

Effect of substrates on the respiration of yeast biology essay



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The aim of this investigation is to examine what effects different substrates have on the respiration of yeast. I will investigate this by measuring the amount of carbon dioxide evolved during anaerobic respiration.

Pilot Experiment:

Before we could test which carbohydrate and type of yeast produced more carbon dioxide, we had to standardise the other variables of this experiment; temperature and concentration. Therefore, in order to find the optimum conditions we carried out a pilot experiment. In this experiment we used a range of temperatures from 10°C to 60°C and three different concentrations of carbohydrate 1%, 5% and 10%. The experiment was carried out as a group experiment with everyone being allocated a different temperature and concentration to test. It was carried out over a standardised period of 5 minutes. The rationale for conducting this pilot experiment was that enzymes are biological catalysts that are made up of globular proteins which are activated to work by temperature. They exist in the yeast and our bodies and therefore work best at 40°C, however, they denature soon after and so our body temperature is kept at 37°C to ensure this does not happen. Denaturation is the irreversible loss of 3D structure of enzymes and can be caused by excess heat or a change in PH. According to the Collision theory however, in order for a reaction to take place a certain level of energy, called the activation energy, must be reached. This energy needs to be reached by the particles colliding in the right way and fast enough, so a reaction can take place. By giving the particles more energy it encourages more to collide therefore the activation energy can be reached and a reaction can happen. The kinetic theory explains the effect of

temperature, volume and pressure on the number of collisions. The theory states that if temperature is increased the particles gain more energy and there are more collisions in a given time. Similarly, increasing the concentration means that there is a higher chance of a collision happening because there are more particles in a given volume. If the concentration of carbohydrate/yeast is increased there are more enzymes known as zymase, produced. This means there are more active sites for the carbohydrate substrate to attach to and the reaction happens faster. Therefore a balance must be reached between temperature so it does not denature the enzymes but is high enough to activate a reaction. Also, having a highly concentrated solution is seemingly advantageous but this can cause osmotic problems, so another balance must be reached, as to avoid this problem, but not to discourage a reaction.

Apparatus:

Beehive shelf Clamp Stand 50ml conical flask

Trough Clamp Thermometer

50cm³ measuring cylinder Heat proof mat Spill

500ml beaker Bunsen burner Delivery tube with bung

Tripod Gauze Stopwatch

25cm³ of baker's yeast 25cm³ of sucrose Electronic water-bath

Method:

25cm³ of baker's yeast and 25 cm³ of sucrose was mixed together and preheated at the required temperature for 15 minutes in an electronic water-bath.

400cm³ of water was preheated to the same temperature as the yeast using the Bunsen burner.

The trough was filled with water and a measuring cylinder was inverted by filling it with water then pressing a piece of paper onto the top to prevent any air bubbles from getting in.

The beehive shelf was placed in the centre of the trough and the measuring cylinder was clamped in place, with the top resting on the beehive shelf, the hole being directly under it.

The yeast was placed in the preheated water-bath and the bung from the delivery tube was replaced.

The delivery tube was inserted into the hole in the side of the beehive shelf and the stop watch was started.

Thirty seconds was timed then the beaker with the yeast/carbohydrate mix was swirled for 5 seconds to mix the yeast/carbohydrate.

This was repeated every thirty seconds for fifteen minutes, with readings being taken at three five minute intervals.

The correct temperature in the water bath was maintained by adding more hot water to it throughout the experiment.

BACKGROUND INFORMATION:

YEAST

Saccharomyces cerevisiae, also known as yeast, is a micro organism that uses saprophytic digestion to break down substrates. This is achieved through releasing specific enzymes to break down specific substrates, but if yeast does not contain a certain types of enzyme then it cannot break down its substrate. The more the enzyme of a particular substrate, the faster the rate of breakdown and therefore the more CO₂ is produced. This will help me to test how much CO₂ each substrate produces. Yeast can also respire aerobically and anerobically depending on the availability of O₂. If there is plentiful of O₂ then yeast would respire aerobically with sugars, producing H₂O and CO₂ as waste products. However, if no oxygen is available then the fermentation would occur which converts sugars into CO₂ and ethanol.

RESPIRATION

Respiration is the process by which energy is released energy from glucose in the presence of Oxygen, forming carbon dioxide and water as waste products. Glucose releases energy in a series of reactions that take place inside components of the cell. The stages are briefly explained below:

GLYCOLYSIS

To get the sugar in a more reactive form it is produced to fructose-1, 6-bisphosphate by the addition 2 phosphate molecules. This process is a phosphorylation reaction. The fructose-1, 6-bisphosphate is then broken

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down into 2 molecules of glyceraldehydes-3-phosphate, which comprises of 3C each. The glyceraldehydes-3-phosphate converted into pyruvate via the oxidation process where each GAL3P molecule releases 2 hydrogen ions and 2 electrons. The electrons are then transferred to NAD to produce NADH (reduced NAD) and the energy is used to produce 4ATP from 4ADP and 4Pi. Finally there is a net yield of 2 molecules of ATP, and 2 molecules of pyruvate which is used in the link reaction and 2 molecules of reduced NAD which carries on to the link reaction.

LINK REACTION

In the link reaction the 2 molecules of pyruvate leave the cytoplasm of the cell and enter the mitochondrial matrix. This is an oxidation reaction where 2 NAD molecules oxidise 2 pyruvate molecules into 2 acid molecules. These 2 molecules of acetic acid then go on to combine with 2 coenzyme-A molecules to form Acetyl Co enzyme A. in the end of this stage 2 molecules of reduced NAD form, 2 molecules of CO₂ is lost and most importantly, Acetyl Co enzyme A is formed through the conversion of pyruvate. This is then used in the next stage of respiration.

KREBS CYCLE

At the start Acetyl Coenzyme A , combines with Citrate Synthase an enzyme as well and a 4 carbon molecule called oxaloacetate, forming Citrate. Then, Citrate goes through the process of oxidative decarboxylation which forms a 5 carbon molecule called oxoglutarate. at this point NADH is produced and CO₂ is removed. In the latter stages of the krebs cycle, the oxoglutarate is changed into a 4 carbon oxaloacetate molecule. NADH is made and 1

molecule ATP is also made. The volume of CO₂ that is produced in the krebs cycle is important as this is the dependant variable.

ELECTRON TRANSPORT CHAIN

In this stage all of the NADH and FADH that has been produced in the previous stages is converted into ATP. This takes place in the cristae of the mitochondria. The NADH and FADH electrons move. When the electrons pass from one carrier to another, a series of reduction and oxidation reactions take place which releases energy in the process. This energy is used to pump H⁺ ions from the matrix into the intermembrane space, thus creating a gradient where the concentration of the H⁺ ions in the intermembranal space is higher than it s in the matrix. The inner membrane contains enzymes called ATP Synthase and The H⁺ ions diffuse through these enzymes causing energy to be released which is used to synthesise ATP through phosphorylation. The process is called because the final terminal electron acceptor is oxygen which picks up the electrons from the chain and the H⁺ ion from the matrix to form H₂O as a waste product. This reaction is catalysed by the enzyme Cytochrome Oxidase

For every NADH which enters the chain and is oxidised by NADH dehydrogenase, 3 ATP are produced. For each FADH that enters the chain, 2 molecules of ATP are made.

ENZYMES

Enzymes are proteins that can effectively increase the rate of a reaction by lowering the required energy (activation energy) needed in order for the reaction to occur. Enzymes have a tertiary structure which decides the shape

of the active site. The substrate must be specific to the active site because if they were not complementary to each other, then the substrate can no longer bind to the active site, thus the enzyme substrate complex does not form. The performance of enzymes can be affected in several ways some of which I have explained below.

TEMPERATURE

An increase in temperature will cause an increase in the rate of reaction because both the enzyme particles and substrate particles have gained kinetic energy. This will result in the particles to move faster, thus increasing collision frequency and the numbers of successful collisions as the particles have the required activation energy. If the temperature rises above the optimum temperature then the enzymes can become denatured. This happens because the enzyme molecule vibrates more causing the weak hydrogen bonds (holding the 3D structure of the enzyme together) to break. This eventually leads to the shape of the active site being altered.

Consequently, the substrate will not be able to bind with the substrate as the shape of the active site is no longer complementary so the substrate enzyme complex can not form. This is important in my experiment because if the yeast (enzyme) was to become denatured then it would not be able to bind with the substrate (e. g. glucose) and the reaction would not be catalysed, preventing any CO₂ from being formed. I must ensure that temperature is kept constant throughout.

PH

Another factor which can affect enzymes is pH. Enzymes also have an optimum pH which is pH enzymes work best at. Changing the pH can change <https://assignbuster.com/effect-of-substrates-on-the-respiration-of-yeast-biology-essay/>

the tertiary structure due to the number of H⁺ ion in an acid or the OH⁻ ions in an alkali. These ions disrupt the hydrogen and ionic bonds between -NH₂ and -COOH. This will cause the tertiary structure to break down and changing the active site in the process. Once again, the substrate will no longer be able to bind with the active site, hence no substrate enzyme complex will form. I intend to use a buffer solution which will resist any changes in pH.

SUBSTRATE CONCENTRATION

Increasing substrate concentration increases enzyme activity as they are more molecules to occupy the active site, thus a faster reaction. If more enzyme substrate complex forms then more CO₂ will be produced. However this occurs only for a certain period until all the active sites are saturated with substrates. Therefore an increase in substrate concentration will not result in an increase in the rate of reaction. Carbohydrates such as glucose and sucrose are too soluble and reactive to be stored as they come as they would present osmotic problems and so they are stored in much more complex, insoluble structures known as polysaccharides. Polysaccharides are macromolecules formed by the joining of many monosaccharides together in condensation reactions. There can be more than 3000 repeating units in a chain, joined by glycosidic bonds, forming many complicated structures, one being starch. Starch is a polymer of alpha glucose, where the hydroxyl group is below the ring, and is made up of 30% amylose and 70% amylopectin. Amylose is a long polymer consisting of over 300 monomers joined by 1, 4 glycosidic bonds. Amylopectin gives starch its compact store of energy property as it consists of monomers of glucose in 1, 4 and 1, 6 linkages

causing the chain to branch out. Amylopectin can contain several thousand monomers and forms a coiled up structure which is a valuable store of energy for living organisms. Starch is suited to storage as it is insoluble in water and therefore cannot move out of the cells during osmosis. However, it can easily be broken down to produce simpler carbohydrates by a hydrolysis reaction via the enzyme zymase produced by yeast. It is broken down firstly into maltose then into glucose then into carbon dioxide and ethyl alcohol.

In this experiment we used two different types of the *Saccharomyces Cerevisiae* ('saccharo' meaning sugar and 'myces' meaning fungus) subspecies of yeast to 'respire' the carbohydrates; baker's and brewer's. Both are made up of small cells, separated by walls of cellulose with a living organism inside called a protoplasm. Yeast cells reproduce by 'budding', and do so every two to three hours under ideal conditions. All types of yeast will respire carbohydrates to make energy in order to reproduce. Therefore, when sugar is added to the Brewer's yeast, the yeast cells secrete the enzyme zymase to begin respiring the carbohydrate substrate according to the following equation:

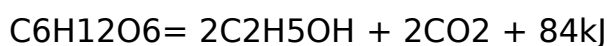


Glucose + Oxygen = Carbon + water + Energy

dioxide

This is known as aerobic respiration due to the presence of oxygen, (defined as 'free or molecular oxygen atoms participating in the respiratory breakdown of organic substances'). Brewers, however, are more interested

in anaerobic respiration, (defined as 'when the respiratory breakdown of organic substrates takes place without the participation of free or molecular oxygen atoms). In yeast, anaerobic respiration is sometimes called fermentation. This happens when the enzyme secreted by yeast, known as zymase, catalyses the break down of glucose to produce ethyl alcohol, in abundance and less carbon dioxide, (which they use to create the 'fizz'). It happens that starch is broken down to form maltose, maltose is broken down into glucose and glucose breaks down according to either the aerobic or anaerobic respiration equation, depending on the conditions. Therefore, Brewer's use anaerobic (airtight) conditions and most of the reaction happens according to this equation:



Glucose = Ethyl + Carbon + Energy

Alcohol dioxide

This produces the desired product i. e. the alcohol and the bi-product of carbon dioxide, which we collect in this experiment.

The Baker's yeast is very similar to Brewer's except it is used for a slightly different commercial purpose i. e. bread making. The yeast respire aerobically in this process as the main function is to 'inflate' the dough to make it softer, and therefore, the desired product comes from the first equation:



Glucose + Oxygen = Carbon + water + Energy

dioxide

The reaction also requires nitrogen from the air to act as a nucleating site for the carbon dioxide to form bubbles against and therefore produces a good yield of carbon dioxide.

In most processes where yeast is used, it will have been cultivated to suit that purpose, e. g. to produce more carbon dioxide or more ethyl alcohol, therefore there will often be a big difference between the behaviour of the two yeasts. We can test this in this experiment as the variable of the condition (whether it is in aerobic or anaerobic conditions) is being standardised by both the experiments being carried out in aerobic conditions. This is due to the fact that there is a good oxygen supply whilst the yeast is preheated and during gas collection, when the system is air tight, it is not left long enough for it to use up the oxygen and respire anaerobically. This is a favourable condition for maximum CO₂ production however as, according to the equation, there are six moles of CO₂ produced aerobically and only two moles of gas produced anaerobically.

Aim:

The aim of the pilot experiment is to investigate the optimum temperature and concentration of carbohydrate, that, when respired with yeast, produces the biggest volume of carbon dioxide.

PLANNING:**THE DEPENDANT AND INDEPENDENT VARIABLE:**

The dependant variable will be the volume of CO₂ produced during respiration and the independent variable will be the substrates that I decide to use in the experiment. These are Glucose, Fructose, Maltose, Lactose and Sucrose.

NULL HYPOTHESIS:

The type of carbohydrate being respired will have no significant effect on the amount of carbon dioxide produced in a given time.

HYPOTHESIS:

In accordance with the information that has been gathered, the following hypotheses were derived;

Hypothesis one: ' When respired by yeast, different types of carbohydrates will produce different amounts of CO₂.'

I believe this because glucose is a monosaccharide which consists of one molecule, sucrose is a disaccharide, which consists of two molecules and starch is a polysaccharide, consisting of many molecules. This means they all have different molecular structures and therefore will break down with different levels of ease. In order to keep an open mind however, the following null hypothesis was also noted;

Null hypothesis one: ' The type of carbohydrate being respired will have no significant effect on the amount of carbon dioxide produced in a given time.'

The second hypothesis was theorised relating to the variable of the yeast:

Hypothesis two; ' Different types of yeast will produce different volumes of CO₂.'

I predict this because the commercial purposes of Baker's and Brewer's yeasts are different and therefore special cultivations might have made the yeast better designed for one purpose than the other. Again a null hypothesis was also adopted;

Null hypothesis two: ' There will be no significant difference between the amount of carbon dioxide produced by the Baker's and the Brewer's yeast.'

Apparatus:

The following apparatus will be used when conducting the experiment:

Beehive shelf

Trough

200cm³ measuring cylinder

500ml beaker

Tripod

25cm³ of baker's yeast

25cm³ of brewer's yeast

25cm³ of sucrose

25cm³ of glucose

25cm³ of starch

Bunsen burner

Gauze

Heat proof mat

Rubber tubing

50ml conical flask

Thermometer

Spill

Delivery tube with bung

Stopwatch

Clamp

Clamp stand

METHOD:

25cm³ of the desired yeast and 25 cm³ of the desired carbohydrate was mixed together and preheated at the required temperature for 1 hour in an electronic water-bath.

400cm³ of water was preheated to the same temperature as the yeast using the Bunsen burner.

The trough was filled with water and a 200cm³ measuring cylinder was inverted by filling it with water then pressing a piece of paper onto the top to prevent any air bubbles from getting in.

The beehive shelf was placed in the centre of the trough and the measuring cylinder was clamped in place, with the top resting on the beehive shelf, the hole being directly under it.

A small piece of rubber tubing was inserted into the beehive shelf through the hole in the side, up into the inverted cylinder, and the other end was attached to the delivery tube.

The yeast was placed in the preheated water-bath and the bung from the delivery tube was replaced.

The delivery tube was inserted into the hole in the side of the beehive shelf and the stop watch was started.

Thirty seconds was timed then the beaker with the yeast/carbohydrate mix was swirled for 5 seconds to mix the yeast/carbohydrate.

This was repeated every thirty seconds for fifteen minutes, with readings being taken at three five minute intervals.

The correct temperature in the water bath was maintained by adding more hot water to it throughout the experiment.

This was repeated using each different type of sugar with each different type of yeast: Baker's glucose, Baker's sucrose, Baker's starch, Brewer's glucose, Brewer's sucrose, Brewer's starch.

Control of the variables of the method:

In order for this experiment to be run fairly there are certain 'controls' that should be taken into consideration:

- The temperature must be kept constant by refilling the water bath with warm water.
- The yeast/carbohydrate solution should be pre-mixed and preheated for the same amount of time i. e. one hour, in each experiment.
- The gas should be collected at three 5 minute intervals for each condition.
- The solution should be swirled every thirty seconds for five seconds to mix the yeast and carbohydrate together.
- The temperature and concentration should be the same for each experiment; 35°C and 7.5% respectively.
- The amount of yeast to carbohydrate should be kept constant i. e. 25cm³ of each.
- The same method should be used; the rubber tube, collection of gas in an inverted measuring cylinder, as certain ways are more accurate than others.

Control of the measurements:

When taking measurements, the following points should be noted:

- When measuring the gas, measure to the bottom of the meniscus of the water.
- Make sure the measuring cylinder is perpendicular to the clamp stand to ensure that the water lies at the correct level.
- When timing, do not shake at 30 seconds by the stopwatch because 5 seconds is added on each time (while it is shaken) and therefore by the sixth minute or so it will require shaking as soon as it has been shaken.
- Keep a constant check on the thermometer to ensure the temperature does not drop.

Results:

A summary table to show volume of gas produced by bakers and brewers yeast with three different carbohydrate substrates:

Amount of CO₂ produced in cm³ in fifteen minutes in each condition

The table above shows the results of our practical, including my own, highlighted in blue. The average volume of gas collected in each separate condition is shown and reveals that most gas was produced in the brewer's glucose experiment, at 115.6 cm³ and the condition that produced the lowest average was brewer's starch, at 9.4. The range of the averages was 94.3, showing there was a large difference between the values.

Analysis of the results:

The graph above clearly shows the difference between the three carbohydrate substrates to be significant. It is clear that the order for most CO₂ produced is glucose, sucrose, starch, the greatest difference being between sucrose and starch. It also appears that the results are closer together for baker's yeast than for brewer's. The average for baker's yeast was slightly higher at 74cm³ than the Brewer's at 71. 2cm³.

Student's T test:

The descriptive statistics above, however, only tell us what has been found, they do not tell us the probability of achieving the scores we did, and therefore an inferential student's t test was applied. The ' student's t test' was devised to analyse smaller sets of samples; to determine whether the results were due to chance or the manipulation of the independent variable. It works out that if the difference between the variance of the sum of the two means (of the two samples) is greater than twice the standard deviation of the difference between the means (of the two samples) then this is unlikely to have occurred by chance, and therefore our data is significant.

Glucose v Sucrose baker's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

103. 6667

93. 25641

Variance

927. 0702

595. 5641

Observations

39

39

Pooled Variance

761. 3171

Hypothesized Mean Difference

0

df

76

t Stat

1. 66608

P(T <= t) one-tail

0. 049907

t Critical one-tail

1. 665151

P(T <= t) two-tail

0. 099814

t Critical two-tail

1. 991675

Glucose v Sucrose Baker's:

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between glucose and sucrose baker's being due to chance was $P > 0.05$. This means the probability that these results are due to chance is more than 0.05 or 5%. Therefore less than 95% due to the manipulation of the independent variable, and are not considered significant.

Glucose v Starch Baker's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

103. 6667

25. 23077

Variance

927. 0702

300. 498

Observations

39

39

Pooled Variance

613. 7841

Hypothesized Mean Difference

0

df

76

t Stat

13. 98055

P(T <= t) one-tail

5. 23E-23

t Critical one-tail

1. 665151

P(T ≤ t) two-tail

1. 05E-22

t Critical two-tail

1. 991675

Glucose v Starch Baker's:

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between glucose and starch baker's being due to chance was $P < 0.001$. This means the probability that these results are due to chance is less than 0.001 or 0.1%. Therefore more than 99.9% due to the manipulation of the independent variable, and are considered very highly significant.

Sucrose v Starch baker's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

93. 25641

25. 23077

Variance

595. 5641

300. 498

Observations

39

39

Pooled Variance

448. 031

Hypothesized Mean Difference

0

df

76

t Stat

14. 19175

P(T <= t) one-tail

2. 29E-23

t Critical one-tail

1. 665151

P(T <= t) two-tail

4. 57E-23

t Critical two-tail

1. 991675

Sucrose v Starch baker's:

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between sucrose and starch baker's being due to chance was $P < 0.001$. This means the probability that these results are due to chance is less than 0.001 or 0.1%. Therefore more than 99.9% due to the manipulation of the independent variable, and are considered very highly significant.

Glucose v Sucrose Brewer's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

115. 641

88. 5641

Variance

1018. 552

568. 1997

Observations

39

39

Pooled Variance

793. 3758

Hypothesized Mean Difference

0

df

76

t Stat

4. 244994

P(T <= t) one-tail

3. 06E-05

t Critical one-tail

1. 665151

P(T ≤ t) two-tail

6. 12E-05

t Critical two-tail

1. 991675

Glucose v Sucrose Brewer's:

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between glucose and sucrose brewer's being due to chance was $P < 0.001$. This means the probability that these results are due to chance is less than 0.001 or 0.1%. Therefore more than 99.9% due to the manipulation of the independent variable, and are considered very highly significant.

Glucose v Starch Brewer's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

115. 641

9. 384615

Variance

1018. 552

30. 66397

Observations

39

39

Pooled Variance

524. 608

Hypothesized Mean Difference

0

df

76

t Stat

20. 4859

P(T <= t) one-tail

5. 59E-33

t Critical one-tail

1. 665151

P(T <= t) two-tail

1. 12E-32

t Critical two-tail

1. 991675

Glucose vs Starch Brewer's.

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between glucose and starch brewer's being due to chance was $P < 0.001$. This means the probability that these results are due to chance is less than 0.001 or 0.1%. Therefore more than 99.9% due to the manipulation of the independent variable, and are considered very highly significant.

Sucrose vs Starch Brewer's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

88. 5641

9. 384615

Variance

568. 1997

30. 66397

Observations

39

39

Pooled Variance

299. 4318

Hypothesized Mean Difference

0

df

76

t Stat

20. 20603

P(T <= t) one-tail

1. 36E-32

t Critical one-tail

1. 665151

P(T <= t) two-tail

2. 71E-32

t Critical two-tail

1. 991675

Sucrose v Starch Brewer's:

Plotting the degrees of freedom against the t stat on the graph of t distribution revealed that the likelihood of the difference between sucrose and starch brewer's being due to chance was $P < 0.001$. This means the probability that these results are due to chance is less than 0.001 or 0.1%. Therefore more than 99.9% due to the manipulation of the independent variable, and are considered very highly significant.

Glucose baker's vs Glucose brewer's:

t-Test: Two-Sample Assuming Equal Variances

Variable 1

Variable 2

Mean

103. 6667

115. 641

Variance

927. 0702

1018. 552

Observations

39

39

Pooled Variance

972. 8111

Hypothesized Mean Difference

0

df

76

t Stat

-1. 69533

P(T <= t) one-tail

0. 047053

t Critical one-tail

1. 665151

P(T ≤ t) two-tail

0. 094106

t Critical two-tail

1. 991675

Glucose baker's vs Glucose brewer's:

Plotting the degrees of freedom against the t stat