Stage 3

Science Investigation

Term 2, 2015

Inhibition of enzyme browning in apple slices

By

Kyle Hubbard

Title

Inhibition of enzyme browning in apple slices

Aim

To investigate different ways and methods, using household items, to stop or slow enzymatic browning in Apple slices. The aim is to find the best inhibitor so I can propose it for lunch break preparation. The resulting apple slice must be still be crisp and fresh with minimal browning.

Research

What is enzymatic browning?

It is a reaction that happens when a specific enzyme (called polyphenol oxidase,) and a certain type of chemical (called phenolic compounds or phenols) combine and react in the presence of oxygen. During this chemical reaction a brown pigment called melanin is produced (the same melanin that gives you your skin tone). It is still perfectly safe to eat but it just looks unappetizing.

The main components of the process.
Enzymes
Phenolic compounds
Oxygen

Enzyme

What is an enzyme?

Enzymes are basically proteins but they have very special jobs.
Enzymes allow chemical reactions to happen that would otherwise never happen because they need too much energy. (Most chemicals like the phenolic compounds (or phenols) are really lazy.) Enzymes are like protein robots inside your cells. So these protein robots take one or two pieces do something to them then let them go. When the robot is finished with the two pieces they move on to the next piece. It is like a robot spray arm in a car factory the robot sprays a car and move on to the next car in the line. The robot only does that one task and nothing else so that robot spray arm can't bolt or lift the car panels enzymes are the same

they can only work on specific molecules and they can only do specific jobs and when the enzyme are finished with the pieces it releases them and the works on the next pieces

Proteins are made from long chains of amino acid molecules. If any one single amino acid is different to the rest it could change the shape, which would stop the enzyme from doing it job because it like a jigsaw puzzle piece. Just like the same robot spray arm configured to spray a mini can't be used to spray a Bugatti Veyron. The car has a different measurements for example the windows are in different places. Enzymes are the same.

So how does an enzyme work?

There are four steps in the process of an enzyme doing its job.

1. The enzyme and a substrate must be in the same area. A substrate is the stuff that the enzymes work on.

2. The enzyme grabs on to the substrate at a special area called the active site. Enzymes fit with other molecules kind of like a puzzle piece so shape means everything it can only fit one way. The active site is like the grasping hand of the robot on an assembly line. It can only pick up one specific part.

3. A process called catalysis happens, catalysis process is when the substrate is changed. It could be broken down or join up with another molecule to make something new.

4. The enzyme lets go and goes back to its original state, ready to work on another molecule of substrate. The first molecule of the substrate that has just been changed is no longer the same and is now the product. The product of enzymatic browning is a dark pigment called melanin (the brown colour) what really happens is that it makes the product Quinone and all the Quinone joins together to make the dark colour.

The enzyme responsible for browning of apples is called POLYPHENOL OXIDASE.

An oxidase is any enzyme (-ase) that oxidizes something. The thing being oxidized is a polyphenol. That is why it is called: polyphenol + oxid + -ase = Polyphenol oxidase!

The enzyme combines with the polyphenols with oxygen from the air and makes Quinone's. They then combine to make the dark coloured molecules called melanin's (the brown colour).

What is the purpose of the enzyme Polyphenol oxidase?

Polyphenol oxidase is part of a defence mechanism, which protects damaged parts of the plant from infection. It converts toxic phenolic compounds into Quinone's, which are not harmful to plants. The compounds it produces kill bacteria and fungi.

Phenols

What are phenolic compounds (or phenols)?

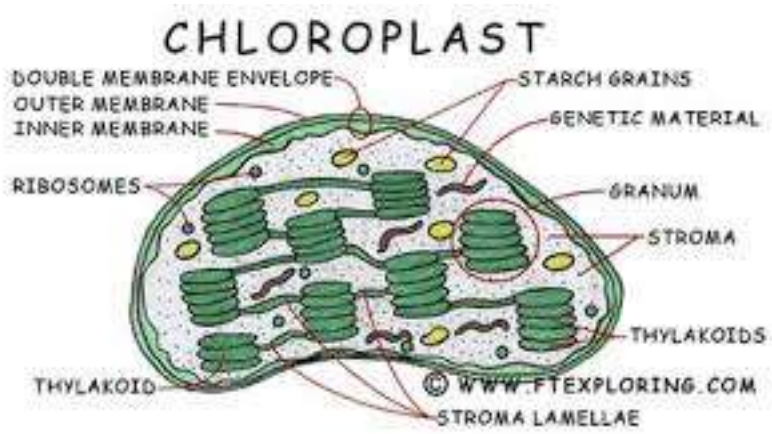
Polyphenols are a large class of chemical compounds naturally found in plants.
In plants polyphenols help defend against attack by insects and give plants their colour and are also important anti-oxidants in plants. Antioxidants clean up free radicals molecules that can harm our cells

Where is the location of the enzymes and Phenols?

The enzyme is stored in the plastids are the place where important chemical compounds used by the cell are produced and stored. Plastids are the double membranes found in plant cells.

The phenols are stored in the vacuoles are storage bubbles found in cells. They store things like nutrients for the cell to survive.

In the Chloroplast picture you can see the double membranes or Plastids.



How does enzymatic browning happen?

Slicing or biting or bruising an apple lets the phenols and enzymes that are normally kept apart by the plastids and Vacuoles to get together.

As soon as this phenol-enzyme mixture is exposed to oxygen the browning process begins. The longer it sits the more brown pigments are formed until all the available phenols (catechol) have been used up and the apple is as brown as it's going to get.

Hypothesis

For enzymes:
Temperature - I think the heat will denature the enzyme and keep the apple slice white for longer. So the 30 seconds will limit the browning but may make the apple mushy
I think the cold will slow down the browning but not completely stop it.

PH Levels - The higher the pH level, the more basic the solution so the apples will be browner due to enzymatic reactions. The low ph level of lemon juice and vinegar should make the enzyme denature.

For the oxygen:
Water – It will slightly slow the process until the apple is dry
Syrup - Sugar water syrup will slightly slow the browning because it leaves a bit of a film.
Antioxidants -Dunking in antioxidants I reckon will make a big difference and I think it will definitely slow the enzyme browning

For stopping the phenols from leaching, I think the calcium will only stop the browning slightly because I read that a lot of the companies already spray picked apples with calcium so the calcium spray has already done its job and I don't know if more calcium will help.

I think the best way to stop the enzymatic browning is a combination of methods. Vitamin C (antioxidant), calcium (stop the Phenols from leeching out) and lemon juice (acidity) will work the best as each does important jobs to stop the enzymatic browning.

Method

Control

For my control I need the time it takes for the natural browning to occur. I left the apple slices for 5 hours 36 minutes to make sure, but most of the browning happened before 2 hours. The browning started at 8 minutes. After that the slices looked the same but they only got drier so at the end they completely dried out. There was a less browning than I expected because I thought they would go very dark brown all over. I have come to the conclusion that the apples I buy from the shop have been sprayed with calcium. My background research proved that the apple companies spray apples with calcium to prevent browning

Variables:

Room temperature: After I did my 1st and 2nd tests I realised there was a problem so because we had one cold week and one warm week the tests varied. So all test 3,4 & 5 will be done on the same day for each experiment.

Size of apples: it is too hard to cut and weigh the apples because of how quick the browning starts. So I tried to cut the apple in equal slices of 8 pieces each. I check the 1kg bag of apples I bought each had 9 apples each so that the apples were pretty much the same size.

Fair test:

I will do all my experiment in the same room. I put all the plates in at the same spot on the countertop so they all had the same air temperature.

I left them all open to the air and not put them in containers so they all had the same amount of oxygen.

I will only use the same type of apple - Granny Smith apples.

I will use the same measurement teaspoon and measurement tablespoon.

Stop the enzyme

Because enzymes are proteins, they denature (change shape) in the presence of heat or acidity. When a protein denature it loses its specific shape and because of that it loses its function. Enzymes work on other molecules by acting like a puzzle piece so it only fits one way. That means the enzyme's shape is critical to do its job. Once it is denatured the enzyme cannot go back to its original shape. That means the effect is permanent.

Things that affect the enzyme activity:
Temperature
Activators (they are molecules that bind to enzymes and make them do their job faster). We don't want this!
PH levels
Inhibitors (molecule that binds to an enzyme and make it go slower)
Genetically modified apples: Arctic Apples genetically modified their brand of apples. Arctic Apples produce almost no PPO so that enzymatic browning reaction never occurs. (http://www.arcticapples.com/blog/julia/how-did-we-make-nonbrowning-apple).
Genetically modified apples will not form part of my experiment.

Temperature:

Proteins change shape as the temperature changes. High enough temperatures will make the enzyme denature. This means that the active site (the bit that looks like a puzzle piece) can't fit around the substrate anymore.

Heat:

Heat can be added by the following methods: Blanch, boil, steam, bake, broil, fry, poach, or microwave. Heat will destroy the enzymes, but it will also cook your apple. So I can't use any of these methods in my project except for maybe a quick blanch otherwise the apply will go mushy. Part of my aim is that the apple must stay crisp.

So to find out if blanching actually works and how long you can blanch it for before the apple gets too soggy I will take the maximum time of 30 seconds because that is how long you cook asparagus for and they should be crunchy after blanching and never be soggy like asparagus I will dip the apple slice in ice water to stop it cooking further. I will record at the start and then 8 minutes because that is when the control experiment showed the apples started to brown.

Variables:
Browning while waiting for blanching of different slices- cut the apple under water to minimise browning while waiting

Fair test: I use a stopwatch to make sure I blanch for exactly the amount of seconds. I dunk all slices in ice-cold water for 5 seconds to stop any further cooking. Have a control.
Materials:
1 x big container to cut apples under water
1 x Pot of boiling water
1 x Bowl of ice water
1 x Thongs
5x Plates
5 x sticky note: 5 sec, 10 sec, 15 sec, and 30 sec, control.

Steps:
> Peel and cut the apple under water to minimize any enzyme browning before I blanch them.
> Cut the apple in 8 equal slices
> Use thongs and drop the apple in for the time
> Grab the apple with the tongs and immediately drop it in the ice-cold water for 5 seconds
> Put it on the place
> Put the control on the countertop.




The times here are the minutes from the time I am putting it on the plate. The photos are of my test 2.

Conclusion

My first test showed browning in the15s and 30s almost immediately but is because I left those pieces of apple on the plate waiting while I boil the others. So I changed my test to cut the apple in water and leave it under water until I put into the boiling water. I asked my little brother to keep the apple slices under the water until I needed each one.
Test 2-5 showed all slices starting to brown around the 10-minute point so keeping the apple in water until the blanching worked. All tests shows that even up to 30 seconds the enzyme does not change shape and browning still happens. After the 30 second blanching it was a bit slimy and mushy.

So I can't use this method because though cooking it for longer will keep it white because the higher heat denatures the apple slice, the end result apple will not be crisp and fresh and not fall within my aim. It does not support my hypothesis because the 30 second blanch will be long enough at high enough temperature for the enzyme to denature. But it was right in that the apple is too slimy and mushy to be a possible solution.

Scientific explanation: High enough temperatures will make the enzyme denature.

Cold:

Refrigerate the apple after cutting it. The enzymes still do their job in the fridge, but they are much slower at low temperatures.
Refrigerate the apple after cutting it. The enzymes still do their job in the fridge, but they are much slower at low temperatures. For this experiment I will do two different type of cooling, one apple will have an ice pack and the other apple will be in the fridge. The ice pack is the method I normally use for school e g. lunchbox fruit break and it is easy

Variables: Size of apple- Cut the apple in 8 equal slices to make the size is close to each other as possible
Room temperature: Did not put the heater on in the lounge so that the kitchen stayed the same temperature

Fair test: Kept the apple slices the same size, kept the room temperate the same, kept a control

Difficulties: because I need to keep taking photos and making observations of the slices I cant keep the slices in containers so that means the icepack can't keep the air around the apple cold.

Steps/Materials
> Peel and cut the apple
> Put apple slice in the fridge
> Put apple slice in on top of an icepack. Remember for a fair test I can't put them in containers so they will be on kitchen towel.
> Put the control on the countertop.

Conclusion:
3 out of 5 experiments show the icepack apple takes longer to go brown then the control, so it only slows it down. (The other test was one a very cold day so I think the air temperature influenced the experiment.) The fridge one takes slightly longer to go brown but it does catch up with the control after 2 hours. I think the fridge slice does better because it is put in an area that is already cold from the start; the icepack takes a bit to cool down the apple.

Scientific explanation: Though the enzyme shape is not affected (denatured) because the temperature is lower the enzymes and molecules will have less energy so move around slower and rate of reactions decreases. So it supports my hypothesis because it only slows the process down but does not stop it.

Difficulties: Up till now I have done 1 experiment test followed by another one but the change in room temperature is giving a problem. We had one very cold week and then one warmer week. So I will conduct all my test 3, 4 and 5 on the same day. I only have enough room on the workbench for 3 sets of tests. I will do this for all my experiments. This should limit the effect of temperature on the results

PH level:

Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are alkaline. The optimal pH for the Phenol oxidase enzyme is between 5 to 7 so **Acidity dentures the enzyme.**

Materials:
4 X cups with 200 ml water each
2 tsp. Cream of tartar
4 tsp. Vinegar
Lemon juice
2 tsp. Sodium bi-carb

Experiment: I will test the acidity and alkaline ph. levels in one experiment
Lemon juice works because of its acidity. Lemon juice's ph. is about **1.5 to 2**. Lemon juice is usually the type of acid used because it tastes nice with apples. You can add sugar if the juice is to tart.
Distilled white vinegar usually measures around **pH 2.4 – 3.6.**
Cream of tartar. **4 PH**
I will use Sodium Bicarbonate solution for my alkaline test. It has a **pH level of 8.4.**

Variables:
Dunking time: Dunk each apple slice in each solution for 2 minutes.

Steps

- Prepare each solution: Pure lemon juice, pure vinegar (20 ml per 100 ml solution), Cream of tartar (1 tsp. per 100 m.) & Sodium Bicarbonate (1 tsp./6g per 100 ml of water for a 6% solution of NaHCO3)
- Slice apples in same size 8s
- Dunk apples for exactly 30 seconds in each solution
- Put slices on kitchen towel

Conclusion:

Lemon juice is the winner by far. At 2 hours it still had almost no browning. The vinegar is slightly less brown then the Cream of tartar. After 5+ hours it was super brown. First I thought it was because the vinegar I used was closer to 3.6 than 2.4 but then that cant be true because it then should be the same as the tartar and not more brown. Cream of tartar is a better inhibitor than bi-carb. After changing the experiment like I said in the difficulties part

Scientific explanation:

Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are alkaline. The optimal pH for the Phenol oxidase enzyme is between 5 to 7 so Acidity dentures the enzyme.

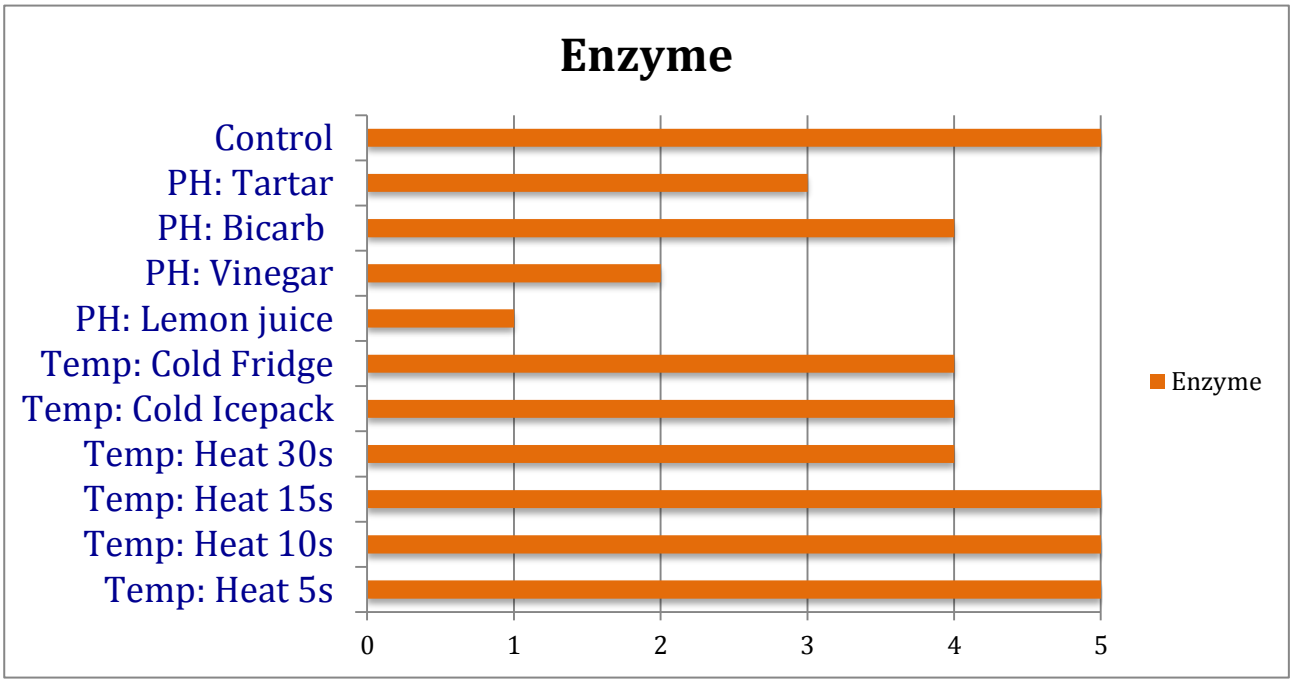
Low pH has more H+ ions and then the enzyme can't grab on to the substrate at the active site because the H+ blocking the binding sites

So it supports my hypothesis that low ph level will inhibit enzyme browining.

Difficulties: I was surprised the vinegar went so very dark brown and did another test will the same result. I guessed it is either because the ph. was closer to 3.6 than 2.4 but then that cant be true because it then should be the same as the tartar and not more brown. It must be because it is undiluted vinegar. I looked for a long time on the internet but couldn't find an explanation on apple slices but then I found the site that said in growing plants that it said that undiluted vinegar will dissolve the cell membrane of the plant so I will use a solution 20ml of diluted vinegar to 100ml of water

So now I will do another test with 1.0% solution. This will be 4 teaspoons 20 mil per 100ml **(5% acetic acid)**. After 5 + hours

Result



Key:

(compared to the control)

1: No Browning present

2: Some brown patches

3: half covered

4: Only stops the browning slightly

5: Same or close to the colour of the control

Stop the oxygen.

Oxygen is needed for the browning reaction to happen. The PPO only act on the Catechol in the presence of oxygen.

 Water:

If you dunk the apple slices in water it will slow down the browning process because the oxygen can get to the cell surfaces of the apple.

 Syrup

If you add sugar to the water and make syrup (it may have a bit more staying power because the syrup leaves a bit of a film. Although it will taste better, so a plus point for taste but but minus point for healthy.

You can also dunk it in water than covering it with sugar but that is too unhealthy so won't form part of my experiments.

 Antioxidants:

When electrons are transferred between molecules the process is called oxidation. So the polyphenol is oxidized because it loses electrons. We say that something is oxidized when it loses electrons, and something is reduced when it gains electrons. A reducing agent is an element that gives way its electron to another element. So a polyphenol is a reducing agent but antioxidants are better reducing agents

Water - because the oxygen can get to the cell surfaces of the apple.

Syrup - it may have a bit more staying power because the syrup leaves a bit of a film.

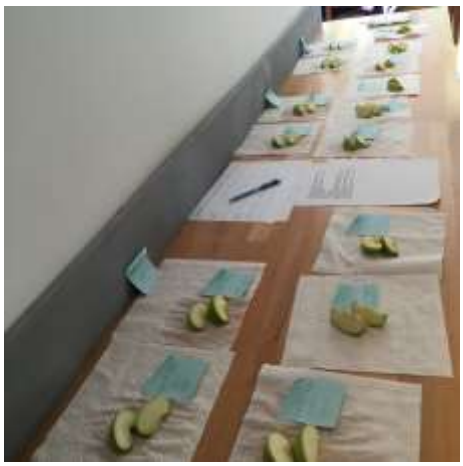
Vit C - because Antioxidants will react with the oxygen and steal it from the enzyme.

Homemade surface glaze: the glaze will have mostly all aspects in stop the oxygen method (except for Vitamin C) the film that it form will have the strongest staying power.

Glaze. In a small pan, add lemon juice, water, sugar, cornstarch and Knox gelatine Whisk all together thoroughly until no lumps remain and cornstarch and gelatine has dissolved

Over medium heat bring to a boil and boil all ingredients 3 to 4 minutes, stirring consistently. Remove from heat. Cool for 3-5 minutes. No longer then 5 minutes, or your glaze with set up.

When glaze has slightly cooled, using a basting brush or a silicone basting brush. Lightly coat over fruit until all is covered.



Materials:
3 tsp. of fresh lemon juice
4 tbsp. water
4 tbsp. white sugar
1 1/2 tsp. cornstarch
1 vitamin effervescent tablet
1 (7 g) small envelope of Knox® unflavoured gelatine

Steps:
> Prepare the solutions: Normal tap water, Syrup (1:2 mixture of sugar: water) and Vit C (1 effervescent tabled in 1 cup of water) & homemade glaze
> Slice the apples in equal parts
> Dunk the apples in the solutions for exactly 2 min
> Put the slices on the paper towel

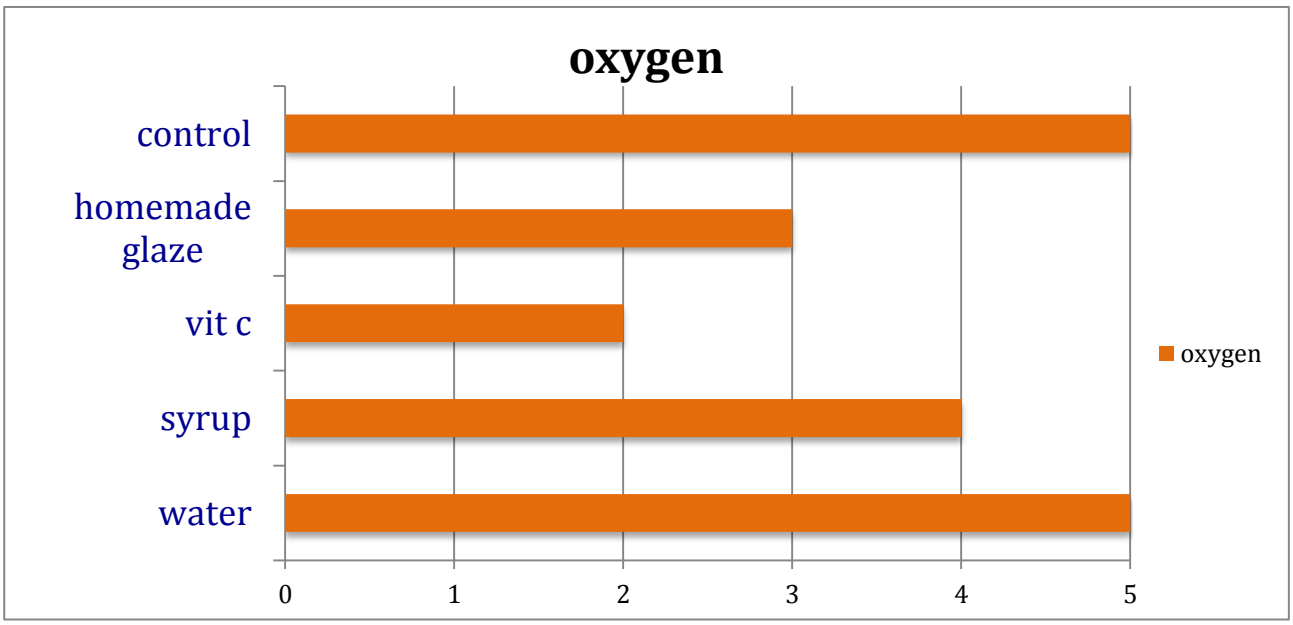
Variables:
The glaze could of set before all the dunking was done but while the glaze was cooling (2 min) down I dunk the rest of the slices in the solutions
Sugar syrup could have had either too much sugar or water so I double check that I have the water and sugar ration right to make the sugar syrup
If an apples is dunked for different times. I got my family to help and dunk the apples all at the same time

Conclusion:
Vitamin C is winner flowed by homemade glaze, which had a small difference but browned a bit earlier than Vitamin C and the best staying power than syrup and water than syrup which I thought was a bit sweet but it did work better than the water and it had better staying power than water but at 20 minutes it eventually wearied off and finally water which I thought did the worst, It stop the browning for two minutes which it started 10minutes so it was okay but good. The glaze inhibited the browning almost as well as the Vit C expect for the top part where I think the glace was runny and did not cover the tip very well.

Scientific explanation: Oxygen is needed for the browning reaction to happen. The PPO only act on the Catechol in the presence of oxygen. If you dunk the apple slices in water it will slow

down the browning process because the oxygen can get to the cell surfaces of the apple. A glace forms a more permanent barrier than water or syrup. Vit C remove the oxygen by using a more powerful reducing agent than the polyphenols. So if you add antioxidants to the apple then it will react with the oxygen and steal it from the enzyme. So it supports my hypothesis that antioxidant will inhibit enzyme browining.

Result



Key:

(compared to the control)

1: No Browning present

2: Some brown patches

3: half covered

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5: Same or close to the colour of the control

Stop the phenol

When you cut the apple the phenol leaches out from the cell walls. Calcium works because it firms the cell walls. If I had calcium to my apple slices it will to firm the cell walls in the apple and slow down the leaching of phenols out of the cut cell structure. It will work like a band-aid.

Materials:
1 x cup
1 x effervescent calcium
1 x cup of water

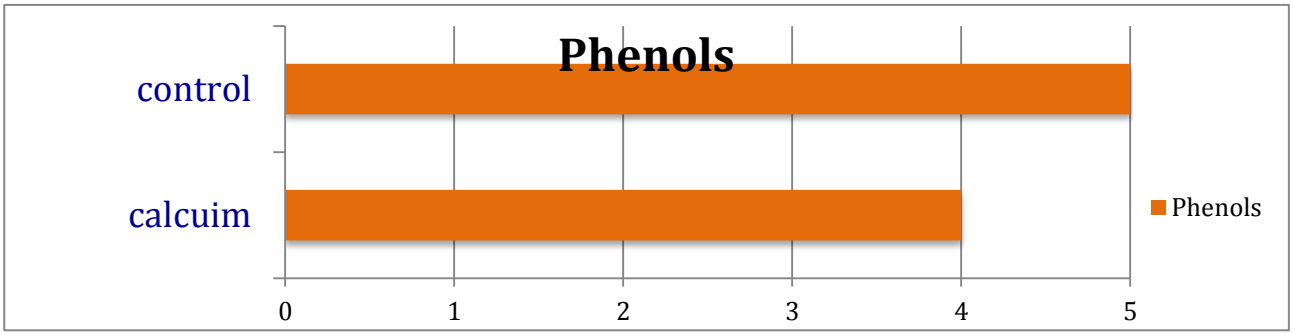
Steps:

Steps:
>Add 1 effervescent calcium tabled to a cup of water.
> Cut the apple
> Dunk the apple slice in the calcium for 2 minutes

Conclusion:

The calcium inhibits the browning very, very slightly. Calcium firms the cells walls and stop the phenols from leaching out so there is less phenols for the enzyme to work on. So it supports my hypothesis that antioxidant will inhibit enzyme browning a little.

Result



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Power method

Combine the winner Rank 1 of each method!

Power solution: Vitamin C antioxidant (stop the oxygen) Calcium antioxidant + strengthen cell structure (Stop the phenols), Lemon juice to lower the pH (stop the enzyme).

Materials:
4 x cups/glasses
Lemon juice
1 x Vitamin C dissolved in 200 ml water
1 x Calcium dissolved in 200 ml water
1 x Power solution: 1 x Vitamin C/Calcium combo effervescent tablet plus 2 teaspoons of lemon juice in 200 ml water

Steps:
> Prepare the solutions disolve the tables
> Dunk apple slices in for 30 seconds
> Put apple slices on paper towel
> Put control on paper towel for the fair test

Conclusion:
I think that the powerhouse combination work the best it was as good an inhibitor as the lemon juice but the pure lemon juice can be sour when eating the apple. The powerhouse tastes not so sour plus it has Vit C plus it has Calcium so is healthier than just the lemon juice

and completely inhibits the browning for the maximum length of 2 hours. So it supports my hypothesis that the combo will inhibit enzyme browning the best.

Overall conclusion

I wrote down the conclusion, analysis and showed the results table after each experiment so that it is easier to follow and is not disjointed)

Best inhibitors:
The optimal pH for the Phenol oxidase enzyme is between 5 to 7 so **low ph. levels** denatures the enzyme because the H+ molecules blocks the binding sites.
Antioxidants are better reducing agents then the substrate. So the antioxidants will react with the oxygen and steal it from the enzyme. **Calcium** will firm the cell walls in the apple and slow down the leaching of phenols out of the cut cell structure.

Overall, I think the Power combo method is the winner because it combines low ph. with antioxidants with calcium. It keeps to my aim where it inhibits the browning for 2 hours and the apple is still fresh and crisp and tastes nice.

Investigation conclusion on use in the wider world:

My homemade glaze had two problems it did not looks so appetizing because the glaze is shiny and may look a bit slimy and it did not cover the apple evenly because it was so runny.

But I found on the web that they use the gum from inside the plant to make a base for something things that we eat like gum, sweets, and jelly. So this might work better then a glaze so if they used the gum as a base and all the best inhibitors to it then it could make a healthy snack that supermarkets can sell.

I sent emails to Woolworths, Coles and Aldi. I asked my mum to help with my grammar.

This is the text in my email:

I am doing a year 6 science project on the best inhibitor of enzyme browning with the aim to promote apple slices as a long-living healthy snack.

Would you consider having packaged ready-to-eat sliced apple products on your supermarket shelves?

My result of my project has shown that Vit C is a great inhibitor because antioxidants are better reducing agents than polyphenols. Lemon juice was excellent in inhibiting enzyme browning because the enzyme denatures with a low ph. Lastly, to stop the oxygen a glaze works well.

Edible plants gums are used as a base for chewing gum. So if you were to combine an edible gum with Vit C and lemon juice and add it to apple slices as a thin film then it would prevent browning and increase shelf-life. Lemon juice enriches the taste of apples and Vit C adds nutrients.

Kids will like it because it looks fresh and crisp and tastes well - no more mushy brown apple. Parents will love it because it is quick and easy to buy and add it to a lunch box and of course because it is healthy with a dose of Vit C. It will be good PR for you because it shows that you care about your customers. So a win-win for everybody!

So to get back to my question is packaged ready-to-eat sliced apple products something that you will seriously consider in the near future? If you have done any trials or taste tests I will be very interested to learn more.

Kind regards,

Kyle H.

Acknowledgements:

Special Thanks to mum for supplying me with the needed items for my experiment. I also asked mum to check my grammar. Thanks to my little brother and mum for being my extra hands when I needed them to hold down the apple slices in the solution for my ph level test.

Thanks to Dad for helping me with the problem of Word splitting my document into multiple files when saving as PDF.

Stage 3

Science Investigation

Term 2, 2015

Log Book

By

Kyle Hubbard

Date	Log Entry
20 – 30 Apr	I looked at multiple options for my project. It came down to a Wi-Fi experiment and the enzymatic browning. I quickly learned that Wife was not a good option because the companies did not respond to my emails asking if I could borrow the Electromagnetic Radiation Detector so I chose the enzymatic browning.
1 – 3 May	I carried on doing some background research on what is enzymatic browning and the different parts that make up the reaction.
4 May	I posted my outline for the investigation.
6,9 & 13 May	I started on my background research and found about 12 websites so far.
16 May	I created my draft skeleton of the investigation. I research where catechol (the polyphenol chemical) and the polyphenoloxiase enzyme is located in the cell. I couldn't find a diagram that clearly showed it. So I printed of a diagram and wrote on where the locations of the phenols and enzymes are. I'll check with Mrs X on Monday
21 May	I couldn't find Mrs X but I searched some more on the internet and it is something called Vacuoles so I will have to look what that is and where it is in the plant cell to figure out in what why they don't normally mix.
23 May	Research what is enzyme browning. Research how does an enzyme work? They are like little protein robots, which is cool because I like robots. Write down what is enzyme browning. IT was hard work.
24 May	Write down how does an enzyme work. This found this diagram that shows POLYPHENOL OXIDASE reaction. It was difficult to understand. It took a long time to write down both of those in my own words.
26 May	Looked for a diagram where it shows the enzyme process and where it looks like a puzzle pieces like in my explanation.
27 May	Research what is the purpose of the enzyme Polyphenol oxidase It took FOREVER to find anything on the internet. I could only find two places where they talk about it. I think most scientists are guessing and nobody really knows why. I put down in my project what everyone thought it was for.
30 May	Background research on What is phenolic compounds (or phenols and where is the location of the enzymes and Phenols? It took a long time to find out that phenols are not in the plant cell membrane but in the Plastids inside the plant cell.
31 May	I did the first experiment for the control: For the control apple I left the apple slices for 5 hours 36 minutes to make sure, but the browning

	stopped at 2 hours. After that the slices looked the same but they only got drier so at the end they completely dried out. There was a lot less browning than I expected because I thought they would go brown all over. I have come to the conclusion that the apples have been sprayed with calcium. My background research proved that the apple companies spray apples with calcium to prevent browning. I wrote down how to stop the phenols and how to stop the oxygen. It is really half background research and half method but I will put it in my background section.
2 June	I finished the background research and finished writing down everything for the background research like Stop the phenol from leaching out. I added nice icon to represent each method
3 June	I wrote down my Hypothesis
6 June	I did a blanching test and recorded all my results I did the blanching test again. I sorted out the photos and copied them into my document and sorted them into a table. It took a long time. I put my results that I had from my previous blanching test into the table.
7 June	I did the 'Stop the oxygen' experiment with water, syrup, antioxidant Vit C, glaze and a control. I wrote down the results at the set times and took photos at the set times. I did the test again.
8 June	I did the pH level test with acidity and alkaline solutions. I wrote down the results at the set times and took photos at the set times. Something is very wrong. The vinegar is not stopping the browning. Maybe it is because the vinegar ph. is more to 3.6 than 2.4.
9 June	I did the pH level test again. Vinegar is still more brown. It can't be vinegar ph. because it is browner than Tartar, which has a ph. of 4. It must be because it is not diluted.
10 June	I looked on the internet to find out what happens when you put apple in undiluted vinegar. I can't find anything on apple but there is one website which talks about the effect on plants.
12 June	Did Stop the Enzyme Temperature test 2. Up till now I have done 1 experiment test followed by another one but the change in room temperature is giving a problem. We had one very cold week and then one warmer week. So from now on I will conduct all my test 3, 4 and 5 on the same day.
13 June	I do the ph. level test again with 1.0% vinegar. This time it works like I expected. Do test 3,4 & 5. I recorded my results and photos and put it in the document.

14 June	Did the Stop the oxygen test 3,4 & 5. I wrote my results and took photos and put it in the document.
16 June	Did the stop the enzyme temperature test 3,4 & 5. I wrote my results and took photos and put it in the document.
17 June	Finished all my conclusions and results if my tests so far. I have written an email to Woolworths, Coles and Aldi about why they don't have ready-to-eat sliced apple products on the shelves. I put in it my findings and suggestions and ask them if they would consider it.
20 June	Finished all my tests in including the Powerhouse. And recorded my results.
21 June	Sent out the emails. My document is a bit confusing now that I moved all the conclusions into one place because it I did all the experiments and by the time you read the conclusion you can't remember what the apple slices looked like so I am asking Mrs X if I can do the conclusion after each experiment. She checked it and said it is fine.
24 June	I still have not heard from any of the supermarkets I sent the email too. So I will send it again to other email addresses. I finished by suggestion in the wider world. I have a big problem when I try to save my word document to pdf because it makes lots of different pdfs and it looks like it makes a new pdf for each section break. I will ask dad to help me.
25 June	Dad fixed the pdf problem and I uploaded it to Mrs X. I am finished yippee!

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