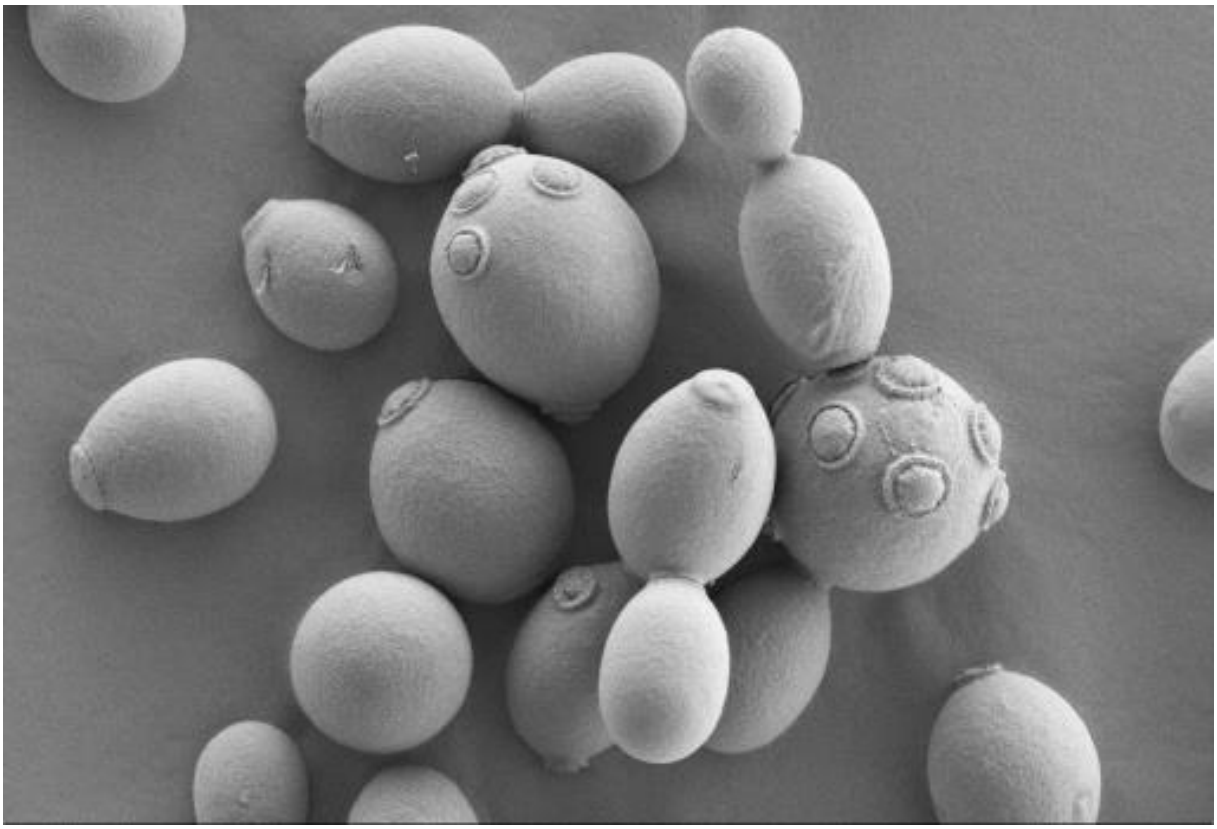
SRP 2016



THE SWEET JOB OF YEAST

An investigation of the metabolism of different sugars using yeast microorganisms

ABSTRACT

Sugars constitute a significant component of our diet and many people are concerned with the characteristics of each variety available for consumption. Clinicians are debating the public health impacts of natural and artificial sugars, and the rising vilification of sugar consumption in general means their health effects require further investigation. This experiment aimed to practically investigate and display the metabolic characteristics of a range of natural, artificial, refined and unrefined sugars in order to determine their benefits and detriments to health. Yeast was used as a cell model of a simplified metabolic process due to its similarity to human cells and unique metabolism of glucose in the form of fermentation. When the yeast respired anaerobically, the sugar was converted into alcohol and carbon dioxide, and the quantitative readings of the amount of carbon dioxide produced and the height of each fermenting mixture were used to compare the fermentation of each sugar in a given time. A second method of metabolic investigation involved determining the growth of spots of yeast-water solution on plates of gelatine made up with different sugars, with increased metabolism resulting in more energy and more cell growth. Results demonstrated that glucose underwent the fastest fermentation and promoted the greatest yeast growth and that the caloric sugars sucrose, raw sugar and brown sugar had similar metabolic properties. It was demonstrated that non-caloric Splenda and Equal sweeteners, though in theory unable to be metabolised, promoted a similar amount of growth and fermentation to sucrose, from which they were structurally modified. The natural sugar Stevia was shown to be metabolised very poorly by yeast, supporting its non-caloric description. In conclusion, glucose, as the basis of metabolism, was the most effective in promoting growth and fermentation, and can be used as a baseline to compare the metabolic properties of other sugars.

INTRODUCTION

This experiment will aim to investigate the metabolic activity of organisms in relation to sugar. This will be done using the unique fermenting properties of yeast microorganisms in terms of their growth when in contact with different sugars, and the rate at which each sugar is fermented by the yeast.

Overview of Sugar

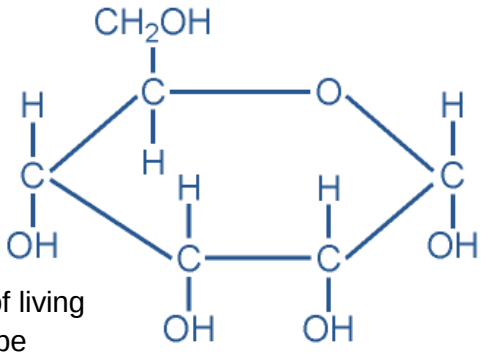


Figure 1: a glucose (monosaccharide) compound *www2.glos.ac.uk*

Sugars are small carbohydrate compounds, consisting of only carbon, hydrogen and oxygen atoms, and along with their fellow carbohydrates make up the most abundant group of organic compounds in nature. These sugar compounds are essential for cellular respiration and various chemical reactions in cells (metabolism) and provide the main source of energy for the cells of living organisms. Carbohydrates, scientifically termed saccharides, can be classed by complexity *depending on their size, chemical structure, and how quickly they are digested and absorbed* (Scientists of National Institute of Health 1997). Monosaccharides are the smallest and most simple compounds, with disaccharides- two monosaccharides joined- and polysaccharides being more complex.

There are also compounds classed as artificial sugars that are *derived from sugars but are structurally modified to elicit a sweet taste whilst not presenting a significant calorie load* (Franklin Marion University Professors 2012). There is much debate among the nutritionist and scientific communities about the effects of different sugars and carbohydrates on human health and their behaviour in the body, and a constant preoccupation of finding the healthiest alternative to satisfy the sweet tooth. This research project will attempt to investigate this by practically demonstrating the effects of complexity and artificiality on the metabolism of different sugars by yeast microbes, in order to discuss firstly how each behaves and interacts with living cells, and also assess their benefits/detriments to human health.

Sugar in the Body

All organisms require an energy source, and sugar is the main source for the cells of the human body and is essential for survival. Sugar is metabolised (used in chemical reactions within cells or organs in the body) by organisms to produce its energy, allow it to grow and to in general maintain cellular function. In digestion, the monosaccharide compound glucose $C_6H_{12}O_6$, the simplest and most common sugar, is isolated after ingested carbohydrates are broken down, enters the bloodstream and is then delivered to every cell in the body to fuel cellular respiration. Hence, it is *the standard by which other carbohydrates are ranked* (Catherine Saxelby 2016) to show how fast they will be metabolised by cells.

Glucose powder is a white crystalline powder with a GI at the maximum of 100, meaning that glucose is rapidly absorbed into the bloodstream (nutritionist Catherine Saxelby 2016).

Figure 2: pure glucose powder: *seniorchem.com*

With our modern Western diet, there is often excess glucose in the body after eating which is not needed as energy at that time, so it is converted into glycogen by the liver and stored as fat somewhere in the body, to which there is no limit.

Larger carbohydrates and sugars that are more complex than glucose have a slower metabolic rate, and take longer to be broken down and converted into energy. This is the basis of the belief that more complex and unrefined carbohydrates (like whole wheat rather than refined white bread) are healthier, as this way the body has more time to use the energy it has been given by food because the glucose contained in it is being released at a slower rate. This also ensures that levels of the hormone insulin, related to blood sugar levels, do not spike immediately after eating and energy lasts longer.

Figure 3: a health food website's description of the healthiness of complex carbohydrates *www.justraisethebar.com*

The human body can also metabolise many other molecules, including starches, lipids, proteins and other sugars like fructose, though these react differently to glucose. However, we are not able to metabolise certain artificial sugar compounds, like those in sugar-free sweet foods- they are deliberately manufactured that way so they cannot provide the body with any energy. Another alternate strategy is having the sugar *modified to have an extremely sweet taste so that only a very small amount is needed* (Franklin Marion University 2012) so its caloric impact is minimal with little or no excess to convert into fat.

Domestic Consumption of Sugar

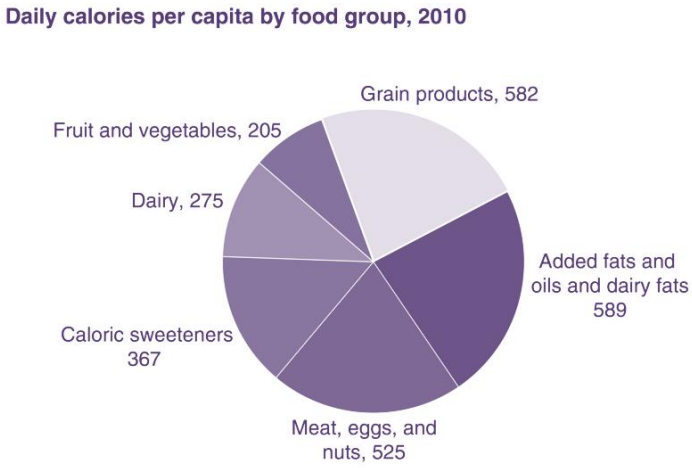


Figure 4: daily caloric intake of average American by food group, caloric sweeteners making up nearly 20%: *http://www.ers.usda.gov/media/1188540/food-availability_fig04.png*

The consumption of sugars is one of great proportions, comprising 10% of total daily calories for the average adult. The World Health Organisation now recommends that sugars make up only 5% of the diet or 6 teaspoons per day in response to the growing epidemics of diabetes and heart disease. This is alarming to *Australians, who on average eat 27 teaspoons of total sugars a day (including natural sugars), according to the 2012 report Sugar Consumption in Australia: A Statistical Update* (writers of ABC 2015). Consumed sugars come in natural, artificial, caloric and non-caloric forms, each presenting its own pros and cons.

Natural consumed sugars include the monosaccharides glucose and fructose (which can both come in powdered or syrup form), the disaccharides sucrose (table sugar- either white, raw or brown) and lactose (milk sugar) and various natural unrefined sweeteners (such as stevia, agave and honey). Many of these unrefined sweeteners like raw sugar and honey, though they contain some beneficial vitamins and minerals which add a small nutritional value, are either mono or disaccharides that are metabolised identically to their refined counterparts. The complex natural carbohydrate starch is a polysaccharide, a long chain of sugar molecules, and is included in this experiment as a compound of greater complexity. Artificial sugars available for purchase include sucralose (Splenda) and aspartame (Equal), which merely pass through the body without being absorbed and are excreted out. They are chemically similar to sucrose, Equal more so, but are structurally different with some added atoms and some absent. Their non-caloric nature means they are beneficial for diets and weight control, but it can be argued that the modified chemicals are harmful for the body in other ways, and can cause side effects and long term damage in large quantities. The fact that the influence of sugar in our diet is so large and is in need of such a great modification means that it is vital to consider each of the many alternatives' behaviour in and effects on the body. This is the backbone of this experiment and its connection to human and real life issues.

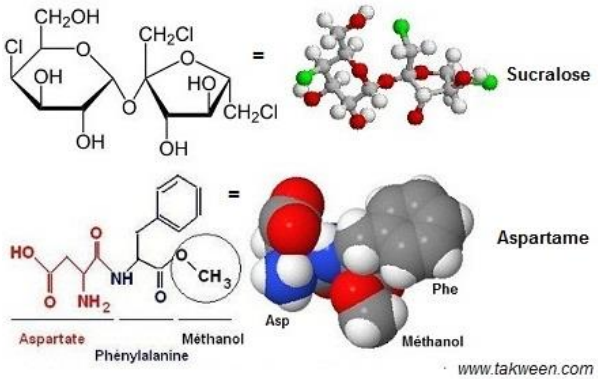
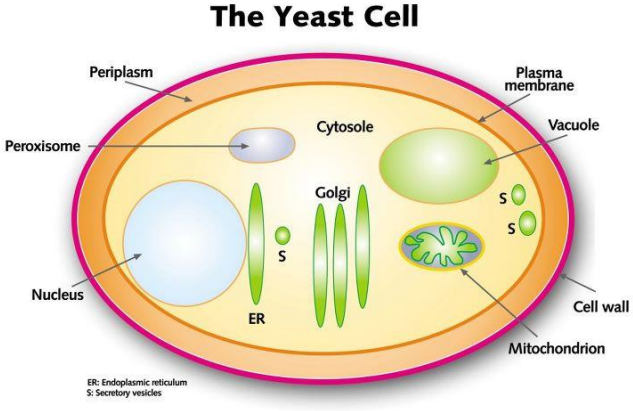
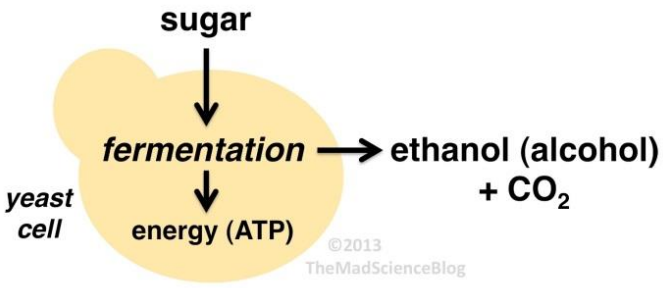


Figure 5: artificial sugar compounds: *www.takween.com*

Yeast and its Relation to Sugar

Yeast is a microbial fungus which derives its energy source from sugar. They are among the smallest eukaryotes, which means they are much larger than common bacteria, have a cell nucleus containing DNA, have organelles for cellular function and have a structure and behaviour similar to eukaryotic human cells. In the presence of yeast, sugar is metabolised anaerobically (without oxygen), converting the sugar to alcohol and carbon dioxide, a process utilised in brewing beer and baking bread. As the simplest eukaryotic organisms to perform this metabolic process of cellular respiration, they are useful models in cell biology. The activity of yeast in the presence of sugar and its similarities to human cells make it ideal for this experiment, as it can not only show which of the tested sugars are the simplest by investigating their effects on fermentation rates but can also simulate a simplified action of these sugars in the human body itself.

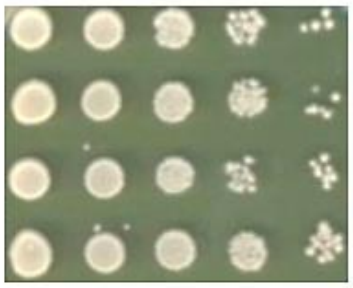




Figures 6 and 7: yeast and its anaerobic metabolism (fermentation) of sugar: *www.themadscienceblog.com*

The more sugar there is, the more active the yeast will be and the faster its growth, up to a certain point - even yeast cannot grow in very strong sugar - such as honey (Science and Plants for Schools (SAPS) UK 2016). An excess of sugar inhibits the growth of the yeast colony, so only small amounts will be used in the tests. There are a number of commercially available yeast products, but the most suitable of these will most likely be a very small amount of a pure yeast culture. Baker's yeast is made using yeast culture and molasses, which is activated in water allowing the yeast to grow. As there is already added sugar in this type of yeast, this would interfere with the experimental method involving growth in different types of sugar. Hence active dry yeast grown on gelatine plates and fermented in test tubes will be used to ensure validity of the tests. Gelatine instead of agar will be used because of its similarities to agar and the ability to create a culture medium specific for yeast growth. The components of nutrient agar are varied and the presence of sugar within it could interfere with the experiment so a gelatine mixture combined with different types of sugar will be used to make the plates for yeast growth. Yeast grows in separate colonies, although when a high concentration of the culture is used, the colonies become confluent and hence the area of yeast on the culture plate can be measured. Like bacteria and most microorganisms, yeast growth begins exponentially (log phase), becomes stationary then steadily decreases in the death phase.

Figure 7: spot plate of yeast cultures in different concentrations: *http://capricorn.bc.edu/bi204/wp-content/uploads/2013/08/4-yeast-culture_2013.pdf*



Spot plate.
Each row on the plate represents a series of 1:10 dilutions of a liquid culture of *S. cerevisiae*. Five µL of each dilution was spotted on the plate. The plate was incubated for two days at 30°C. Individual colonies are apparent at the highest dilution of each extract.

The activity of yeast relies on glucose. *Each sugar (fed to the yeast) needs to be converted to glucose to enable it to feed into respiration (see fig.8) and it is this process which produces the gas* (SAPS UK 2016). Yeast produces enzymes which break down sugars to release glucose, much like in the human body, and then uses it as energy to anaerobically respire (ferment). As a result, the simplest carbohydrates- those closest in structure to monosaccharide glucose- are metabolised fastest, and those requiring the most enzymatic breakdown will take the longest, resulting in a slower growth and fermentation. This will be the basis of the investigation into the different sugars.

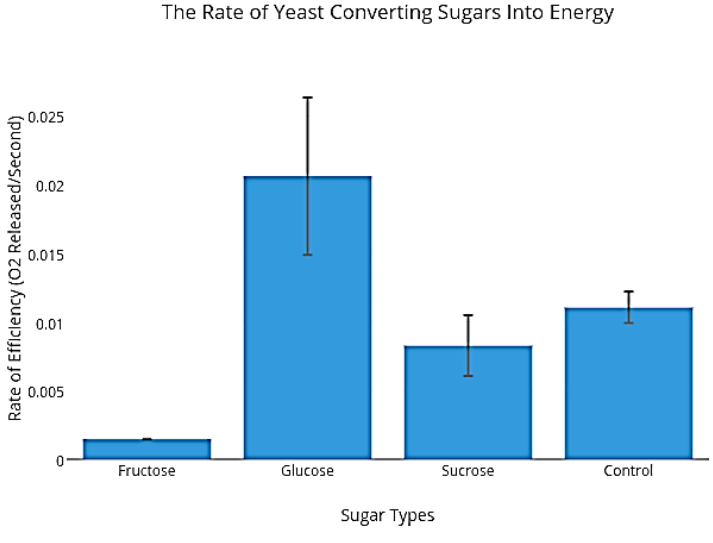


Figure 8: yeast's conversion of different sugars into energy (by metabolism), glucose is fastest: *https://plot.ly/~sb17909/43/the-rate-of-yeast-converting-sugars-into-energy/*

AIM: to determine which type of sweetener is metabolised fastest by yeast and promotes the most microbial growth and fermentation, in order to evaluate different sugars' metabolic properties and benefits and detriments to human health

HYPOTHESIS: pure glucose powder will cause the most yeast growth and fastest fermentation

EQUIPMENT

5x packet active dry yeast
1x electronic balance
1L distilled water
12g gelatine powder
1x small cardboard box
1x micropipette
10g pure glucose powder
10g white table sugar
10g raw sugar
10g brown sugar
10g cornstarch
100mL measuring cylinder
10g Splenda sweetener
10g Equal sweetener
10g Stevia sweetener
9x stoppered test tubes
2x test tube rack
10mL measuring cylinder
1x stopwatch
1x carbon dioxide data logger
1x millimetre ruler
1x spatula
1x fridge

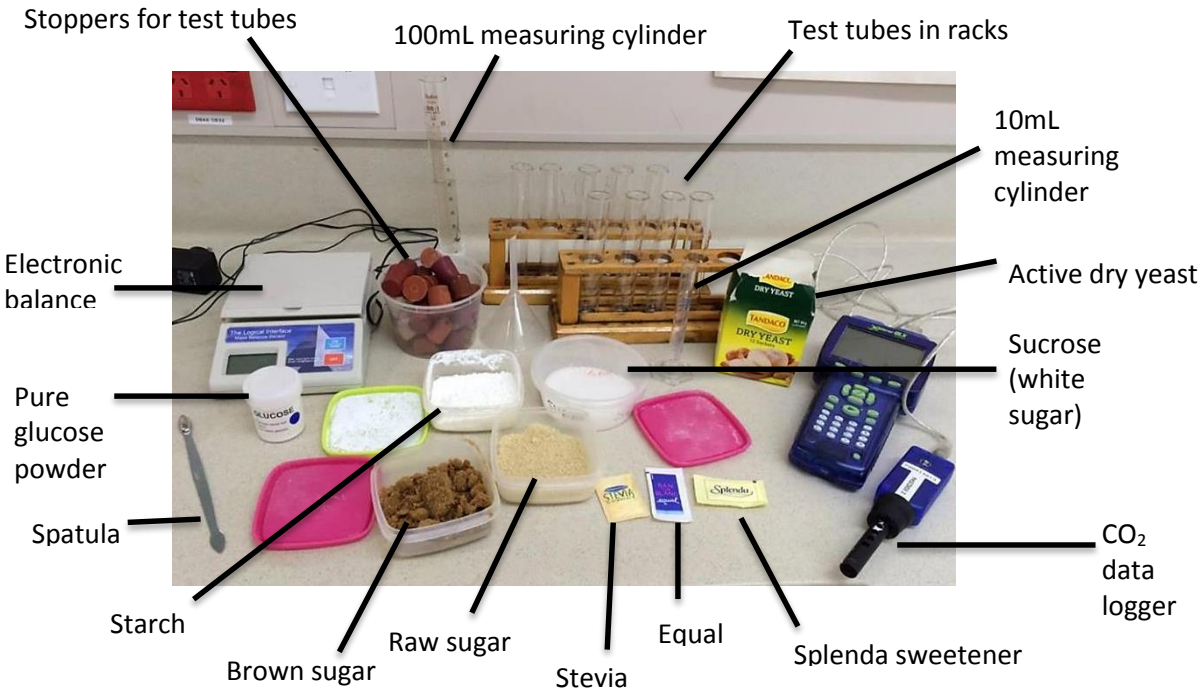


Figure 9: experimental setup and equipment for test tube fermentation trial

METHOD

Gelatine plates and size of growth trials

Figures 10 & 11: making the gelatine plates

1. A mixture of 750mL distilled water, measured with the 100mL cylinder, and 12g gelatine powder, using the electronic balance, was prepared in a saucepan on a stove and was heated until the powder completely dissolved. *(fig 10)*
2. A benchtop was sterilised by wiping down with ethanol.
3. An empty petri dish was placed onto this benchtop and filled only with 20mL gelatine-water mixture as a negative control.
4. 2g of pure glucose powder was placed in an empty petri dish and 20mL of gelatine mixture was poured in. The contents of the dish were stirred until powder dissolved. *(fig 11)*
5. Step 4 was repeated for each petri dish, each with 2g of a different sugar (raw, brown, cornstarch, Splenda, Equal, Stevia).
6. The 9 gelatine plates were labelled according to their sugar contents and placed in the fridge for 12 hours.
7. A mixture of 5g active dry yeast *(fig 12)* and 10mL water, measured with the 10mL measuring cylinder was prepared to form a 50% concentration paste, and was left to stand for 10 minutes for the yeast to activate.
8. The set plates were taken out of the fridge. Using the micro-pipette, 10µL of yeast mixture was dropped onto each gelatine plate, and the diameter of each yeast spot was measured with the millimetre ruler.
9. The 9 prepared plates were sealed with tape and were placed into the small cardboard box. *(fig 13)* The box was placed in the corner of a room with a low ambient temperature variation, kept constant at 21°C with air conditioning.
10. After 1 hour the plates were taken out of the box, and without opening them the diameter of the yeast spot on each plate was measured again. The plates were then returned to the box which was replaced in the room.
11. Step 10 was repeated after 2 hours, 12 hours and 24 hours.
12. The difference in initial size and size after 24 hours was calculated for each spot.
13. The experiment was repeated twice for reliability and reproducibility.

Figure 12: weighing 5g of dry yeast to mix into a paste

Figure 13: prepared gelatine plates with yeast spots in the containing cardboard box

Spot of active dry yeast and water mixture

Petri dish

Small cardboard box

Mixture of gelatine powder and water

Test tube and carbon dioxide probe fermentation trial

Figures 14 & 15: weighing the yeast and sugar

1. 9 test tubes were each filled with 5mL of distilled water, measured by the 10mL measuring cylinder, and were placed in test tube racks.
2. 0.5g of active dry yeast measured with the balance on a watchglass *(fig 14)* was emptied into each test tube.
3. 0.5g of pure glucose powder *(fig 15)* was added to the first test tube and then it was stoppered, labelled and replaced into the test tube rack.
4. The remaining sugars used in the agar growing experiment were each allocated a test tube. 0.5g of each sugar was added to its corresponding tube and each was placed in the rack. No sugar was added to the final test tube as a negative control.
5. The test tube racks were left to stand at a constant room temperature of 21°C and the yeast left to ferment.

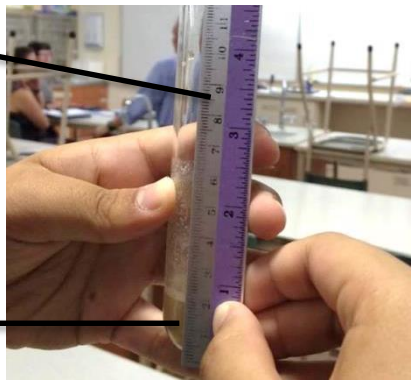


Figure 16: measuring the height of the yeast mixture in the test tube

6. The height of the liquid mixture in each test tube was measured using the millimetre ruler every 3 minutes for 15 minutes *(fig 16).*
7. After 20 minutes the amount of carbon dioxide produced by the fermentation of the pure glucose was measured using the CO_2 probe by immediately inserting it into and plugging the opened test tube. *(fig 17)*. The same was done for each of the other sugars.
8. The carbon dioxide and mixture height measurements were recorded, tabulated and analysed.
9. The experiment was repeated twice to test for reliability and reproducibility.

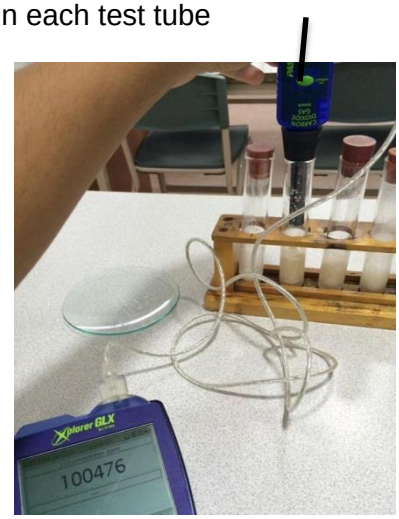


Figure 17: measuring the carbon dioxide concentration in each of the test tubes after sugar fermented

Figure 18: a fermentation trial after 15 minutes, showing the differing heights of the mixtures

VARIABLES

Independent- the type of sweetener given to the yeast to grow or ferment

Dependent- the size of each yeast colony after growth on agar, the amount of carbon dioxide produced by each yeast mixture after growing and fermenting AND the height of the yeast/water mixture at various intervals during fermentation

Controlled- initial amount/concentration of yeast in each trial, conditions of preparation environment, conditions of yeast growing environment, amount of sugar or sweetener applied to each yeast trial, amount of time between measurements of yeast cluster size, amount of gelatine and sugar in each plate, amount of water in each test tube, dimensions of each test tube, amount of time each trial is open or exposed to air, sterility of gelatine plates

RISK ASSESSMENT

How do you assess the risk? For each hazard identified in Step 1, answer A then answer B. Then add A and B together to determine Risk and Action required

A **What is the potential impact or consequence of the hazard?**	**B** **What is the likelihood of the event happening?**	**Add the numbers in columns A and B together**	**How to assess the risk**	**Action**
1 = MINOR First Aid required with little or no lost time	1 = LOW It could happen but only rarely		1 – 2 = LOW RISK	Proceed with caution
2 = MODERATE Medical treatment required, some lost time	2 = MODERATE It could occasionally happen		3 – 4 = MODERATE	Consult with teacher
3 = SERIOUS Medical treatment required, extended lost time	3 = HIGH It could frequently happen		5 – 6 = HIGH	Reassess the need to perform practical/ consult with teacher

(see next page)

Science Student Research Project Risk Assessment

Activity Description: Growing and fermenting yeast in gelatin and in test tubes

Activity Description: Growing and fermenting yeast in agar and in test tubes

Step 1: Identify the hazard	CSIS user code	Step 2: Strategies to minimise the hazard	Step 3: Assess-ment of risk (see table below)	Step 4: What if something goes wrong?	Step 5: Packing up
Glassware can break and cause cuts	n/a	Place glassware away from edges of benches and keep outside dry to minimise slipperiness	1+2=3= MODERATE	In case of breakage consult teacher. Empty glassware, brush up and place in 'broken glass' bin. Wipe up any spills. If cuts occur, seek first aid.	Clean, dry and pack away carefully
Some yeasts are pathogenic under certain conditions when ingested	n/a	Use gloves, do not eat, clean and disinfect workspace after	2+1=3= MODERATE	If a yeast infection is apparent after experiment is conducted, consult a doctor and/or visit a specialist	Wrap up and throw away yeast in rubbish bin and avoid direct contact
Bunsen burner can cause burns or start a fire	n/a	Don't touch flame, push burner to back of the bench	1+2=3= MODERATE	Run burned area under cold water, bandage and consult teacher if burns are more serious	Turn off gas tap, run extinguished matches under water and return burner to cupboard
Ethanol is flammable and toxic if ingested in large amounts	C_2H_6O	Make sure there is no ethanol near the Bunsen burner, don't ingest and try not to inhale too much	2+1=3= MODERATE	Extinguish flame by covering or using fire extinguisher if too large or dangerous	Pour away down sink
Any excess bacteria growing on agar could be harmful if ingested	n/a	Do not touch agar plate after growth	1+1= LOW	Wash hands thoroughly after touching, if symptoms occur alert a teacher	Dispose of agar in the bin carefully and with gloves

Mandatory precautions: Covered shoes, safety glasses, hair exceeding shoulder length tied back.

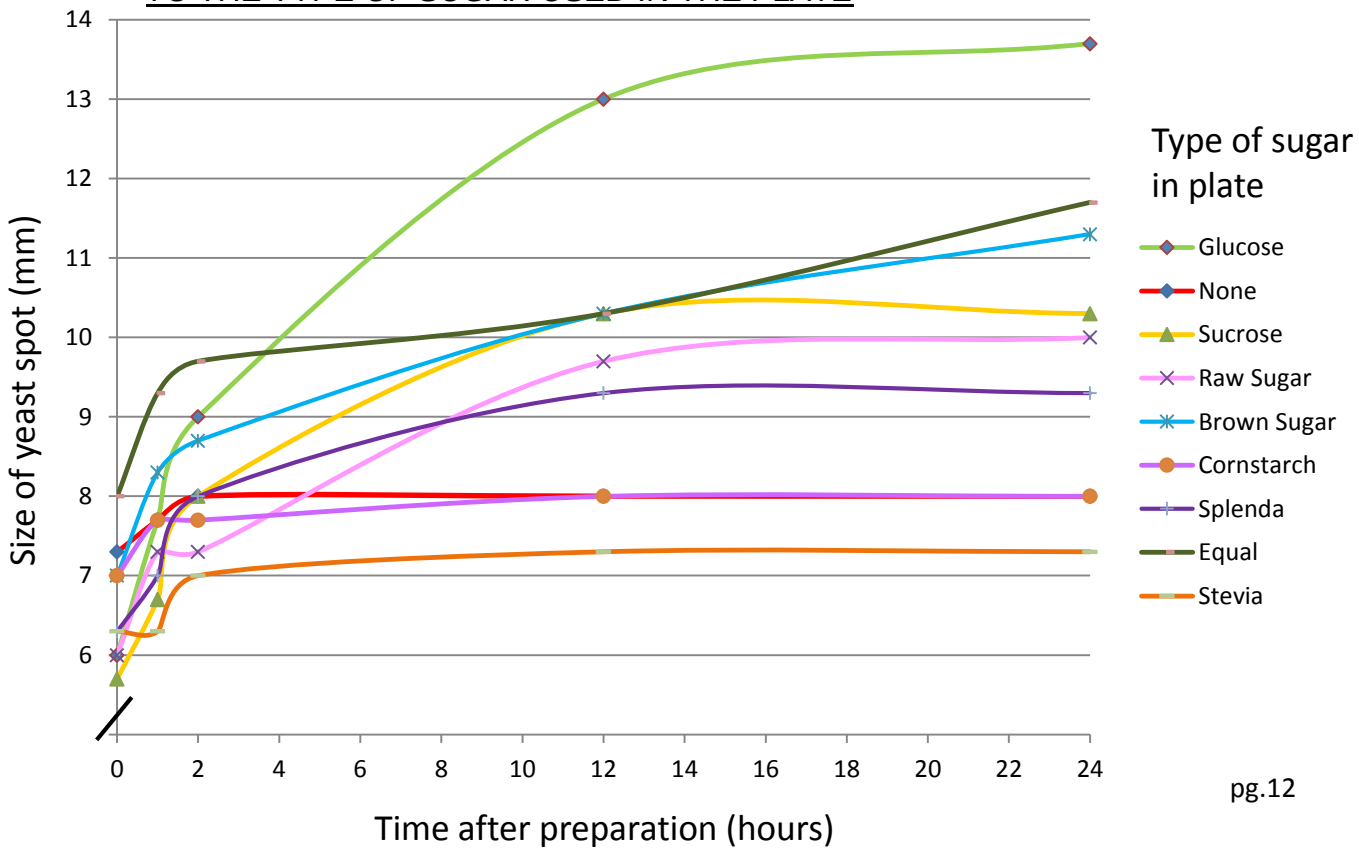
Date: 5/2/15 Student Signature: Sabena Bhadri

RESULTS (see appendix for raw data)

THE AVERAGE GROWTH OF YEAST ON GELATINE PLATES IN RELATION TO THE TYPE OF SUGAR USED IN THE PLATE

Type of sugar in plate	Time after preparation (hours)					Total growth (mm)
	0	*1*	*2*	*12*	*24*	
	Diameter of yeast spot (mm)					
No sugar	7.3	7.7	8.0	8.0	8.0	0.7
Glucose	6.0	7.7	9.0	13	13.7	7.7
Sucrose	5.7	6.7	8.0	10.3	10.3	4.6
Raw sugar	6.0	7.3	7.3	9.7	10.0	4.0
Brown sugar	7.0	8.3	8.7	10.3	11.3	4.3
Cornstarch	7.0	7.7	7.7	8.0	8.0	1.0
Splenda	6.3	7.0	8.0	9.3	9.3	3.0
Equal	8.0	9.3	9.7	10.3	11.7	3.7
Stevia	6.3	6.3	7	7.3	7.3	1.0

THE AVERAGE GROWTH OF YEAST ON GELATINE PLATES IN RELATION TO THE TYPE OF SUGAR USED IN THE PLATE



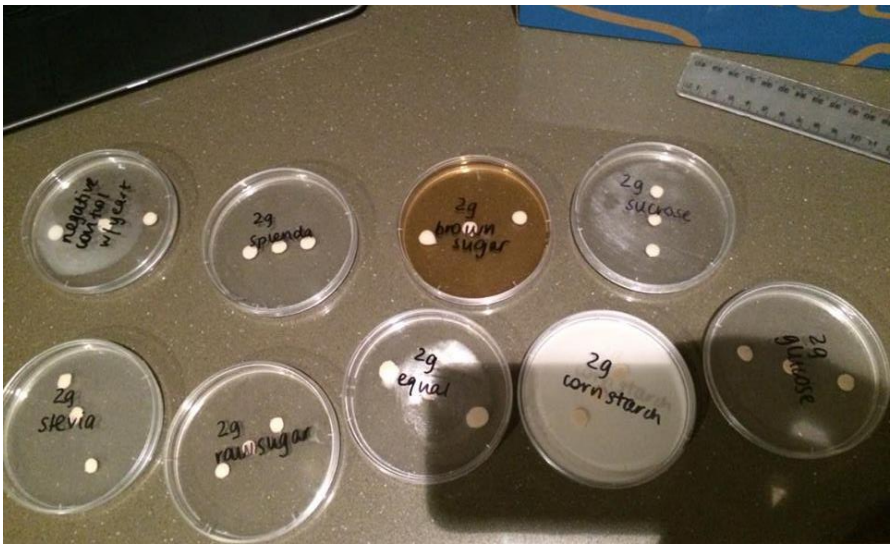


Figure 19: gelatine plates immediately after preparation- all spots have been measured and their diameters are within a 3mm range (5-8mm)

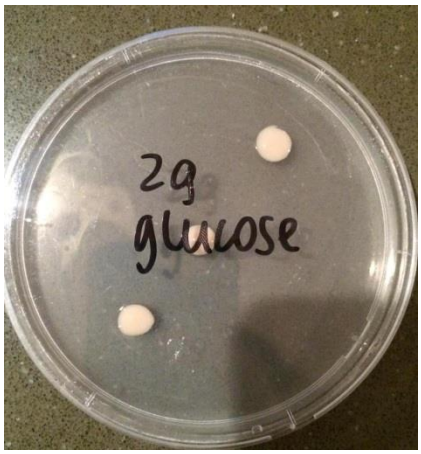


Figure 20: the glucose plate immediately after preparation

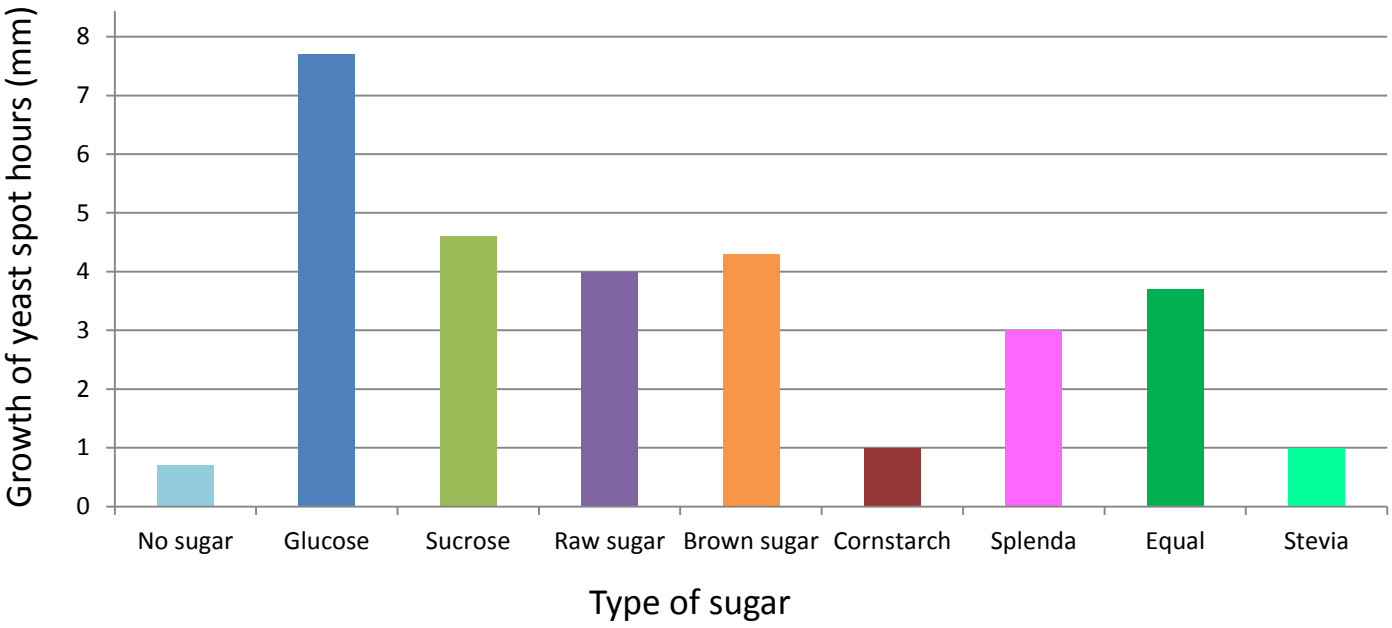
Figure 21: measuring the initial diameter of the yeast spot

Figure 22: gelatine plates 24 hours after preparation- some spots have grown, some dispersed, some sunk into the gelatine mixture leaving only a small measurable section on the surface (eg brown sugar), some have remained the same (eg cornstarch)

Figure 23: measuring the glucose fed yeast spot diameter of 13mm after 12 hours of growth

Figure 24: the Equal fed yeast spots, seen to have sunk slightly into the mixture (the sunken yeast having fermented inside the plate seen in bubbling) and grown and dispersed irregularly, as was the case for a few of the sugars

THE AVERAGE CHANGE IN SIZE OF YEAST SPOTS ON SUGAR GELATIN PLATES AFTER 24 HOURS IN RELATION TO THE TYPE OF SUGAR IN THE PLATE



THE AVERAGE CHANGE IN HEIGHT OF YEAST MIXTURE IN TEST TUBES IN RELATION TO THE TYPE OF SUGAR FERMENTING

Type of Sugar added	**Fermentation time (mins)**					
	0	*3*	*6*	*9*	*12*	*15*
	Height of yeast/water mixture (mm)					
No sugar	20.0	19.7	20.0	20.0	20.0	20.3
Glucose	20.0	23.7	27.0	31.0	35.7	40.0
Sucrose	20.0	22.7	24.7	27.0	30.0	34.3
Raw sugar	20.0	21.3	24.7	27.3	29.7	34.3
Brown sugar	20.0	22.3	24.7	28.7	31.7	35.3
Cornstarch	20.0	21.0	20.7	21.3	21.6	22.0
Splenda	20.0	21.0	23.0	25.0	26.7	29.0
Equal	20.0	21.0	22.7	24.3	26.3	29.3
Stevia	20.0	20.7	20.7	21.3	22.0	22.3

THE AVERAGE CHANGE IN HEIGHT OF YEAST MIXTURE IN TEST TUBES IN RELATION TO THE TYPE OF SUGAR FERMENTING

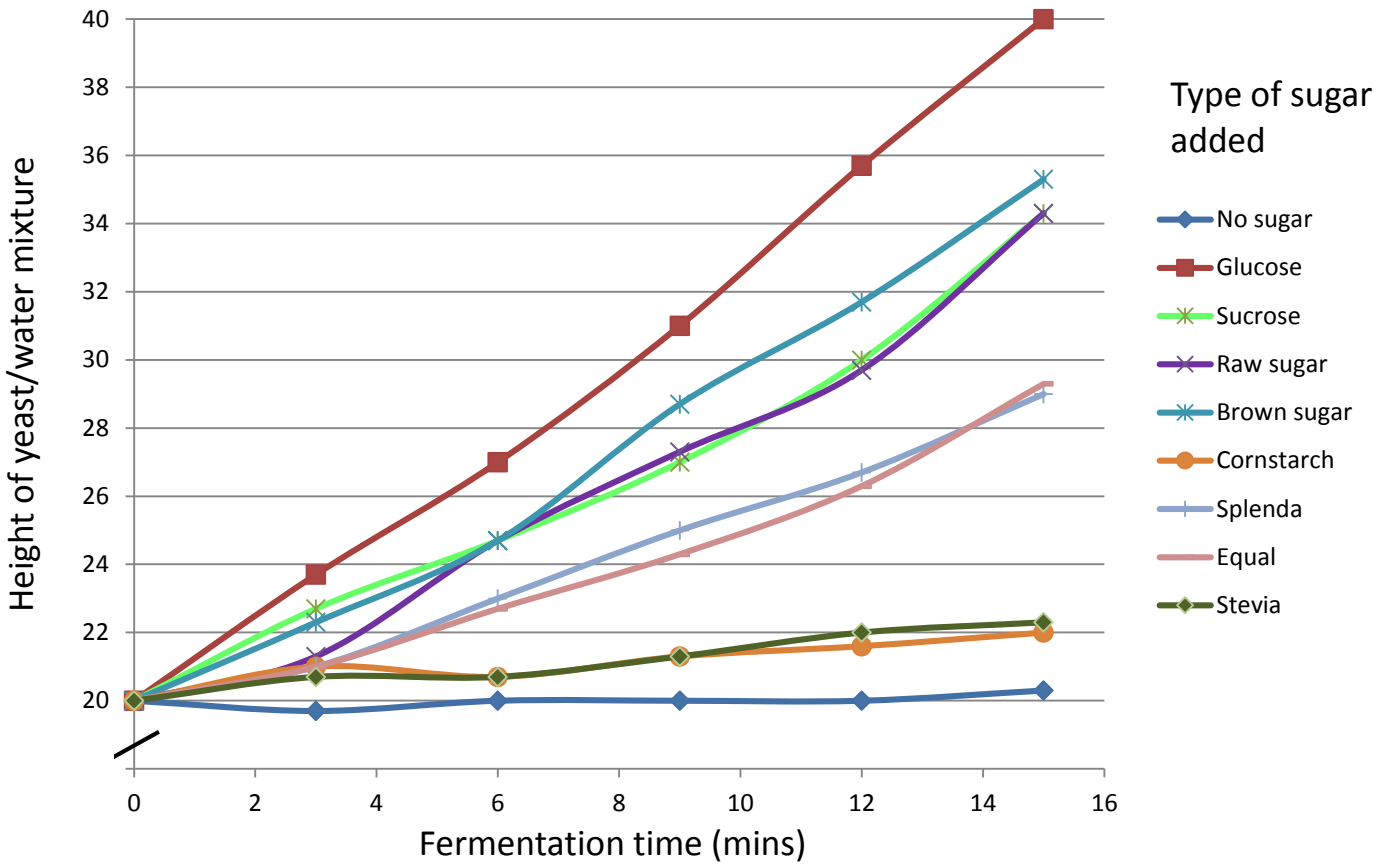


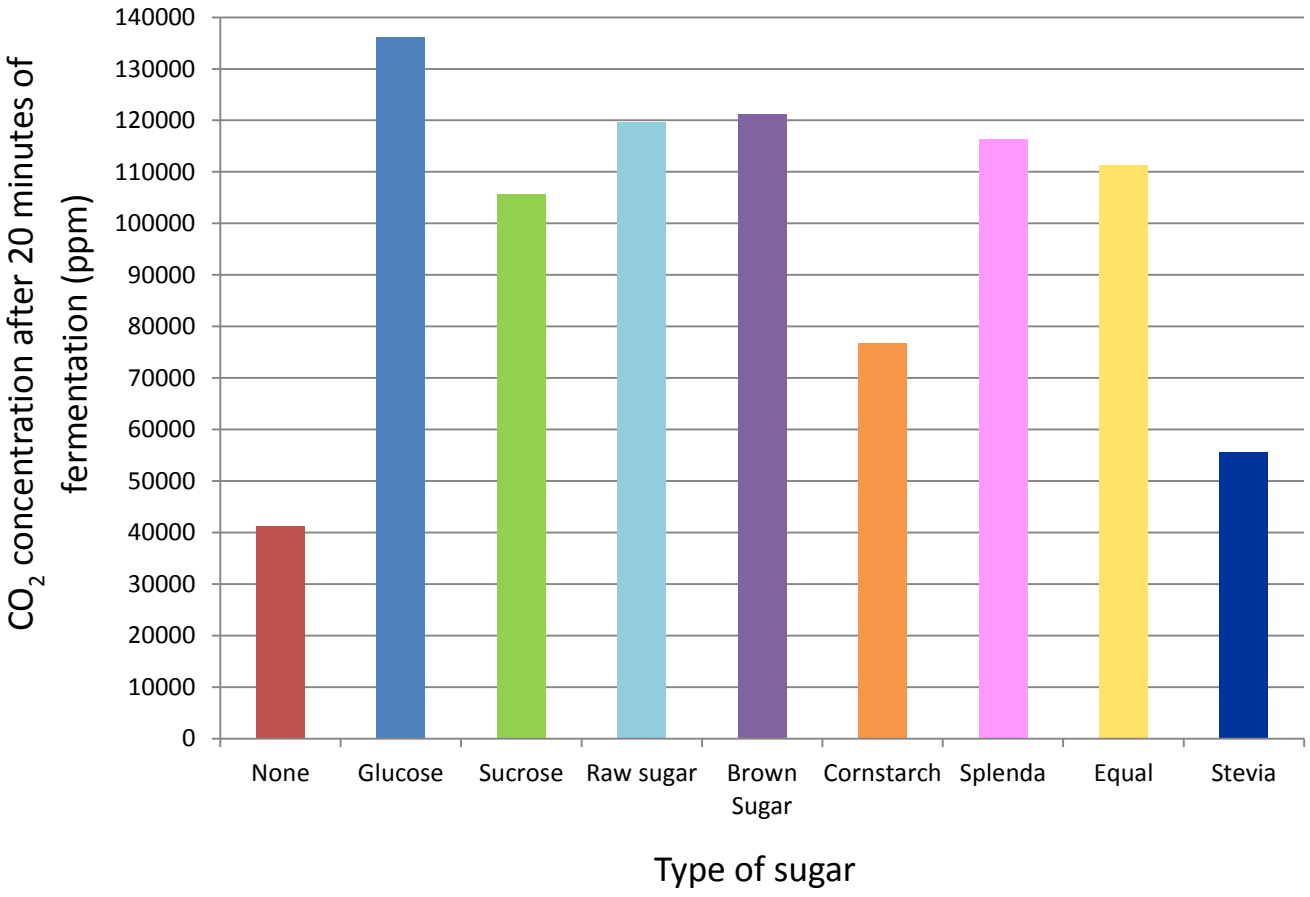
Figure 25: the test tubes before fermentation, each containing 5mL of water, 0.5g yeast and 0.5g of their respective sugar

Figure 26: the test tubes after yeast fermentation, all at different heights, glucose having increased in height the most

THE AVERAGE CONCENTRATION OF CARBON DIOXIDE INSIDE TEST TUBES OF YEAST MIXTURE IN RELATION TO THE TYPE OF SUGAR FERMENTING

Type of sugar fermenting	**CO_2 concentration in test tube after 20 minutes of fermentation (ppm)**
None	41 194
Glucose	136 083
Sucrose	105 729
Raw sugar	119 597
Brown sugar	121 298
Cornstarch	76 637
Splenda	116 323
Equal	111 250
Stevia	55 584

THE AVERAGE CONCENTRATION OF CARBON DIOXIDE INSIDE TEST TUBES OF YEAST MIXTURE IN RELATION TO THE TYPE OF SUGAR FERMENTING



(see appendix for full raw/unprocessed data)

DISCUSSION

This experiment was valid as the measurements taken were as accurate as possible, were testing the hypothesis and were measuring the intended variables. These are the amount of yeast growth, which can be measured mechanically by the size of the yeast cluster, and the amount of fermentation by the amount of carbon dioxide produced and height of the mixture at various times. The accuracy of the carbon dioxide measurements was very high as the apparatus being used is electronic so there is no degree of subjectivity. The height and size readings are less so, as only a millimetre ruler can be utilised as more precise forms of measuring distance, such as callipers, cannot be used correctly in these trials for mechanical reasons. The results were also valid in that they were gathered using appropriate methods of mechanical measuring, with the same ruler each time, and electronic measuring with the carbon dioxide probe, and were not interfered with by uncontrolled variables.

Reliability was fairly high, though the final test tube fermentation trial was moderately slower than the other two fermentations, but didn't dramatically change the averages overall. The results for the gelatine plate growth trials were also very consistent and reliable, with corresponding trial readings within a 1mm range. The fermentation trial readings, in the case of the glucose fermentation, were within a 14mm range, widened by the slower third trial. This suggests that this aspect of the method was less reliable potentially due to inconsistencies in the nature of the yeast, the temperature of the room for each trial or the amount of yeast/sugar/water in each trial. This also shows that to increase reliability the trial should be conducted again to see if results are consistent with the others and therefore eliminate the slow trial as an outlier. The carbon dioxide concentration measurements were fairly consistent across the three trials, again except for the third slower fermentation which in turn produced less carbon dioxide.

The measurements were accurate as they were made using the most precise equipment available, as millimetre rulers were the smallest scaled instruments that could have been utilised and the carbon dioxide logger the most advanced and exact technology available. Similar results were not found in any secondary sources, though according to information gathered the results agreed with theory, strongly supporting their accuracy. In terms of important secondary sources used to gather information, they were valid as they related to the investigation, reliable due to their repute, objectivity and currency and accurate as they presented information that was consistent across many sources. The sites used were mainly educational, governmental and organisational sites, or scholarly articles and textbooks which are known for good repute.

The averages presented in the results section of the report were in some ways expected, as for glucose, and less so but also interesting in other ways, in the case of the artificial sugars. Glucose, as the sugar that is the easiest for yeast to metabolise, was indeed the quickest to ferment and allowed for the most yeast growth. Its fermentation, on average, produced a mixture 19.7mm higher than the negative control, and grew its yeast spot 7mm larger than the negative control. The concentration of carbon dioxide in its test tube was also a dramatic 94889ppm greater than the negative control, and 30354ppm greater than the next highest concentration in the sucrose test tube. Sucrose and raw sugar as expected had very similar fermentation rates, both creating a 34.3mm high mixture and their CO_2 concentrations

differing by only 13000ppm.They also promoted a similar degree of yeast growth, their spots differing by only 0.6mm. Raw sugar produced a slightly greater CO_2 concentration and yeast spot, suggesting that unprocessed forms of sugars are fermented slightly better by yeast. This is supported by the growth and fermentation of the yeast in contact with the even less refined brown sugar, which fermented faster than raw sugar, produced more carbon dioxide and grew a 0.3mm larger yeast spot. However, in terms of growth, sucrose produced a slightly larger 4.6mm spot rather than the 4.3mm for raw sugar, showing that refined disaccharide sugars (like sucrose) may promote a marginally greater amount of growth but overall are processed by the yeast very similarly to their unrefined counterparts (raw and brown sugar), as they are structurally the same. Cornstarch was included in this experiment to demonstrate the slower metabolism of a more complex polysaccharide carbohydrate by yeast, demonstrated by its promotion of a mere 1mm of yeast spot growth, 30,000ppm difference in CO_2 concentration compared to the control and only a 2mm mixture height increase- as expected.

The artificial sugars Splenda and Equal in theory should not have promoted much growth or fermentation due to their structural modification. However, in the experiment, the two artificial sugars grew their yeast spots to a size only 1mm smaller than that of sucrose and with a similar degree of fermentation. They are structurally modified from sucrose, so this infers that their modification isn't to drastically change the metabolic properties of the sugar, although in theory Splenda and Equal are meant to be significantly poorer at being metabolised. Instead, the modification could be for altering the taste, making the artificial sugars a lot sweeter than sucrose, meaning fewer calories need to be ingested for the same degree of sweetness. Stevia, being a natural non-caloric sugar and its metabolic properties still unknown, had a very low fermentation and created very little yeast growth. Its results were similar to that of the negative control, supporting its claims of being unable to be metabolised by cells.

In regards to the health benefits and detriments each sugar presents, of which some can be practically examined in this experiment by their metabolism by yeast, it is seen that glucose is the simplest sugar, due to its very fast fermentation and growth promotion. In the body, this would translate to a very fast metabolism and a sharp peak in energy, which if unused will convert into fat, so would only be beneficial when in need of an instant energy boost and would otherwise contribute to weight gain and unhealthy blood sugar levels. The belief that unrefined sugars like raw and brown sugar are healthy due to the small amount of nutrients they contain are disproved practically by this experiment, in that refined sucrose, raw sugar and brown sugar are metabolised very similarly by yeast and would thus have very similar metabolic impacts on the body. Though they are slower to metabolise than pure glucose, they are still simple carbohydrates that produce an energy peak resulting in an insulin spike that converts glucose to fat if not burned in a given time. Cornstarch by comparison is much more complex, seen in its very minimal fermentation and yeast cell growth promotion, and would be converted into energy a lot slower. This means for the human body more calories are utilised and less are left to store as excess as fat. The benefits of these sugars and carbohydrates is that they are naturally occurring and do not present any chemical or toxic dangers to the body like artificial sugars are said to. The latter present no useful calories, which can be seen as a good thing for combatting obesity, but their structural modifications are argued by some health professionals to be toxic. In regards to sucralose (Splenda): "*Its preparation involves chlorinating sucrose. Chlorine a known carcinogen."*

(globalhealingcenter.com 2015.) There are also risks associated with aspartame (Equal): "*Aspartame has been investigated as a possible cause of brain tumours, mental retardation, birth defects, epilepsy, Parkinson's Disease, fibromyalgia, and diabetes."* Although these sugars do not promote body fat in the short term, the synthetic nature of artificial sugars means that their potential long term toxicities are unknown. This experiment also showed that sucralose and aspartame are indeed fermented by yeast, meaning that they might be metabolised in the body and have a caloric impact anyway, even if very small. Stevia's effects and composition are unknown, but there have been health concerns about the long term deleterious effects it can have on the body. That being said, this experiment proved practically that Stevia cannot be metabolised well by organisms, supporting it being non-caloric. In conclusion, different sugars have different metabolic properties and thus effects on the body, and should be selected for consumption depending on the needs and desired effects of the individual.

Flaws in this experiment included the weakness of the gelatine plates, the inconsistency in the final fermentation trial, the unpredictability and changeability of the mixture heights and the unreliable growth of the yeast on the gelatine. The homemade plates themselves were created so that the sugar content of each was directly known, however the gelatine was not as strong as conventional agar and began to liquefy when out of the fridge for more than 12 hours. This made the readings at 24 hours hard to take as the yeast spots had somewhat sunk into the melted gelatine and had begun fermenting in the liquid, and the trial had to be stopped after this time though was originally meant to last 48 hours. For future, plain agar should be used instead of gelatine, as it will not melt but also doesn't pre-contain nutrients so can be mixed with different sugars and still be controlled, though for these trials it was unavailable. The third and final fermentation trial, as addressed, impaired the reliability of the experiment and was perhaps a result of quantity and condition inconsistencies. The heights of the mixtures were also unreliable to measure, as a small amount of shaking of the test tube could disrupt the air bubbles being created inside the mixture, and dramatically change the liquid level. The measurements would also be easier to make if the liquid level changed more rapidly and more dramatically. This height reading should therefore be made on stationary test tubes, without lifting them up and accidentally shaking them, and be conducted in narrower test tubes so that the height increase is more noticeable. Finally, the growth of the yeast on agar was not entirely accurate, as it was fermentation rather than cellular growth that was being measured. It still produced results, and a comparative measure of how much each sugar multiplies a volume of yeast mixture, though the gelatine plate rather than cultivating the microorganisms just provided the yeast with a medium to ferment uniformly outwards on, perceived as growth. The spots grew outwards from the increase in volume of yeast mixture, as a result of both cells multiplying and production of gas, but if true cellular growth is to be measured, a microscopic yeast culture must be used.

Investigations into different sugars could be furthered with a number of different methods. Using a refractometer or Brix meter to measure the remaining sugar content of different yeast/sugar mixtures after a period of fermentation could further demonstrate the amount of fermentation each sugar underwent. For more technical biological investigations, a pure yeast culture, in the form of microscopic cells, can be used to streak agar plates containing different types of sugar and examining the amount of yeast growth in colonies on each plate after incubation. This method would have been conducted for this experiment if not for time constraints and equipment accessibility.

CONCLUSION

Glucose powder promoted the fastest and greatest amount of yeast growth and fermentation, and can be used to compare the metabolic properties of other sugars and weigh their benefits and detriments to human health.

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APPENDIX

THE GROWTH OF YEAST ON GELATINE PLATES IN RELATION TO THE TYPE OF SUGAR USED IN THE PLATE

GELATINE PLATES TRIALS 1, 2 AND 3

Type of Sugar in plate		**Hours after preparation**					**Total growth (mm)**
		0	1	2	12	24	
No sugar	**Diameter of yeast spot (mm)**	7	8	8	8	8	1
Glucose		6	8	10	13	13	7
Sucrose		6	7	8	11	11	5
Raw sugar		6	7	7	10	10	4
Brown sugar		7	8	9	11	11	4
Cornstarch		7	7	8	8	8	1
Splenda		6	7	8	10	10	4
Equal		8	10	10	11	12	4
Stevia		6	6	7	7	7	1

Type of Sugar in plate		**Hours after preparation**					**Total Growth (mm)**
		0	1	2	12	24	
No sugar	**Diameter of yeast spot (mm)**	8	8	8	8	8	0
Glucose		6	7	8	13	13	7
Sucrose		5	6	8	10	10	5
Raw sugar		6	7	7	9	10	4

Brown sugar		7	8	9	10	12	5
Cornstarch		7	8	7	8	7	0
Splenda		6	7	8	9	9	3
Equal		8	8	9	10	11	3
Stevia		6	6	7	8	8	2

Type of Sugar in plate	**Diameter of yeast spot (mm)**	**Hours after preparation**					**Total Growth (mm)**
		0	1	2	12	24	
No sugar		7	7	8	8	8	1
Glucose		6	8	9	13	13	7
Sucrose		6	7	8	10	10	4
Raw sugar		6	8	8	10	10	4
Brown sugar		7	9	8	10	11	4
Cornstarch		7	8	8	8	8	1
Splenda		7	7	8	9	9	2
Equal		8	10	10	11	12	4
Stevia		7	7	7	7	7	0

THE CHANGE IN HEIGHT OF YEAST MIXTURE IN TEST TUBES IN RELATION TO THE TYPE OF SUGAR FERMENTING

TEST TUBE FERMENTATION TRIALS 1, 2 AND 3

Type of Sugar added		**Fermentation time (mins)**						**CO2 concentration (ppm) after 20 mins**
		0	3	6	9	12	15	
No sugar	**Height of yeast/water mixture (mm)**	20	20	20	20	20	21	41 000
Glucose		20	26	31	38	44	47	160 000
Sucrose		20	24	28	32	37	41	145 700
Raw sugar		20	23	29	31	36	40	142 000
Brown sugar		20	25	29	36	40	43	148 200
Cornstarch		20	22	22	21	21	22	106 600
Splenda		20	22	27	30	32	34	138 000
Equal		20	21	25	29	31	35	134 000
Stevia		20	21	21	23	23	24	66 000

Type of Sugar added		**Fermentation time (mins)**						**CO2 (ppm) after 20mins**
		0	3	6	9	12	15	
No sugar	**Height of yeast/water mixture (mm)**	20	19	20	20	20	20	34675
Glucose		20	23	27	30	35	40	139160
Sucrose		20	23	25	27	29	35	94686
Raw sugar		20	21	23	28	30	36	106056
Brown sugar		20	21	23	27	31	37	116004

Cornstarch		20	21	22	22	23	23	62101
Splenda		20	20	21	24	26	31	108962
Equal		20	21	22	23	25	28	98272
Stevia		20	21	21	21	22	22	50213

Type of Sugar added		**Fermentation time (mins)**								**CO2 concentration (ppm)**	
		0	3	6	9	12	15	20	25	after 20mins	after 2 hrs
No sugar	**Height of yeast water mixture (mm)**	20	20	20	20	20	20	20	20	45912	47908
Glucose		20	22	23	25	28	33	36	41	95 696	109090
Sucrose		20	21	21	22	24	27	29	35	61 456	76802
Raw sugar		20	20	22	23	26	27	28	33	63 000	110734
Brown sugar		20	21	22	23	24	26	29	34	40 964	99890
Cornstarch		20	20	20	21	21	21	22	22	35 800	61209
Splenda		20	21	21	21	22	22	24	28	39 000	102008
Equal		20	21	21	21	21	23	25	27	36 000	103478
Stevia		20	20	20	20	21	21	21	22	24 840	50540

- slight outlier, fermentation here a bit slower than the other trials