Biology Internal Assessment

Purpose
My interest in the ripening of fruit developed from an observation that fruits bought in my local supermarket do not always ripen effectively. This stimulated me to find out more about the process of ripening in fruits. I chose nectarines as my material because they were in season and they seemed to be the worst affected by the problem of ripening.

Research Question
How do two different methods of fruit ripening affect the metabolism of starch to glucose in nectarines (*Prunus persica*) over 7 days?

Introduction

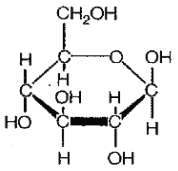

Figure 1 Glucose molecule
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Glucose is one of the most important carbohydrates in biochemistry and is pivotal in the key biological processes of photosynthesis and cellular respiration. In the ripening process, starch molecules (polysaccharides) are broken down by digestive enzymes to glucose (monosaccharide). This process is made possible by the induction of ethene gas.[2][3]
Ethene gas is biological hormone that is used in plants to stimulate key processes, for example the germination of seeds, fruit abscission and the ripening process. It is more readily produced by some fruit, in particular bananas and apples, and will hasten the ripening of fruit when in a contained environment, for example inside a plastic bag or box. Another method suggested is to bury the fruit in rice. It is supposed to retain the ethylene gas produced by the fruit longer.[4]

This experiment aims to simulate three different ripening conditions, all of which are presumed to induce the ripening process. In the first trial, a banana will be placed with a nectarine in a closed bag. In the second, a nectarine will be placed under rice in a plastic box. Thirdly, a control whereby a nectarine is placed alone in a plastic bag, will be set up as the null hypothesis, supporting the assumption that the production of ethene gas and the concentration of glucose are independent of one another. It is important that all three trials be conducted in closed environments, which favour the retention of ethene gas.

The presence of glucose has been used in this experiment to indicate the extent to which ethene gas has affected the metabolism of starch and the concentration of simple sugars in nectarines.

The detection of glucose concentration is possible through the use of a coloured indicator composition of potassium permanganate ($KMn0_4$) solution and an acid, in this case sulphuric acid (H_2SO_4). A strong oxidising agent, $KMn0_4$ solution is used to convert alkenes to glycols and thereby quantitatively test for the presence of unsaturated bonds within a sample. The $KMn0_4$

[1] http://mwsu-bio101.ning.com/profiles/blogs/the-molecules-within-you-1
[2] http://www.newton.dep.anl.gov/askasci/bot00/bot00553.htm
[3] J.H.LaRue & R.S.Johnson (1989) Peaches Plumbs and Nectarines U Cal Google Books http://books.google.fr/books?id=0EEtgcbJaAIC&pg=PA163&lpg=PA163&dq=starch+in +nectarines&source=bl&ots=8Iab1znGzd&sig=bjD1Nk0gCGTwj3zlbRenFlbREms&hl=en&sa=X&ei=wzo6T_C3LYfL0QW Kk42QCw&redir_esc=y#v=onepage&q=starch%20in%20nectarines&f=false
[4] Matthew Rogers 14/06/11 http://lifehacker.com/5811686/ripen-fruit-faster-by-burying-it-in-rice

solution is pink in colour and its discolouring demonstrates the metabolism of starch to glucose. The time taken for the pink colour to disappear is demonstrative of the concentration of glucose in the filtrate sample, e.g. the smaller the amount of time taken for the colour to disappear, the higher the concentration of glucose in the sample.

Prediction
It is expected that the nectarines exposed to the rice packaging trial will ripen the fastest. The contained environment in which they are placed will favour the retention of ethene gas around the nectarine. As a result, there will be a faster decrease in the concentration of polysaccharides (starch) and a faster increase in the concentration of monosaccharides (glucose) in this trial. The nectarines kept with the banana will also ripen faster than the control as the ethane produced by the banana will supplement that produced by the nectarines themselves.

Method

Materials
- 36 nectarines
- 12 bananas
- Snap lock bags, plastic containers
- Basmati Rice (approximately 3kg)
- 560ml Sulphuric Acid 1M (H_2S0_4)
- 230ml Potassium Permanganate solution 0.01M ($KMnO_4$)
- Knife, cutting board, food processor, sieve
- Stop watch
- Syringes - 3ml, 5ml and 10ml
- 4x 750ml beaker (each repeat)
- 12x 50ml beaker (each repeat)

This experiment aims to determine how ethene gas affects the concentration of glucose in nectarines. In order to come to a conclusion, two common methods of fruit ripening, i.e. banana packaging and rice packaging, were tested together with a control. The methods below correspond to these different conditions.

Due to the subjective nature of the 'end point' of the solution, i.e. when the pink colour disappears and the stop-watch is stopped, it was decided that measures should be taken to eliminate as much as possible this error. On each day of the different conditions (banana, rice and control), 4 nectarines were pulverised and effectively, tested. The filtrate of each nectarine was tested three times. This was done so as to eliminate any error that might be associated to the - stirring of the solution and avoid disparity in the results.

On Day 1 of the experiment the following were set up:
(a) one banana and one nectarine were placed into a snap-lock bag. The air inside the bag was removed and the bag was sealed
(b) one nectarine was placed into a plastic box. The container was filled with rice until the nectarine was fully covered and the box was sealed
(c) one nectarine was placed into a snap-lock bag. The air inside the bag was removed and the bag was sealed.

This was repeated in four trials for each treatment.
One untreated nectarine was retained on Day 1 to establish the initial glucose levels.

The fruit were left for 3, 5 or 7 days in room temperature conditions. At the end of the period the

nectarines were removed and qualitative observations and measurements of the glucose levels were made in the following way.

1. The flesh of the nectarine was removed and placed into a food processor. 500ml of distilled water were then placed in the same processor and pulsed for 30 seconds. The liquid was filtered, through a sieve, into a 750ml beaker.

2. 10ml of the nectarine filtrate was placed into a 50ml beaker. In addition to this, 2ml of $KMn0_4$ solution and 5ml of H_2SO_4 solution were added into the beaker simultaneously. The stopwatch was started immediately. The solution was swirled in a constant motion and at a constant speed.

3. When the pink colour of the solution had disappeared, the stopwatch was stopped and the time taken was recorded.
   This was repeated three times from the filtrate from each nectarine.

Variables

Variable	Identify variable	How to control variable
Independent	Conditions that the nectarines are exposed to, i.e. banana packaging, rick packaging and controlled environment	
Dependent	Time taken for the pink colour of potassium permanganate solution to disappear (demonstrative of glucose concentration)	
Controlled	Source and age of nectarines	All the nectarines were picked on the same day and sourced from the same supplier. When chosen, it was observed that they were of similar colour, size and firmness.
	Source and age of bananas	All the bananas were picked on the same day and sourced from the same supplier. When chosen, it was observed that they were of similar colour, size and firmness.
	Indicator composition	Remained constant. The ability of $KMnO_4$ solution to react with impurities meant that the same solution had to be maintained throughout trials.
	Same concentration of $KMnO_4$ and H_2SO_4	Ensures consistency. Pour a standard solution at beginning of experiment and use throughout
	Initial concentration of glucose	One nectarine was tested and used as an initial value. This value was used across all my trials.
	Nectarine sample	The entire nectarine flesh was pulverized to a filtrate on all repeats.
	Judgement of end point	The 'end-point' of the experiment had to be decided on. Therefore same person had to conduct the experiment to ensure valid results.
	Constant temperature	Temperature affects enzyme activity, i.e. will affect the rate of ripening. Conduct experiment in closed environment.
	Closed environment	Mold and other microorganisms require oxygen to grow, therefore, restricting the amount of oxygen in samples will restrict the development of mold.

Risk Assessment

All apparatus was labelled with relevant information (name, date class nature of materials and experiment)
All unnecessary materials were cleared away from the work space.
Glassware is fragile it was used towards the centre of the bench with stable supports.
Sharp cutting tools and the blender were used with care.

Electrical apparatus

The connections of the balance, magnetic stirrer and blender, were kept away from running water and trailing cables were avoid
Spills were cleaned up

Chemicals

Sulphuric acid is corrosive and toxic.
$KMnO_4$ is a powerful oxidiser and can cause fires.
Eye protection, gloves and lab jacket were worn when handling these chemicals.

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Results

Table showing the observations of the three methods on the ripening process

	Banana	Rice	Control
Day 1	One nectarine was used for all of the trials to ensure that the initial concentration of all the repeats was constant. All nectarines on Day 1 where firm, white/yellow in colour and had no visible mould on their surfaces.		
Day 3	Nectarines 1 and 4 showed signs of developing mould. The bananas of these nectarines were discolouring and condensation was visible inside the snap lock bags.	Nectarines were 90-100% covered by the rice. There was minimal condensation inside the box. No mould present.	No mould. No condensation. White/yellow in colour. Firm.
Day 5	All nectarines were softer. Signs of mould. White residue on nectarine 4. Flesh was noticeable darker. Condensation inside of bag.	All nectarines were mouldy, with nectarines 2 and 4 showing the largest mould colonies. White residue. Condensation inside the box. Nectarines were mostly covered by rice, one nectarine was only 75% covered.	No mould. Minimal condensation. Pinkish in colour.
Day 7	All nectarines are at least partially covered by mould and are emitting white residue.	All nectarines at least 9.0% covered in mould. The flesh is a deep brown colour. White residue.	Pink and white in colour. No mould. 'Bruising' patches (soft spots on surface).

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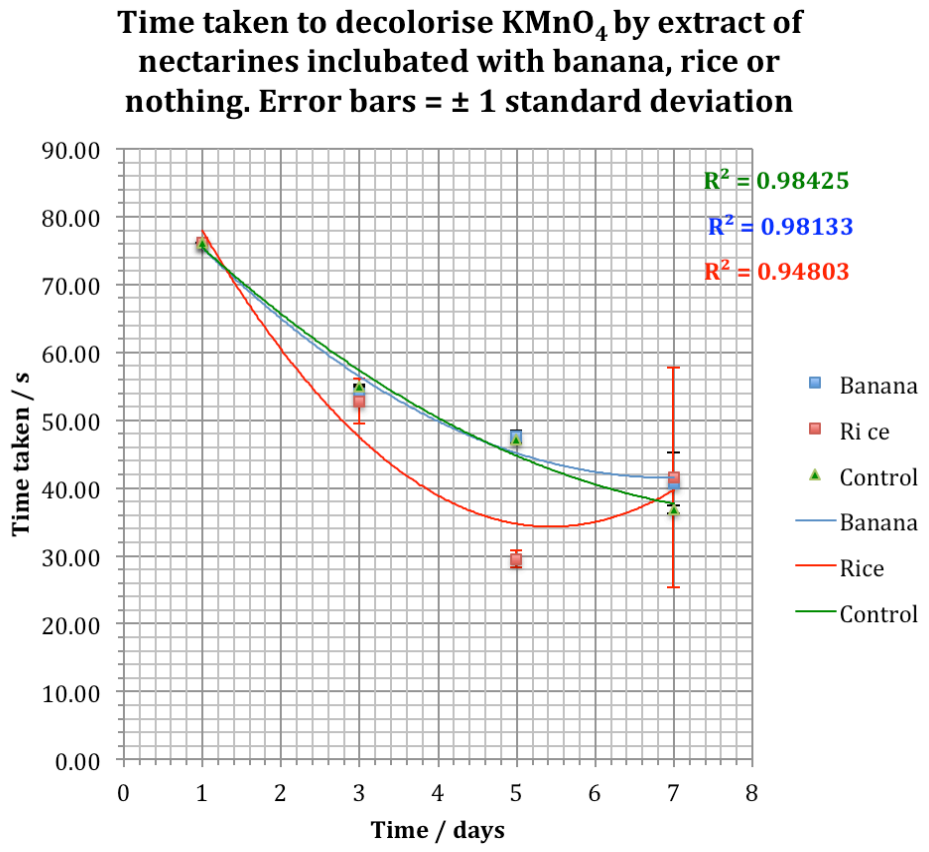
Table showing the amount of time taken for the pink colour of the potassium permanganate solution to disappear

	Time for $KMnO_4$ colouration to disappear / s ± 0.05s											
	Banana				Rice				Control			
Day	1	3	5	7	1	3	5	7	1	3	5	7
Trial 1	76.23	52.37	47.00	33.03	76.23	56.09	30.57	56.78	76.23	54.33	47.13	36.96
		52.98	48.87	34.31		54.59	31.00	57.23		54.67	46.98	36.78
		52.66	47.96	35.97		54.35	30.76	57.13		55.13	47.96	35.98
Trial 2		54.34	48.28	44.53		50.50	30.19	25.19		54.78	46.56	37.23
		55.65	47.88	45.66		49.86	28.20	26.63		54.65	46.78	37.65
		54.23	48.53	45.17		50.06	29.37	24.78		55.07	46.99	37.98
Trial 3		54.75	47.76	44.27		48.98	29.22	26.78		55.02	47.12	36.87
		54.17	48.22	43.18		49.43	30.45	25.87		55.34	47.56	36.45
		54.23	47.89	44.73		49.56	30.76	26.98		54.69	47.32	36.22
Trial 4		53.98	45.66	38.97		56.33	28.25	57.43		54.79	46.98	36.87
		54.37	46.76	39.24		57.19	27.91	56.91		54.99	47.51	36.98
		54.21	46.23	39.58		56.74	27.65	56.50		55.34	47.35	36.56
Mean	**76.23**	**54.00**	**47.59**	**40.72**	**76.23**	**52.81**	**29.53**	**41.52**	**76.23**	**54.90**	**47.19**	**36.88**
St Dev	**0.00**	**0.91**	**0.97**	**4.50**	**0.00**	**3.32**	**1.25**	**16.18**	**0.00**	**0.30**	**0.38**	**0.56**

N.B There is only one value for Day 1 as only one nectarine was used to test for the initial concentration of glucose. This value was used as the initial value (Day 1 value) for all of the subsequent trials.



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t-test
The data for the banana treatment and the control do not show much difference for the time taken except after 7 days. The control looks as though it has a higher glucose content than the banana treatment at Day 7. I decided to see if this difference was significant.

Null Hypothesis = there is no difference between the results for the banana treatment and the control on Day 7

Alternative Hypothesis = There is a difference between the results for the banana treatment and the control on Day 7
t-test equation

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

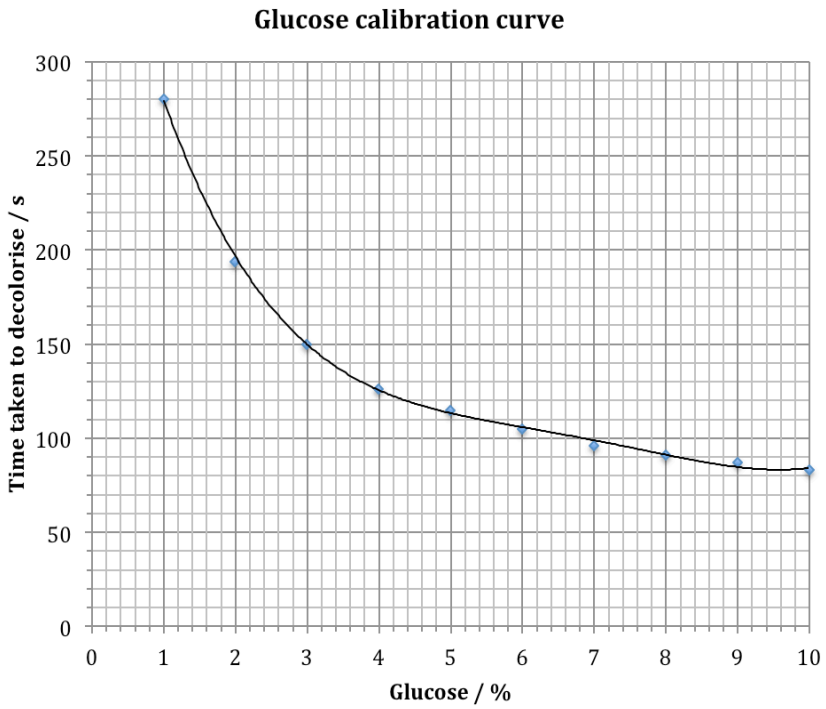
t_{calc} = 2.93
For p = 0.05 using a two tailed test
t_{crit} = 2.07

There for there is a significant difference the alternative hypothesis is retained the null hypothesis is rejected. However, this difference is not great, it is only significant to p = 0.01

Standard Reference Curve for Glucose Concentration

Glucose calibration

Glucose / %	Time taken / s ± 0.05s
1	280.00
2	194.00
3	150.00
4	126.00
5	115.00
6	105.00
7	96.00
8	91.00
9	87.00
10	83.00



Unfortunately the data obtained was outside of the range of the standard curve so curve could not be used to obtain an estimate of the glucose content of the filtrate.

Error and Limitations
It was acknowledged that the method for this experiment contained certain flaws and that the results obtained from the trials were subject to error. Error-reducing methods were implemented where possible.

Uncertainties were accounted for and are recorded below:

Identify uncertainty	Degree of uncertainty
Stopwatch	Reaction time ± 0.05 s
3ml syringe	± 0.1ml
5ml syringe	± 0.1ml
10ml syringe	± 0.2ml
Beakers	± 1.0ml

Because the glassware used in the experiment was not altered from trial to trial, the level of uncertainty in each trial would have remained constant. Care was taken to measure exact values, for example the amount of water added to the food processor and the volume of sulphuric acid, potassium permanganate solution and nectarine filtrate added to each trial. The stopwatch would have caused the greatest amount of uncertainty in the method as it relied on the reaction time of the person conducting the experiment. While the observer was constant throughout all of the trials, a number of different factors could have affected how quickly the stopwatch was started/stopped and subsequently, the time that was recorded. In improving the method, the 'end-point' could be objectively tested for using colorimetric methods. A standard solution could be passed through the colorimeter and the time taken for the solution to reach a certain percentage of light absorption recorded. Each trial would be tested for in a similar way.

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Potassium Permanganate, which was used as the indicator solution for this experiment, is a strong oxidising agent. With the ability to convert alkenes to glycols and thereby detect the presence of unsaturated bonds in a solution, the potassium permanganate could have reacted with impurities in the nectarine filtrate. In such a case, this would have affected the results considerably as the time taken for the pink colour of the potassium permanganate solution to disappear might not have been just testing for glucose. Thus the person conducting the experiment was in reality testing for another variable, the metabolism of impurities in the filtrate, which had not been accounted for in the method. In order to reduce this error, another indicator solution, which does not react with impurities to the same extent as potassium permanganate, could be used, for example iodine solution. Deep blue in colour, iodine solution detects the presence of starch in biological samples. Recognising that starch hydrolyses into glucose molecules, iodine could be used to show the concentration of starch in the nectarine filtrate, diminishes with the ripeness of the fruit. Alternatively a specific glucose test such as that used by diabetics could be used.

In the method, it was decided that each individual fruit should be tested three times, i.e. the time it took for the pink colour of potassium permanganate solution to disappear when placed with the filtrate was tested three times using a constant solution. Due to the subjective nature of the 'end-point' test, where we look for a change in colour to indicate the metabolism of carbohydrates to glucose, testing each solution three times limited any error that might be associated to the stirring of the solution and minimised the possibility of outliers in my results.

Each repeat was independent of one another, i.e. the nectarines from Day 3 and Day 5 trials had no relation to one another. A variety of different factors, which were not accounted for in this experiment and which could have been present in the repeats, for example the presence of pesticides and artificial ripening agents, or a former exposure to ethene gas, could have influenced the results. In effect, this meant that the method relied on commonalities between all of the nectarines in determining a relationship between the production of ethene gas and glucose concentration. The standard deviations remain reasonable except for the rice packaging treatment on Day 7. In general the standard deviation increased with the duration of the ripening. This might be expected as the fruits will vary at slightly different rates.

The abscission zone, or the region the closest to the stem of the fruit, has been shown to contain higher concentrations of glucose[5]. In order to minimise this factor, when pulverising the nectarines into a filtrate, the person conducting the experiment made use of all of the flesh of all the nectarines. This meant that the variation of glucose concentration within the fruit would remain constant throughout the experiment.

The biodegradation process, whereby microbes chemically digest materials, was one of the largest sources of error in this experiment. Mould, which develops as a result of an excess of moisture in an environment, was observed on all nectarines in the banana and rice trials after Day 5. The extent to which the propagation of mould had on the results can be seen in the calculated standard deviation values for the rice packaging trial. Day 7, in particular, had a massive standard deviation (16.18s), indicating that there was an enormous spread of data. Furthermore, because chance was a major factor in these results, they are not reliable and could probably not be reproduced again. The reproduction of microorganisms is affected by temperature. Therefore, the maintenance of a constant and relatively low (around 15°C) temperature would restrict the development of microorganism reproduction without significantly affecting the temperature required by the ripening process (remembering that the enzymes

[5]Studies on locating the signal for fruit abscission in the apple tree. J. Beruter and Ph. Droz, Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, CH-8820 WadenswilSwitzerland, Accepted 8 October 1990- Available online 14 October 2003

involved in the conversion of polysaccharides to monosaccharides work within a specific and narrow temperature range). The contamination of the fruit by microbes might be reduced by making sure the fruit is thoroughly cleaned on its surface before use. A sterilisation solution might be used.

Certain measures were taken to achieve environmental controls, for example temperature and exposure to light. The experiment was conducted in room temperature conditions, with the temperature of the laboratory being recorded twice each day. It was observed that the temperature fluctuated between 28°C and 29.5°C during the day. No recordings were taken between 3pm and 8am, There would have been great variation at night; however, this could not be controlled by the observer due to practical reasons, ideally, the experiment would be left in a consistently controlled environment, for example an incubator, where a constant temperature could be maintained.

The standard reference curve for glucose concentration that was produced proved to be irrelevant for the data. The data obtained was outside of the range of the standard curve. It was not possible to extrapolate the standard curve to cover the range of outcomes and therefore to infer the glucose concentration arising from the experimental trials. A calibration curve using higher concentrations of glucose would have to be reproduced.

Due to time constraints each trial was only repeated four times. In order to be able to draw concrete conclusions, 20 repeats would be required. This was taken into account when processing the results and it was acknowledged that any conclusions drawn from this experiment may or may not be wholly accurate.

Evaluation and Conclusion
It was hypothesised that the nectarines exposed to the rice-packaging trial would contain the highest concentration of glucose. It was thought that the rice would be conducive to the retention of ethane gas produced by the nectarines themselves around the fruit, hastening the ripening process and increasing the rate at which starch metabolised to glucose. In addition, the rice and nectarine were stored in a container from which air had not been removed. By contrast, the air had been removed from the plastic bags containing the fruit from the other two trials. It is possible that the higher concentration of oxygen in the box would have helped promote the metabolic process and the propagation of mould.

Bananas are used in both traditional and industrial situations to induce the ripening of fruit, due to their ethene-producing characteristics. This assertion, however, cannot be seen in the results. Whilst the bananas might have produced a small amount of ethene, on Day 7 of the experiment the control trial had a higher concentration of glucose though the results are not very different from the banana treatment though this difference is significant according to the t-test carried out on these data. The fact that the nectarines placed into plastic bags individually ripened at a faster rate than the nectarines that were placed with the bananas points to two possible conclusions. Firstly, methodological error meant that the conditions in which the bananas were placed were not conducive to the production of ethene. Or, secondly, that the nectarines used in the control trial were affected by factors that were not accounted for in this experiment, for example they contained higher concentrations of glucose at the beginning of the experiment.

It can be seen in Figure 1 that in all three of the trials the nectarines increased their glucose concentration at a similar rate from Day 1 to Day 3. We can thus assume that in this time period, the nectarines metabolised starch at a similar rate and produced similar amounts of ethene gas. It can be seen in the Qualitative Data Table that on Day 3 there were no definitive signs of mould, except on Nectarines 1 and 4 of the banana trial.

On Day 5 of the experiment, the banana and controlled trials continued to increase their glucose concentrations at a similar rate, albeit slower than the rate increase from Day 1 to Day 3. The rice packaging trial, however, had continued to increase its glucose concentration at the same rate, demonstrating a linear relationship between the concentration of glucose (y-axis) and time (x-axis). All of the nectarines subjected to these conditions were mouldy and were secreting a white residue. This was not the case with the nectarines in the banana and controlled trials, which showed little to no mould. One can deduce that it was the presence of mould that caused the sharp increase in glucose concentration. The enzymes from the mould are probably hydrolysing the starch of the nectarines.

As the nectarines in the banana and controlled trials continued to increase their glucose concentrations from Day 5 to Day 7, the nectarines in the rice packaging trial began to decrease in glucose concentration. Probably consumed by the microbes. At the same time, it was observed that all of the nectarines in this trial had become increasingly mouldy -all were at least 90% covered in mould - and that all nectarines were secreting a white residue. One possible conclusion that can be drawn from this observation is that there exists a 'threshold' whereby the increasing glucose concentration is counteracted by the increasing development of mould colonies. As large starch molecules are metabolised there will be a rise in the concentration of glucose. This process develops parallel to the growth of mould and bacterial colonies, which will feed off the increasing concentration of simple sugars and 'spoil' the fruit. From the results obtained in this experiment, it can be seen that the glucose concentration corresponding to the 29.53 seconds it took for the pink colour of the potassium permanganate solution to disappear is the highest attainable concentration of glucose. After this, the amount of glucose consumed by the microbial colonies outnumbers the amount of glucose being produced by the hydrolysis of starch, and thus a decrease in glucose concentration can be observed. As seen in all three of the trials, the development of mould before this 'threshold' does not have a significant affect on the increasing glucose concentration.

The only differentiating factor that could be observed in this experiment was the removal of air (oxygen) from the plastic bags. On Day 5, the controlled and banana trials possessed relatively similar glucose concentrations and in both of these trials, the air had been removed. Therefore it is unlikely that ethene gas produced by the banana was a significant factor in the conversion of starch to glucose. In the rice trial, where air was not removed from the box, the glucose concentration was significantly higher. The hypothesis that the presence of rice caused the ethene to be concentrated around the fruit does not hold up as ethene gas would equally have been retained around the fruit in the control trial. It is more likely that it was the presence of air, and oxygen in particular, that promoted both the growth of mould and the higher glucose concentration.

All of trials produced more or less the same outcome (the final values all lay within a 4 second period except Day 7 of the rice treatment). Qualitatively, all of the nectarines were observed as being rotten and covered in mould. The large standard deviations that were calculated from these results emphasised the wide spread of data around these three points and demonstrated the unreliability of the data on Day 7 of the rice treatment. The R^2 values remain high for the control and banana treatment remain high but the rice treatment R^2 is lower, reflecting the problems with these fruits.

As the nectarines were observed as being covered in mould and at this stage, it was likely that other significant chemical reactions were taking place within the fruits. The rice packaging trial had a standard deviation of 17.8 seconds, producing an error bar that encompassed all of the experimental results of the other trials (see Figure 1). The results of the experiment are in part due to processes that were not initially anticipated.

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