foods, feeds, pharmaceuticals, cosmetics and chemicals contain added vitamins or vitamin-related compounds and single or multivitamin preparations are commonly taken or prescribed. Furthermore, vitamin-enriched and medicated feed is used worldwide to procure healthy livestock (Wilson et. al, 1979).

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1.3 Sources of Vitamins

Although vitamins are consumed in the form of supplements by a growing fraction of the population, the food supply generally represents the major and most critically important source of vitamin intake. Foods, in their widely disparate form, provide vitamins that occur naturally in plant, animal and microbial sources as well as those added in fortification. In addition, certain dietetic and medical foods, enteric formulas and intravenous solution are formulated so that the entire vitamin requirements of the individual are supplied from these sources (Belitz & Grosch, 1999).

Regardless of whether the vitamins are naturally occurring or added, the potential exist for losses by chemical or physical (leaching or other separations) means. Losses of vitamins are, to some degree, inevitable in the manufacturing, distribution, marketing, home storage and preparation of processed food and losses of vitamins can also occur during the post-harvest handling and distribution of fruits and vegetables and during the post-slaughter handling and distribution of meat products. Since the modern food supply is increasingly dependent on processed and industrially formulated foods, the nutritional adequacy of the food supply depends, in large measure, on the understanding of how vitamins are lost and on the ability to control these losses (Wilson et. al, 1979).

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1.4 Objectives

The objectives of the study are:

1. To determine the amount of ascorbic acid (vitamin C) available in commercial fruit juices.
2. Comparison between the spectrophotometric method with the titration method in ascorbic acid determination.

1.5 Justification and Significance

The purpose for the study is to determine the amount of ascorbic acid present in commercial fruit juices for comparison with labelled content. It is important for consumers to be informed of nutritional facts in commercial food products. In addition, ascorbic acid is an essential nutrient to the human body. It is important to be able to get enough supplement of this nutrient to prevent deficiency. Other than that, comparison of the spectrophotometric method with the titrimetric method can confirm the consistency and accuracy of the spectrophotometric method.

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CHAPTER 2

LITERATURE REVIEW

2.1 Vitamin C

Vitamin C (*L-3-keto-threo-hexuronic-acid-γ-lactone*) or also known as ascorbic acid or ascorbate is a water soluble compound similar to glucose. It is an essential vitamin for human health. It is widely obtained from dietary sources, primarily vegetables and fresh fruits. In human beings deprived of vitamin C, the life-threatening nutritional deficiency disease scurvy develops. That ascorbic acid is the essential nutrient for the prevention and treatment of scurvy has been accepted medical wisdom since the 1930s, when Albert Szent-Gyorgyi was awarded the Nobel Prize for its discovery and isolation. But very little else about this contentious substance has achieved such unanimity of medical opinion (Richards, 1991).

Vitamin C is a water soluble, highly unstable sugar acid that is an essential nutrient. Its reduced form is readily oxidised thus serving as an antioxidant and reducing agent. This reversibility is probably the key to its importance as a vitamin. Most animals can synthesise vitamin C; however, humans and other primates, guinea pigs and a few

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other species, lack the necessary enzymes to synthesise it and thus require a dietary source, such as fruit and vegetables (Gropper, 2002).

Daily consumption of vitamin C-rich foods is recommended since vitamin C is water soluble and not stored in the body to any great extent. Citrus fruits are best sources but other fruits and vegetables also contribute, depending on their freshness and post-harvest handling. The instability of the vitamin molecule requires careful handling – it is deactivated by cooking and exposure to air. Vitamin C is absorbed from the intestine by active transport; it is found primarily in the adrenal and pituitary glands with small amounts distributed among other organs. Vitamin C and its metabolites (e.g. diketogulonic acid, oxalic acid and ascorbate 2-sulfate) are excreted primarily in the urine. Ingestion of large (mega) doses of vitamin C has resulted in uricosuria, increased iron absorption, impaired leukocyte activity and hypoglycaemic-type effects. Abrupt withdrawal of megadoses of vitamin C can result in symptoms resembling scurvy in some individuals. There is no definite evidence that megadoses of the vitamin prevent or cure the common cold, although few studies have suggested that vitamin C may reduce the frequency and severity of such cold (Gropper, 2002).

2.2 Structure and General Properties

Vitamin C appears as white crystals that readily dissolve in water. When dry, vitamin C crystals will undergo inactivation when exposed to air, heat, light or metals such as copper and iron. The vitamin is unstable in alkali medium but relatively stable in an acid

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one (Wilson et. al, 1979). L-ascorbic acid (Figure 2.1) is a carbohydrate like compound whose acidic and reducing properties are contributed by the 2,3-enediol moiety. This compound is highly polar; thus, it is readily soluble in aqueous soluble and insoluble in less polar solvent. L-ascorbic acid is acidic in character as a result of ionisation, dissociation of the C-3 hydroxyl, is much less favourable (pK_{a1} = 4.04 at 25°C). A second ionisation, dissociation is much less favourable (pK_{a2} = 11.4 at 25°C). Two-electron oxidation and hydrogen dissociation convert L-ascorbic acid to L-dehydroascorbic acid (Figure 2.2). L-dehydroascorbic acid exhibits approximately the same vitamin activity as L-ascorbic acid because it is almost completely reduced to L-ascorbic acid in the body (Fennema, 1996).

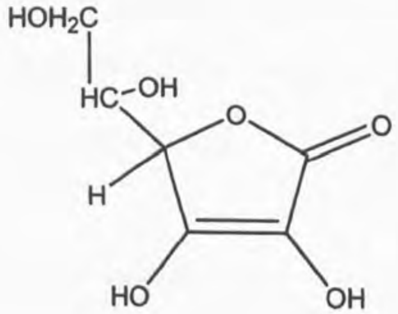

Figure 2.1 Chemical structure of L-ascorbic acid.

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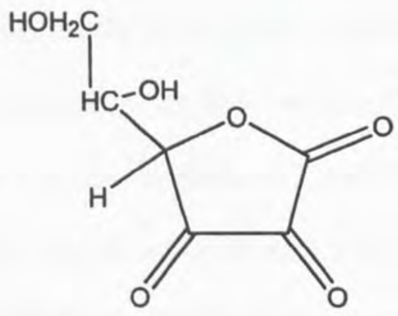

Figure 2.2 Chemical structure of L-dehydroascorbic acid.

L-Isoascorbic acid (Figure 2.3), the C-5 optical isomer and the D-ascorbic acid, the C-4 optical isomer, behave in a chemically similar manner to L-ascorbic acid but these compounds have essentially no vitamin C activity. L-Isoascorbic acid and L-ascorbic acid are widely used as food ingredients for their reducing and antioxidative activity (e.g., in the curing of meats and for inhibiting enzymatic browning in fruits and vegetables) but D-ascorbic acid has no nutritional value (Fennema, 1996).

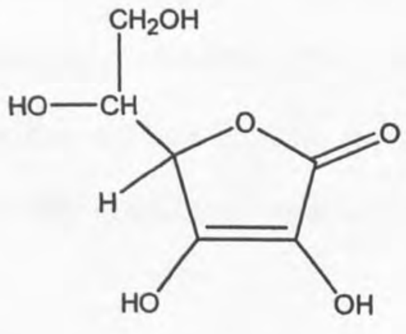

Figure 2.3 Chemical structure of L-Isoascorbic acid.

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L-ascorbic acid occurs naturally in fruits and vegetables and to a lesser extent, in animal tissues and animal-derived products. It occurs naturally almost exclusively in the reduced L-ascorbic acid form. The concentration of L-dehydroascorbic acid found in food is almost always substantially than L-ascorbic acid and is a function of the rates of ascorbate oxidation and L-dehydroascorbic acid hydrolysis to 2,3-diketogulonic acid. Dehydroascorbate reductase and ascorbate free radical reductase activity exists in certain animal tissues. These enzymes are believed to conserve the vitamin through recycling and contribute to low L-dehydroascorbic acid concentrations. A significant but currently unknown fraction o the L-dehydroascorbic acid in foods and biological materials appears to be an analytical artefact that arises from oxidation of L-ascorbic acid to L-dehydroascorbic acid during sample preparation and analysis. The instability of L-dehydroascorbic acid further complicates the analysis (Fennema, 1996).

L-ascorbic acid may be added to foods as the undissociated acid or as the neutralised sodium salt (sodium ascorbate). Conjugations of L-ascorbic acid with hydrophobic compounds confer lipid solubility to the ascorbic acid moiety. Fatty acid esters such as ascorbyl palmitate and ascorbic acid acetals are lipid soluble and can provide a direct antioxidative effect in lipid environments (Fennema, 1996).

Oxidation of L-ascorbic acid takes place as either two one-electron transfer processes or as a single two-electron reaction without detection of the semihydroascorbate intermediate. In one-electron oxidations, the first involves transfer of an electron to form the free radical semihydroascorbic acid. Loss of an additional electron

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yields dehydroascorbic acid, which is highly unstable because of the susceptibility to hydrolysis of the lactone bridge. Such hydrolysis, which irreversibly forms 2,3-diketogulonic acid, is responsible for loss of vitamin C activity (Fennema, 1996).

L-ascorbic acid is highly susceptibility to oxidation especially when catalysed by metal ions such as Cu^{2+} and Fe^{3+}. Heat and light also accelerate the process, while factors such as pH, oxygen concentration and water activity strongly influence the rate of reaction. Since hydrolysis of L-dehydroascorbic acid occurs readily oxidation to L-dehydroascorbic acid represents an essential and frequently rate-limiting aspect of the oxidative degradation of vitamin C (Fennema, 1996).

A frequently overlooked property of L-ascorbic acid is it ability, at low concentrations, to act as a pro-oxidant with high oxygen tension. Presumably this occurs by ascorbate-mediated generation of hydroxyl radicals ($OH^{\cdot}$) or other reactive species. This appears to be of minor importance in most aspects of food chemistry (Fennema, 1996).

2.3 Stability of Vitamin C

Because of the high solubility of vitamin C or ascorbic acid (AA) in aqueous solutions, the potential exists for significant losses by leaching from freshly cut or bruised surfaces of fruits and vegetables. Chemical degradation primarily involves oxidation to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and

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