Vitamin c effect on apple browning | experiment

This experiment was designed to investigate the effect of Vitamin C concentration on the enzymatic browning of apples. Apple slices were dipped into Vitamin C solution of different concentration ranging from 0 mg to 100 mg. They were left under room temperature and conditions for 24 hours before the browning index was measured. There was a general decrease in browning index as Vitamin C concentration increased with 80 mg showing the lowest browning index. Pearson’s product-moment correlation coefficient established a strong negative correlation between the two variables investigated with a 5% confidence level. The results supported the experimental hypothesis.

Research and Rationale

The browning of certain fruits such as apples, pears, peaches and bananas is caused by an oxidation process known as enzymatic browning which requires three factors: substrate, which consists of polyphenolic compounds; a polyphenol oxidase (PPO), an enzyme that can catalyze the first step in the reaction; and oxygen, a reactant.[1, 3] Enzymatic browning is mostly undesirable but is inevitable when these fruits are subjected to mechanical injuries or processing.

When fruits are sliced or the skin of the fruit is pierced open, the enzyme PPO which exists in the cells are released and exposed to the surrounding air. Upon contact with oxygen in the atmosphere, PPO reacts with oxygen and starts catalyzing the conversion of polyphenolic compounds into quinones. Quinones are not dark in color but are readily polymerized to form complex brown polymers. [12]

369words Prevention of undesirable enzymatic browning is of utmost importance to the food processing industries because browning of fruits will cause deterioration of quality, alteration of flavor and color of fruit products, causing industries to incur losses and wastage of food. Preventive steps include inactivation of enzyme by heat denaturation, the use of acid to inhibit enzyme activity and the use of bisulfites to interfere with browning.[1] In this practical, I am looking at the function of Vitamin C in preventing enzymatic browning.

The function of Vitamin C as a natural antioxidant to the browning of sliced apples was first discovered by Dr. Szent Gyorgi, a Nobel Prize Winner. Vitamin C, a strong reducing agent and reactive species, prevents enzymatic browning by reacting with oxygen, inhibiting the PPO enzyme and disallowing oxygen to react with the phenolic compounds. Borenstein (1965), Sapers and Dougles (1987) and Sapers and Ziolkowski (1987) stated that ascorbic acid is a more effective inhibitor of enzymic browning than are sulfites or erythorbic acid. Besides that, Taeufel and Voigt (1964) stated that ascorbic acid is the most significant inhibitor of PPO because it has no detectable flavour at the concentration used which would interfere with the acceptability of the final processed product. Also it has no corrosive action upon metals, in addition to its vitamin value. The biggest advantage of using Vitamin C as a browning inhibitor is it is natural and healthy. [6]

Vitamin C is the most widely used as a food additives in inhibiting discoloration due to enzymatic browning because Vitamin C has quite powerful reducing properties together with its obvious physiological acceptance and safety. Moreover, Vitamin C becomes a very common food

additive due to its worldwide legislative acceptance and many of its technical uses. The availability of crystalline Vitamin C to a very high standard of purity in industrial quantities has undoubtedly been of assistance in the growth of the market. [2]

Apples are chosen as a material in this experiment because apple is a very common fruit used in the food industry and is always included in our daily diet for its high nutritional value. Moreover, apples brown rapidly after being bruised due to their high polyphenolic compound contents. The pale coloration of apples gives an easily observable and clear indication of the amount of browning. Vitamin C is applied on the surface of cut apples to slow down the oxidation process. In the experiment, the amount of browning was indicated by the browning index of the apples. The higher the browning index, more enzymatic browning that took place.

According to Eskin et al. (1971)[6] reported that food material must be treated with an adequate amount of Vitamin C to totally halt the browning process, otherwise browning is only slightly delayed, up to the point at which all the Vitamin C is oxidized. Therefore, the aim of experiment is to investigate the relationship between Vitamin C concentration and enzymatic browning of apples, determining the ideal concentration which inhibits enzymatic browning most effectively.

Experimental hypothesis

The higher the concentration of Vitamin C, the slower is the enzymatic browning of apples.

Null hypothesis

There is no significant correlation between the concentrations of Vitamin C and the enzymatic browning of apples.

Planning

A trial experiment was conducted to help me choose the most suitable methods to conduct the experiment, the best 2 quantification methods and the range of concentration of Vitamin C to be used in the real experiment.

Methods involved

- Preparing Vitamin C of different concentration

A standard Vitamin C solution of concentration 4mg/ml was prepared by dissolving two 500mg Vitamin C tablets in 250ml of distilled water and homogenised in a volumetric flask. Six different concentrations of Vitamin C solutions of 20, 40, 60, 80 and 100mg with the same total volume of 25 ml were prepared by serial dilution method. To make Vitamin C solution of 20mg, 5ml of the stock solution was mixed with 20ml of distilled water. To make 40mg, 10ml of stock solution was mixed with 15ml of distilled water and so on. 5ml of buffer at pH6. 5 was added into each solution. A control solution of only distilled water was also prepared.

- Preparing the apple cubes

A total of 30 freshly cut apple cubes of dimension 2. 0-2. 0 cm were prepared. Each five apple cubes were dipped into one of the solutions prepared. The apple cubes were then placed on separate Petri dishes and left at room temperature and conditions for 24 hours.

Collecting data

The apple cubes were examined with 3 different methods. Firstly, the intensity of the colour of the browned apple cubes was examined using a colour scale from 1 to 9. The percentage coverage of browned parts of the apple cubes was also examined using a 2-2cm quadrat. Lastly, the apple cubes are homogenised in a blender for 2 minutes, followed by centrifuging at 10000 rpm. After filtering the solution, the browning index of the clear fruit juice obtained was determined by measuring the absorbance at 420nm using a spectrophotometer.[7, 5]

Trial results

From the results obtained, I learnt that increasing Vitamin C concentration does have an effect on the browning of apples and the most preferable method of quantification was measuring the browning index. The results obtained from measuring the percentage coverage and colour intensity were less significant and these observations were very subjective.

Besides that, I realised that the trial results were inconsistent and did not show significant difference in all the quantification methods used. This may be either caused by errors and limitations occurred during the trial experiment or the concentration of Vitamin C used was not high enough. However, according to Linus Pauling Institute in Oregon State University, the recommended daily allowance of Vitamin C for normal healthy adult above age 19 is around 75 to 95mg.[4, 9] Thus, increasing the Vitamin C concentration was not advisable.

Therefore, subsequent experiments were carried out by using the same Vitamin C concentrations but steps were taken to ensure the errors and limitations were minimised. Vitamin C stock solution was heated to ensure complete dissolving but the temperature of heating cannot be too high to prevent breakdown of Vitamin C. Procedures were repeated once to get more reliable results and the number of apple cubes used were increased from 5 to 10. This could help eliminate any browning happening due to chance. Besides that, all the apparatus used for the cutting and handling of the apple cubes were cleaned thoroughly and free from rust as rust can accelerate the browning of apples.

Apparatus

Petri dishes, measuring cylinder, glass rod, beaker, boiling tubes, filter funnel, normal laboratory spectrophotometer, cuvette, pestle and mortar, water bath, knife, dropper, volumetric flask, blender, centrifuge, knife, marker pen

Materials

Distilled water, apples, Vitamin C tablets, label stickers, filter paper

Variables

Manipulated: Concentration of Vitamin C (mg)

Serial dilution technique was used to make the same volume of lime juices containing different Vitamin C concentration. The Vitamin C concentrations used were 0, 20, 40, 60, 80 and 100mg. The control was distilled water to allow comparison to be made.

Responding: Browning index (ABS)

The browning index was determined by blending, centrifuging, filtering and measuring the percentage absorbance at 420nm of the oxidised apple cubes.

Controlled: Types of apples, pH of Vitamin C solution, surrounding temperature

All the apples used in the experiment were of the same type, same origin and were bought from the same supermarket at the same time. The pH of the solutions was kept constant using a buffer at pH6. 5. The apple slices were placed in separate Petri dishes in the laboratory under normal room temperature.

Real Experimental Procedure

Six different concentrations of Vitamin C of 0, 20, 40, 60, 80 and 100mg were prepared. Sixty apple cubes were cut with 10 apple cubes dipped into each concentration. The apple cubes were placed in different Petri dish and left at room temperature and condition for 24 hours. The apple cubes were then homogenised in a normal blender for 2 minutes centrifuged at 10000rpm and filtered. The absorbance of the clear apple juice at 420nm was measured using a spectrophotometer and the reading of the spectrophotometer was recorded as the browning index. The experiment was repeated once.

Safety Precautions

Gloves were worn when dealing with Vitamin C tablets to prevent the tablet from being contaminated.

Vitamin C solutions were prepared in situ and sealed after the preparation to prevent any loss of Vitamin C due to oxidation.

Care was taken when cutting the apple into cubes with knife to avoid cutting the hand.

Knife and all the apparatus involved in the handling of apple cubes were cleaned thoroughly before the experiment and were make sure to be free from rust as rust can accelerate enzymatic browning.

Apples were made sure to be healthy and not bruised when they were purchased.

All glassware and apparatus were clean and free from laboratory chemicals. Special glassware and equipment, stored away from all sources of laboratory chemical contamination, and reserved only for food experiments was used. [8]

Data Collection in Main Study

Statistical Analysis

There is a quite clear negative correlation between Vitamin c concentration and the browning index. This relationship was further proven by using Pearson product-moment correlation coefficient[10, 11] to test the linear dependence between the two variables being investigated.

The correlation coefficient, r ranges from -1 to 1. A value of 1 implies that a linear equation describes the relationship between X and Y perfectly, where as Y increases, X increases. A value of -1 implies that when Y decreases, X increases. A value of 0 implies that there is no linear correlation between the two variables.

The negative value of r indicates a negative correlation between the two variables investigated.

The critical value for 5% confidence level = 0. 811, which is smaller than the value of r calculated from the data of the real experiment. Therefore, the PMCC analysis showed that there is a statistically significant negative correlation between the concentration of Vitamin C and the browning index.

The null hypothesis can be rejected.

Data Analysis

The graph showed that the increasing Vitamin C concentration brings about an overall decrease in the browning index of the apple cubes by 69 %. From 0 mg to 80 mg of Vitamin C concentration, the browning index decreases almost linearly. Although there was a slight increase in browning index from 80 mg to 100 mg, the value of Pearson product-moment correlation coefficient, r = – 0. 942 re-affirmed that there is a strong negative correlation between Vitamin C concentration and browning index.

The browning index is an indication of the proportion of oxidised phenols[7] during apple storage of 24 hours in the experiment. A lower browning index indicates a lower proportion of reacted phenolic compounds. In other words,

the lower the browning index, the lesser the enzymatic browning taking place in the apple cubes.

When the apples were cut into cubes, they were exposed to oxygen and enzymatic browning was initiated. By coating the apple cubes with a layer of Vitamin C, also known as ascorbic acid (AA) which is a very strong reducing agent, the process of enzymatic browning was inhibited as AA prevented O-quinone from being converted to its colored end product. Besides, AA competed with polyphenol oxidase (PPO) to react with oxygen. AA also formed a barrier to prevent oxygen from reacting with the substrate of the enzymatic browning process. The mode of AA action upon phenols can be summarized as follow: [6]

O -diphenol + ½ O2 O – quinone + H2O

O -Quinone + AA O- diphenol + dehydro AA

A A + ½ O2 dehydro AA + H2O

With increasing Vitamin C concentration, more Vitamin C was made available to prevent the oxidation of phenols, thus the amount of oxidised phenols which contributed to the absorption at 420 nm was reduced and the browning index decrease.

From the graph, the Vitamin C concentration that gave the lowest browning index was 80 mg. The browning index at this concentration was 86% lower than that of 0 mg. This showed that 80 mg was the ideal concentration which inhibits enzymatic browning happening in the apple cubes most effectively.

Vitamin C concentration of 0 mg was used as a control in this experiment to show that Vitamin C did play a role in inhibiting enzymatic browning.

The browning index at 100 mg was higher than that at 80 mg when it was expected to be lower. This may be due to another type of browning, known as non-enzymatic browning taking place in the apple cubes in which ascorbic acid was found out to play a role in some non-enzymatic browning. The non-enzymatic browning may have produced end products which also caused absorption at 420nm. As ascorbic acid affects non-enzymatic browning, the higher ascorbic acid concentration at 100 mg contributes to more non-enzymatic browning than that at 80 mg, contributing to a slightly higher browning index. [1]

Evaluation

In food processing industries, the average effective level of Vitamin C used for apple halves is 660mg/kg[2], which is also equivalent to 0. 66mg/g. The total mass of all the apple cubes used in the experiment for each concentration was 45 g, so the ideal concentration of Vitamin C should be 29. 7mg which almost three times lower than the ideal concentration obtained in the experiment. This may be due to errors and limitations which had arisen during the experiment.

Apples are very porous and thus very difficult to treat. It is difficult to get ascorbic acid solution into sufficiently intimate contact with the fruit, which is important to maintain the quality of the fruit.[2] In this experiment, the apple cubes were only dipped into the Vitamin C solution and the solution did not have time to diffuse completely and reach every cell in the apple cubes.

Besides that, this experiment was carried out under normal room temperature in which enzymatic browning occurs at a faster rate. The apple cubes were left at normal room conditions and constantly exposed to air, thus more ascorbic acid was needed to inhibit enzymatic browning as compared to vacuum packed processed apples. Moreover, a delay in adding ascorbic acid after the cutting of apples will cause permanent browning as the enzyme was not inactivated rapidly enough to prevent any appreciable oxidation to occur before ascorbic acid was added. [2]

3074 words The apples may be subjected to mechanical injuries during handling which may contribute to browning of the apples. Therefore, the apple cubes were handled as carefully as possible. A stainless steel knife was used to make sure there was no rust which could accelerate browning of the apple cubes. Different types of apples have different amount and types of phenolic compounds, substrates of enzymatic browning, which would affect the rate and amount of browning happening.[1, 2] Apples of the same origin and type were bought at the same time at the supermarket to minimise this limitation. Further enzymatic browning may occur during the blending of apple cubes which may affect the final browning index. To prevent this, during blending, 50cm^3 of Vitamin C solution of that particular concentration which the apple cubes where dipped into was added.

The quantification method used in this experiment has been simplified as the materials and apparatus available in my college laboratory was limited. The experiment can be improved using a more accurate and advanced method, polyphenol oxidase (PPO) assays [6, 7] which measures the effect of Vitamin C on the activity of PPO directly so that the results obtained would be more

reliable. Other improvements of this experiment include soaking the apple cubes longer in the Vitamin C solution and cutting the apples into smaller pieces to increase their total surface area to volume ratio for rapid diffusion of Vitamin C solution into the apples.

Further studies on the type of food additives that affect the enzymatic browning of apples could have been investigated and their effectiveness compared. [6]

Conclusion

There is a negative correlation between Vitamin C concentration and enzymatic browning with 80 mg as the ideal concentration of Vitamin C which significantly reduced enzymatic browning by 86%. This was determined by the decreasing browning index as the Vitamin C concentration increases.

Sources Evaluation

Sources 1 to 4 are published books by food experts and also accredited universities. Moreover, books which are published are usually reviewed by other experts from that particular field prior to publication. Therefore, the information from these sources is reliable and factual.

Sources 5 to 7 are online journals about Vitamin C and its effect on enzymatic browning, the quantification method of browning and the factors affecting enzymatic browning. These journals are peer-reviewed and so should contain sound scientific information. Furthermore, the information in these journals has been found to correlate each other, implying that they are highly regarded as reliable sources.

Cumulative word count: 3349 words Sources 8 to 12 are websites which are well established and have many viewers. Therefore, the information they provide must have been screened and reviewed by experts to ensure they are sound and correct.