Determination of vitamin c in tablets

INTRODUCTION

Nowadays, health has become the most important property of human's life. Commonly, diets with high contents of fruits are protective against several human diseases such as cardiovascular diseases and even cancer. Therefore, people are putting more and more attention on antioxidant substances such as vitamin C which is also known as ascorbic acid or more specifically L-ascorbic acid. Vitamin C is probably one of the most highly well known. Furthermore, people have become more aware to the importance of vitamin C. Hence, this causes the global market flooded with vitamin C fortified foods (Arya, Mahajan and Jain, 2000).

The term of vitamin C is used as generic term for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The molecular structure of vitamin C is C6H8O6 and the molecular weight is 176. 1 (Ball, 2006). Vitamin C is highly polar and readily soluble in aqueous solution and insoluble in less nonpolar solvents (Fennema, 1996). It is an acidic compound due to the facile ionization of hydroxyl group on carbon 3 (pK1 = 4. 17) while the hydroxyl group on carbon 2 is much more resistant to ionization (pK2 = 11. 79). The structure of L-ascorbic acid is shown in Figure 1 (Ball, 2006).

Ball (2006) also stated that ascorbic acid is easily and reversibly oxidized to dehydroascorbic acid, forming the ascorbyl radical anion which is also known as semidehydoascorbate as an intermediate as shown in Figure 2. Dehydoascorbic acid possesses full vitamin C activity because it is readily reduced to ascorbic acid in the animal body. However, dehydoascorbic acid is not an acid in the chemical sense, as it does not have the dissociable protons that ascorbic acid has at carbon 2 and carbon 3 positions.

One of the most important properties of vitamin C is that it is an antioxidant. Nevertheless, it has a wide range of antioxidant properties outside the body and can quench most biologically active radicals. It scavenges superoxide, nitroxide, hydroxide, hydrogen peroxide and will reduce vitamin E (Hickey and Roberts, 2004). It is also found to be a strong antioxidant as it helps to neutralize harmful free radicals (Izuagie and Izuagie, 2007).

Vitamin C is an almost odorless white or pale yellow crystalline powder with a pleasant sharp taste and melting point of about 190°C. It is not a carboxylic acid but a lactone and ease of oxidation to the presence of an enediol grouping (Izuagie and Izuagie, 2007). Vitamin C is highly susceptible to oxidation, especially when catalyzed by metal ions such as copper(II) ion and iron(III) ion. The functions and activities of vitamin C are based on its properties as a reversible biological reductant (Hickey and Roberts, 2004).

Vitamin C participates for the growth and repair of tissues in all parts of the body (Kleszczewski and Kleszczewska, 2002). Vitamin C is a natural antioxidant that mostly found in fruits and vegetables. The main sources of vitamin C are citrus fruits, strawberries, peppers, tomatoes, cabbage, and spinach. Vitamin C plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism (Gazdik et al, 2008).

Vitamin C can help to prevent and treat common cold, mental illness, infertility, scurvy, cancer and acquired immune deficiency syndrome (AIDS) (Yusuf and Gurel, 2005). It is reported to lower cancer risk and also said to have important interactions with other vitamins. For example, excessive

intake of vitamin A is less toxic to the body when vitamin C is readily available (Izuagie and Izuagie, 2007). Due to the great importance of vitamin C in human beings, the quantitative analysis of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical and food applications (Yusuf and Gurel, 2005).

Vitamin C is also used as an index of the nutrient quality for fruit and vegetable products. This is because it is much more sensitive to various modes of degradation in food processing and subsequent storage (Ozkan, Kirca and Cemero, 2004). It is well known that vitamin C is easily oxidized to dehydroascorbic acid in alkaline solutions, while it is relatively stable in acidic solution. Vitamin C of fruit juices is readily oxidized and lost during staying of the juices (Kabasakalis, Siopidou and Moshatou, 2000).

In the food industry, vitamin C is used as food additive (Mai and Mohammed, 2004). It preserves and protects food from any colour changes and act as an important component of our nutrition as well. Vitamin C helps to prevent the degradation of soft drinks and juice which helps to retain their flavors. Hence, it increases the quality of food and nutritional value as well (Burdurlu, Koca and Karadeniz, 2005).

Degradation of vitamin C undergoes both anaerobic and aerobic pathways. Qxidation of vitamin C in aerobic pathway occurs mainly during the processing of food whereas anaerobic degradation of vitamin C mainly during storage. Hydroxymethylfurfural (HMF) is one of the decomposition products of vitamin C and acts as precursor of brown pigments (Burdurlu, Koca and Karadeniz, 2005). Vitamin C degradation in packaged fruit juices

depends mainly on storage temperature, pH, dissolved oxygen level, residual hydrogen peroxide, H2O2 left after the sterilization of packaging material and trace metal ions (Ozkan, Kirca and Cameroglu, 2004).

Consequently, studies on vitamin C content in foods are important in relation to the control of nutritional labels, the update of food databases and the establishment of dietary reference intakes. Orange juice is probably the most globally accepted fruit juice and it is recognized worldwide as a good source of ascorbic acid (Sharma, Singh and Saxena, 2006).

In addition, there are many analytical methods used to determine the concentration of vitamin C in the pharmaceutical samples which are colorimetric method, titration, enzymatic method, flow injection analysis (FIA) and high- performance liquid chromatography (HPLC) (Arya and Mahajan, 1997). Reflectometer is an instrument that can used to analyze many different types of test which include ascorbic acid test that is concerned in this project. It provides a simple and rapid determination of vitamin C content in many pharmaceuticals product.

LITERATURE REVIEW

Various methods used in determination of Vitamin C

In recent years, the determination of vitamin C has become an important subject in the field of biochemistry and commercial foods. This is because vitamin C plays an important role in maintaining human health (Chen and Sato, 1995). Due to the importance of vitamin C in human beings, the quantitative analysis of vitamin C has gained a significant increase in several

areas of analytical chemistry such as pharmaceutical and food applications (Yusuf and Gurel, 2005).

There are numerous methods for the determination of vitamin C in a variety of natural samples, biological fluids and pharmaceutical formulations. The methods for the determination of vitamin C are spectrophotometric methods and non-spectrophotometric methods (Arya and Mahajan, 1997). For non-spectrophotometric methods are such as high-performance liquid chromatography (HPLC), titration, enzymatic method and fluorometry (Arya, Mahajan and Jain, 2000). Direct spectrophotometry also has been applied to determine the vitamin C content in soft drinks, fruit juices, and cordials after correction for background absorption in the UV region.

Flow-injection analysis (FIA)

In FIA, there is no air segmentation and it is not necessary for a state of chemical equilibrium to be reached. The sample is introduced into a carrier stream as a discrete plug. The presence of a sample-carrier interface allows diffusion-controlled dispersion of the sample as it is swept through narrow-bore tubing to create a concentration gradient. The flow-through detector monitors the change in concentration of the reaction product, which is displayed as a well-defined peak (Ball, 2006).

Flow-injection analysis permits a simple, rapid and sensitive method for the determination of vitamin C where its systems allow faster sampling rates and consumed fewer reagents compared with segmented-flow analysis (Kleszczewki and Kleszczewska, 2002). Memon, Dahot and Ansari had proposed a method by using mono 1, 10-phenanthroline-iron(III) complex as

oxidant. This experiment was based on its reducing reaction on mono(1-10-Phenanthroline)-iron(III) to tris(1, 10-Phenanthroline)-iron(II) (ferroin) and the absorbance of ferroin was monitored at 510nm through spectrophotometer equipped with a flow through cell (Memon et al, 2000).

In this analysis single channel manifold is used as shown in Figure 3. The reagent stream is pumped at the flow rate 1. 1mL/min via a peristaltic pump equipped with PVC pump tubing. The vitamin C sample is introduced into the reagent stream via a rotary teflon valve. A calibration curve for vitamin C in the range 0-50ppm was plotted from the results obtained by Memon, Memon, Dahot and Ansari which are shown in Figure 4. They also studied about the effect of reaction coil and reagent concentration. From the graph (Figure 5), the maximum intensity was observed at 50cm reaction coil. While the results of the effect of reagent concentration obtained is shown in Figure 6 indicating that the maximum signal could be obtained at 35% reagent (Memon, Memon, Dahot and Ansari, 2000).

This method can be improved within certain limits by increasing the volume of the injected sample in flow injection analysis. The sensitivity is increased two fold with the increase of sample volume. As conclusion, since the time required for sample preparation is short and reagent consumption is low, hence the method is highly economical and is suitable to use on routine basis for determination of ascorbic acid in pharmaceutical preparations (Memon et al, 2000).

Ultraviolet (UV) spectrophotometry

Direct ultraviolet spectrophotometry is a fast, simple and reliable method for the determination of vitamin C. This method can be done through alkaline treatment and the maximum absorption of vitamin C falls at 243nm at pH2 (Yanshan, 1997). The absorption of UV light by the sample matrix was the major problem in this method. Therefore, alkaline treatment method was found to be used as background correction in blank. This is because more than 95% of vitamin C will be destroyed in 10 minutes after alkaline treatment which is in the range of pH 12 to 13 (Salkic and Kubicek, 2008). UV spectrophotometry method was found to be applicable for most fruits, fruit juices and soft drinks except those that are unstable to alkaline treatment, and were deeply colored, or contained high concentration of caffeine, saccharin, caramel and tannic acid (Yanshan, 1997).

To determine the total content of vitamin C in food samples, a well-established method was investigated by Khan, Rahman, Islam and Begum, 2006 by using the 2, 4-dinitrophenyl hydrazine methods (DNPH). This is a simplified method for the simultaneous determination of total vitamin C employed coupling reaction of 2, 4-dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination. The spectrophotometric method involves the oxidation of ascorbic acid to dehydroascorbic acid by the action of bromine solution in the presence of acetic acid. Reaction between dehydoascorbic acid and 2, 4-dinitrophenyl hydrazine at 37 °C temperature for three hours will form an osazone. The solution is treated with 85% H_2SO_4 to produce a red color complex. The absorbance of all standards was measured at 521 nm by using a UV-

spectrophotometer. The results obtained were taken to contruct a calibration curve (Khan et al, 2006).

The calibration curve was constructed by plotting the concentration versus the corresponding absorbance as shown by Figure 7. The molar absorptivity, ? can be obtained using Beer-Lambert plots. The reliabilty of this method was justified by the calculations of the % of standard deviation and it was found to be varied within the range from 0. 20 to 2. 45%. The reliability of this method was also confirmed from the consideration of the following expected interferences (Khan et al, 2006).

There are a few interferences that might affect the results. First, the interference was due to the diketogulonic acid. At higher pH, destructive oxidation hydrolysis might occur. This results in the opening of the lactone ring of the ascorbic acid and loose the vitamin activity. These processes are naturally occurred in fruits and some amounts of diketogulonic acid are presence in the fruits. Besides that, diketogulonic acid has keto group that might form osazone when react with DNPH. Hence, there is a chance of error in this method which may give false results (Khan et al, 2006).

Another interference was due to the extracted glucose which contains similar structure like vitamin C. Therefore, some of the glucose may be extracted in the meta-phosphoric acid during the extraction of ascorbic acid from sample. Glucose may also cause the formation of colored complex with DNPH and gives the false result in the determination of vitamin C. This was proven in Figure 8 where there is no absorption peak around the interested peak at 521nm (Khan et al, 2006).

As conclusion, the method is simple and excellent for the determination of total vitamin C in fruits and vegetables (Khan, Rahman, Islam and Begum, 2006).

Fluorometric Method

Fluorometric analysis has been used for ascorbic acid assay in pharmaceutical preparations, beverages, special dietary foods and even for human serum (Arya, Mahajan and Jain, 2000). This method had been reported to have successful application to a wide range of foodstuffs, including liver, milk, fresh and canned fruit, raw and cooked vegetables, and potato powder (Ball, 2006). Previously, fluorometric determinations of vitamin C have been developed based on condensation reactions of vitamin C with o-phenylenediamine and on the oxidation with mercury (II) of vitamin C to form quinoxaline derivative. The reaction products of these methods exhibit fluorescensce (Yusuf and Gurel, 2005).

Figure 9 shows the reaction of the dehydroascorbic acid with 1, 2-phenylenediamine dihydrochloride to form the fluorescent quinoxaline derivative 3(1, 2dihydroxyethyl) furol[3, 4-b]quinoxaline-1-one. The blank can be prepared by complexing the oxidized vitamin with boric acid to prevent the formation of the quinoxaline derivative. It is used to reveal any fluorescence due to interfering substances (Ball, 2006).

Yusuf and Gurel have described a method by using Methylene Blue (MB) for the determination of vitamin C. This experiment was run by using a spectrofluorimeter to record the spectra and carry out fluorescence measurements. This method was used to determine the amount of vitamin C

in the purified materials, specifically vitamin C tablets. MB is a member of thiazine dye group. It is widely used in many different areas. For example, a photo sensitizer is used to produce singlet oxygen in photodynamic therapy for the treatment of cancer. The highly colored oxidized form of MB can be reduced to be colorless leuco form, Leuco-Methylene Blue (LMB) which is shown in Figure 10. LMB is the reduced and colorless form of methylene blue (Yusuf and Gurel, 2005).

According to Yusuf and Gurel, the fluorescence bands of MB were obtained at 664nm for excitation state and 682nm for emission peaks. This was proven by the other researchers who also examined the emission bands at 682nm for MB and 452nm for LMB. In Figure 11, the emission peak of MB at 682nm increased due to the increase of its concentration. A linear relationship between MB concentration and intensity was obtained over the concentration range of mol L-1 MB (y= 49. 082x + 94. 46, r2= 0. 9969). The excitation peak of MB at 664 nm also linearly increased depending on the increase of its concentration (Yusuf and Gurel, 2005).

The studies of the effect of vitamin C on the fluorescence of MB is made to avoid any errors that might affect the accuracy of the results. In order to examine the effect of vitamin C on the fluorescence of MB at 664 nm, mol L-1 MB solutions, each solution was added with different concentration of vitamin C and were prepared under nitrogen (N2) atmosphere. This was shown in Figure 12 where the spectra were recorded at 664nm (Yusuf and Gurel, 2005).

Figure 12 above shows the excitation intensity of mol L-1 without adding vitamin C was about 1000. 0 and above. The intensity was decreased by the increase of vitamin C concentration in MB solutions (Yusuf and Gurel, 2005).

Figure 13 shows the emission spectrum of mol L-1 MB as a function of time. Each spectrum was recorded at 1 minute intervals. The results showed that the fluorescence was not changed with time, reflecting that the fluorescence spectrum of MB was highly stable with time (Yusuf and Gurel, 2005).

In the redox reaction between ascorbic acid and MB, the ascorbic acid is oxidized to dehydroascorbic acid, while MB was reduced to colorless LMB as shown in the following:

The calibration curve was made based on the concentration of MB (mol L-1). The results indicate that the fluorescence intensity of the system is a linear function of vitamin C concentration in the range of mol L-1 and the regression coefficient is 0. 9941 as shown in Figure 15 (Yusuf and Gurel, 2005).

Table 1 below shows the tolerance towards different compounds that might cause interferences in this method. These compounds are usually present in most vitamin C tablets. The experimental results showed that the presence of hundred-fold excess of the all contaminant compounds and twenty-fold excess of citric acid did not significantly influence the determination of vitamin C using this method. Therefore, it can be concluded that there is no major interference caused by these compounds (Yusuf and Gurel, 2005). So it is possible to use this method for direct determination of vitamin C in pharmaceuticals without separating the interfering materials.

Table 2 lists the results obtained by the proposed method with triiodide method. It can be clearly seen that the results are in good agreement with the triiodide method (Yusuf and Gurel, 2005).

Thus, the proposed method provides a simple and sensitive fluorimetric procedure by using MB for the determination of vitamin C. This experiment also shows that MB could be used for fluorimetric determination of vitamin C in vitamin C tablets although it has only slightly fluorescence property compared to LMB. Therefore, as conclusion, it can be explained that the fluorescence intensity of MB was more sensitive to determine vitamin C concentration (Yusuf and Gurel, 2005).

Stability of Vitamin C in Orange Juice

Vitamin C is very susceptible to chemical and enzymatic oxidation during the processing, storage, and cooking of food. The catalyzed oxidation pathway of vitamin C degradation is the most important reaction pathway for the loss of vitamin C in foods. Therefore, vitamin C of orange juice is readily oxidized and lost during staying of the juice (Ball, 2006). On the other hand, there are several factors that will also affect the stability of vitamin C in orange juice. The factors are such as the effect of vitamin E, pH, and parameters which include air, heat, water as well as prolonged storage and overcooking (Kabasakalis, Siopidou, and Moshatou, 2000).

According to Ball, a meta-oxygen-ascorbate complex is formed in the presence of molecular oxygen and trace amounts of transition metal which particularly are copper (II) and iron (III). This complex contains a resonance form of a diradical that rapidly decompose to give the ascorbate radical

anion, the original metal ion, and hydrogen peroxide. This radical anion will in turn reacts with the oxygen to give dehydroascorbic acid (DHAA). For anaerobic pathway of vitamin C which occurs in the absence of free oxygen, the degradation is caused by the formation of diketogulconic acid. As the rate of degradation is maximum at pH 3 to pH 4, therefore this pathway is mostly responsible for anaerobic loss of vitamin C in canned grapefruit and orange juices (Ball, 2006).

Effect of vitamin E on the stability of vitamin C in orange juice

Vitamin E is a fat soluble antioxidant that has four tocopherols and four tocotrienols. In nature, these four tocopherols and four corresponding tocotrienols are designated as alpha-(?), beta-(?), gamma-(?) and delta-(?) according to the number and position of methyl substituent in chromonal ring (Ball, 2006).

The vitamin E functions as a biological antioxidant by protecting the vital phospholipids in cellular and subcellular membranes from peroxidative degeneration. Vitamin E mostly accumulates in body which are liver and pancreas. But unlike vitamins A and D, vitamin E is essentially nontoxic (Ball, 2006).

Nagymate and Fodor (2008) have designed a method to study the effect of vitamin E on the stability of vitamin C. In this experiment, vitamin E stock solution was prepared by dissolving ?-tocopherol in absolute ethanol. The orange juice which contained vitamin E and vitamin C was used as sample. The storage temperature of the vials was 4°C and they were covered with aluminium foil to prevent the effect of sunlight. Besides, two different

temperatures were used to examine the effect of vitamin E at that temperature which half of the samples were stored at 20°C. On the other hand, the additive effect of these vitamins was also examined but only cool samples (4°C) were used for this experiment. Two samples were prepared which one contained vitamin E stock solution and vitamin C stock solution while another contained only vitamin C stock solution. The samples were analysed once a week for five weeks (Nagymate and Fodor, 2008).

The results of the stability of vitamin C show that the presence of vitamin E influenced the decay of vitamin C. Figure 17 shows that there were differences between samples with or without vitamin E. From figure 17, it can be clearly seen that the concentration of vitamin C without vitamin E fell down to 1. 2mg/L on the second day. However, in the presence of both vitamins, the decay was also observed, but it was lesser. The concentration of vitamin C in the orange juice with vitamin E was 13mg/L in the fifth week.

As a result, it seems that vitamin E stabilized vitamin C in orange juice at a determined concentration. This is because vitamin E delay the oxidation of vitamin C thus, enhances the stability of vitamin C in orange juice. The combination of vitamin C with vitamin E makes the orange juice more stable and slower the degradation of orange juice. This concluded that orange juice with vitamin E addition is a good way to preserve the vitamin C content during storage (Nagymate and Fodor, 2008).

Effect of temperature on the stability of vitamin C in orange juice

Vitamin C of fruit juice is readily oxidized and lost depends on the conditions of storage. There are studies about the determination of the amounts of

vitamin C content in fruit juices under different storage conditions. Kabasakalis, Sipadou and Moshatou had done an experiment to determine the rate loss of vitamin C with respect to time and temperature of storage. A long-life and short-life commercial orange juice 100% without preservatives and fresh orange juice were used for analysis. In this experiment, the days before the expiration date were recorded in Table 3 and Table 4 to observe the loss of vitamin C in short-life and long-life orange juice 100% as the expiration date was approached (Kabasakalis, Siopidou and Moshatou, 2000).

Table 5 shows the loss of vitamin C from fresh and long-life commercial orange juice 100% during a 31 days period, with measurements made every 1 to 3 days. The samples were refrigerated into containers which after the initial measurement remained either open or with closed cap until the next measurement. Based on the results shown in table 5, the magnitude of vitamin C did not differ significantly between open and closed cap for both juices. The commercial orange juice lost higher amounts of vitamin C compared with fresh orange juice. As reported, decreases of vitamin C upon storage did not correspond to increases of dehydroascorbic acid levels. In fact, there was an increase of dehydoascorbic acid levels in aseptically packaged orange juices. This means that the overall nutritional quality of orange juices is affected upon storage (Kabasakalis, Siopidou and Moshatou, 2000).

The loss of the vitamin C in a commercial long-life orange juice 100% stored in refrigerator and non-refrigerated for a period of 10 days in open

containers were shown in Figure 18 (Kabasakalis, Siopidou and Moshatou, 2000).

According to Figure 18, non-refrigerated samples show higher percentage loss of vitamin C as compared to refrigerated samples. This is because the dehydoascorbic acid, the oxidized form of ascorbic acid was more stable at lower temperatures. Thus, the vitamin C, in the form of dehydroascorbic acid for refrigerated orange juice was well retained than non-refrigerated orange juice (Kabasakalis, Siopidou and Moshatou, 2000).

Effect of hydrogen peroxide on the stability of orange juice

Hydrogen peroxide, H2O2 is the primary chemical for sterilization of plastic packaging material used in aseptic system. Aseptic packaging technology is widely used by fruit juice industry for the production of shelf-life stable fruit juices. A Food and Drug Administration (FDA) regulation currently limits the residual of H2O2 to 0. 5ppm, leached into distilled water, in finished food packages which stated in Code of Federal Regulations, 2000. However, during the sterilization of aseptic chambers or packaging material with H2O2, some residues will still be left on the packaging material or vapors generated during drying may get trapped inside the package upon sealing. These residues will then cause the degradation of vitamin C (Ozkan, Kirca and Cemeroglu, 2004).

An experiment was proposed by Ozkan, Kirca and Cemeroglu to determine the rates of vitamin C degradation in orange juice with or without addition of H2O2 at various storage temperatures. In this experiment, the orange juice sample was thawed at room temperature and sodium benzoate was added to

prevent spoilage. The degradation studies were done at H2O2 with 0. 5ppm concentration at 20°C, 30°C and 40°C respectively. At regular time intervals, samples were removed from the water bath or incubator (Ozkan, Kirca and Cemeroglu, 2004). Then, the predetermined amounts of diluted sodium hydroxide solution were added rapidly to the samples to halt the reaction between H2O2 and vitamin C. The samples were then rapidly cooled by plugging into an ice water bath and held at -30°C until analyzed for vitamin C content. Vitamin C concentration was measured by using HPLC method. Qzkan, Kirca and Cemeroglu had modified the method by blending the orange juice sample with metaphosphoric acid. The sample was filtered through a membrane filter and was analyzed using HPLC (Shimadzu brand) (Ozkan, Kirca and Cemeroglu, 2004). Vitamin C contents of orange juice were plotted for various temperatures at 0. 5ppm H2O2 concentration which is shown in Figure 19 below.

From Figure 19, the results show that at higher temperature, the rate of vitamin C degradation also increased. The addition of 0. 5ppm H2O2 did not greatly increase the degradation of vitamin C. However, raising H2O2 concentration from 0. 5ppm to 5ppm resulted in a tremendous increase in degradation rates which was recorded in Table 6. At 0. 5ppm H2O2, the antioxidant substances in orange juice which was flavonols reacted with H2O2, thereby preventing the autoxidation of vitamin C. The protective mechanism of flavanols was mainly due to chelation of metal ions and action of antioxidant. Flavanols function as antioxidants by donating the hydrogen ions to reactive free radicals which may otherwise cause the autoxidation of vitamin C (Ozkan, Kirca and Cemeroglu, 2004).

Ozkan, Kirca and Cemeroglu also studied the degradation of vitamin C in the absence of H2O2. In this case, the activation energy, Ea was taken into account to determine the stability of vitamin C in orange juice. The temperature dependence of the degradation of vitamin C in orange juice was compared by calculating Ea and temperature quotients (Q10) at 20° to 40°C from the following equation:

These results clearly indicate that the rate of vitamin C degradation in the presence of H2O2 was slower at 30°C to 40°C than 20°C to 30°C. This indicates that at 30°C to 40°C, the least effect of temperature rise on vitamin C degradation. The results obtained for Ea shows that higher Ea in the presence of H2O2. This means that higher energy needed for the degradation of vitamin C. Therefore, the reaction time is slower and the degradation of vitamin C also slower. As conclusion, the effect of temperature on the degradation rates of vitamin C in orange juice was more pronounced at higher H2O2 concentrations. Therefore, greater vitamin C losses should be expected as residual H2O2 concentration and storage temperature increase in aseptically packaged fruit juices (Ozkan, Kirca and Cemeroglu, 2004).

Effect of pH on the stability of vitamin C

pH is a measure of acidity or basicity of a solution. pH is one of the primary factor that would affects the stability of vitamin C in orange juice. Hence, the pH value of the matrix has an influence on the stability of vitamin C. According to FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, Bangkok, Thailand, 1998, the vitamin C will decay if the pH higher than 4 (Nagymate and Fodor, 2008).

Vitamin C is unstable in neutral and alkaline environments, therefore the higher the pH value and the longer the exposure, the greater the loss of vitamin C. This is because the higher the pH value, the faster the oxidation reaction of vitamin C and causes the degradation of vitamin C. Besides that, the increase in pH also related to deterioration of fruit characteristic which in this literature review, orange juice is more concerned. Table 8 below shows the pH value of the fruit juice with storage time (Ajibola, Babatunde and Suleiman, 2009).

In this Table 8, the pH values of the orange juice were higher at room temperature and keep increasing from week to week. This study concluded that, though pH was significant for the stability of vitamin C, it was not the sole factor in controlling the deterioration of vitamin C in orange juice with storage life (Ajibola, Babatunde and Suleiman, 2009). On the other hand, the loss of vitamin C activity during oxidative degradation of vitamin C occurs with the hydrolysis of the dehydroascorbic acid lactone to yield 2, 3-diketogulonic acid. This hydrolysis is favored by alkaline solution. Dehydroascorbic acid is most stable at pH 5. 5 but decrease in stability as pH increases which is more than pH 5. 5 (Fennmena, 1996). For example, half-time values of dehydroasorbic acid hydrolysis at 23°C were 100 and 230 minutes at pH 7. 2 and pH 6. 6 respectively as shown in Figure 20. At pH 5. 0 or below, dehydroascorbic acid was quite stable which decayed by less than 3% over 4 hours. This experiment evaluated the effect of hydrogen ion concentration on delactonization of dehyroascorbic acid over the range of pH 3. 0 to pH 8. 0. The possible influence of the presence of oxygen was done by equilibrating the reaction mixture before and during the incubation with

100% oxygen or with 100% nitrogen. The results indicated no change in the decay rate of dehydoascorbic acid was obvious with these alterations of atmospheric conditions. The rate of dehydroascorbic acid hydrolysis markedly increases with increasing temperature but was unaffected by the presence of oxygen (Bode, Cunningham and Rose, 1990).

Other researchers had proposed a method to determine the effect of pH on the degradation of vitamin C in orange j