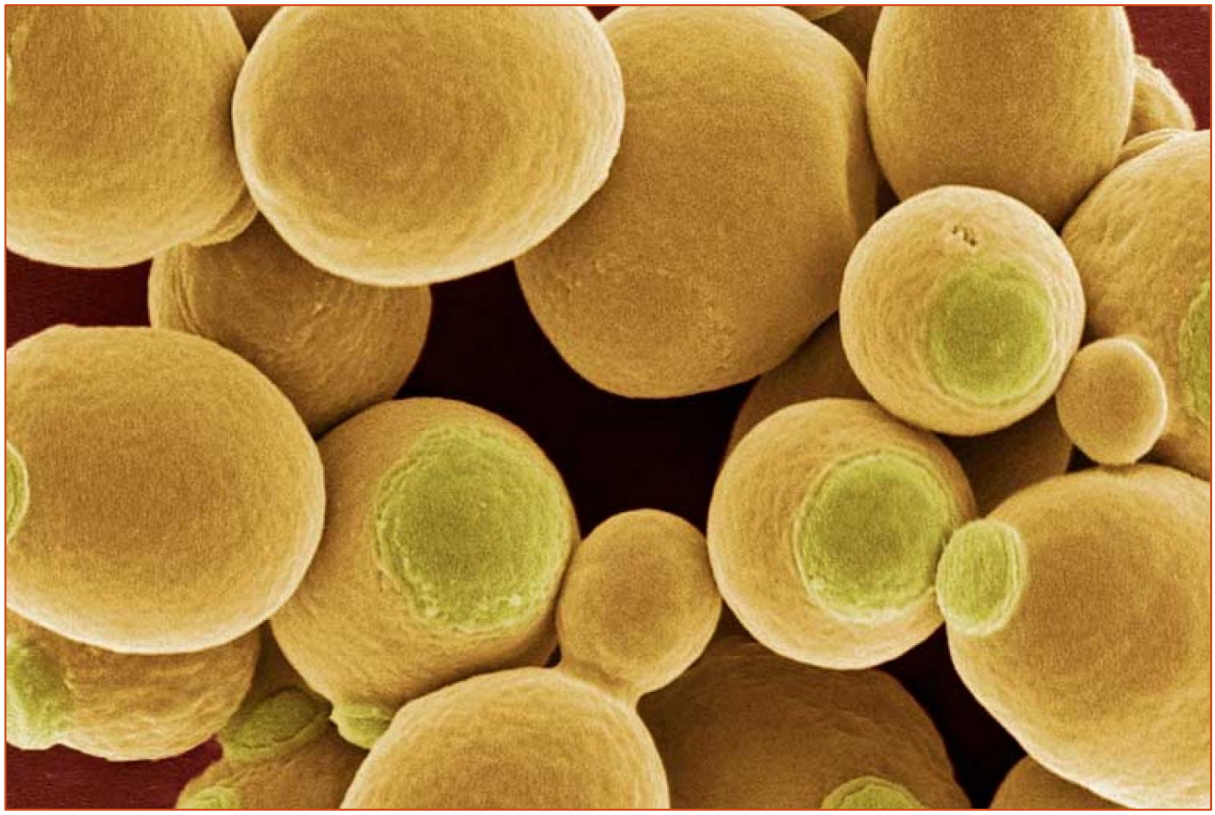
Stage 2 Biology

SUMMATIVE COMPLETION PRACTICAL

FACTORS AFFECTING YEAST FERMENTATION



(FINE DINING LOVERS, 2012)

Introduction:

Respiration is a metabolic process occurring in cells through which chemical energy stored in the bonds of fuel molecules, such as glucose, is released. This energy is used to synthesise adenosine triphosphate molecules, where it is stored and used in cell metabolism. Several enzymes catalyse individual reactions within respiration.

Respiration can occur in the presence or absence of oxygen. Anaerobic respiration without oxygen and is less efficient than aerobic respiration.

Yeast is a fungus which acts on sugar in an anaerobic respiration process known as alcohol fermentation. This process produces ethanol and energy, as well as carbon dioxide gas, which can be used to measure the rate of yeast fermentation.

Yeast fermentation is influenced by factors such as temperature, pH and sucrose concentration. This experiment will focus on effects of temperature on yeast fermentation. The rate of yeast fermentation initially increases with temperature, as temperature controls the kinetic energy of yeast and their enzymes, causing them to respire at a faster rate. High temperatures may cause enzymes within yeast to denature or kill the yeast, resulting in a decrease in the rate of yeast fermentation.

Aim:

The purpose of this experiment is to investigate effects of temperature on rates of anaerobic respiration in yeast. This was observed through measuring the volume of CO_2 gas bubbles produced.

Hypothesis:

I predict the rate of anaerobic respiration in yeast will increase sharply with temperature, with 40^{o}C achieving optimum temperature, as this will presumably greatly increase the kinetic energy of the yeast and its enzymes.

Materials:

- Fresh Defiance Instant Dry Yeast – 2 boxes of 12 packets (each packet = 8g)
- Granulated sucrose sugar
- 4x Thermometers
- Timer
- 2x Stirring rods
- 4x 100mL measuring cylinders
- 1x 10mL syringe
- 1x 100mL syringe
- 2x 250mL beaker
- 4x 600mL beaker
- 1x Orange lab tub
- Retort stand and clamp
- CO_2 gas collecting apparatus
- 4x Large 50mL test tube
- Kettle
- Approx. 20 mL of ice

Procedure:

Initial Set-up –

A. Set up gas collection water tub:

1. Fill orange lab tub 2/3 full of water.
2. Fully submerge 4x 100mL measuring cylinders and ensure no air bubbles are trapped inside.
3. Lift the measuring cylinders so that it is inverted, and the open end is still submerged in the water. The cylinders should be full of water with no air bubbles.
4. Attach a clamp to a retort stand and fasten the clamp so that it holds each measuring cylinder in an upright inverted position.

B. Make up warm yeast suspension:

1. Warm 150mL of water to 35˚C in a 250mL beaker by using kettle and tap water.
2. Pour in one 8g packet of dry yeast.
3. Stir well. Set aside.

C. Make up 10% sucrose solution (standard):

1. Warm 100mL water to 35˚C in a 250mL beaker by using kettle and tap water.
2. Pour in 10g of sucrose.
3. Stir well. Set aside.

D. Prepare warm water baths:

1. Fill each 600mL beaker 2/3 with water. This will provide a bath for the test tube (once prepared) to rest in to help maintain its warm temperature for the yeast reaction.
2. Set up each of the 4 beakers so you can maintain a chosen temperature in each. Use thermometer and hot water from kettle to heat one beaker to 40°C and one to 55°C. Use ice to cool one beaker to 5°C. Use tap water to achieve 20°C in final beaker.
3. Throughout process, maintain temperature of the water bath as close to the set temperatures as possible by monitoring with a thermometer, and extracting water and adding warmed or tap water using a large syringe.

Gas Collection –

1. Using small syringe, put 20mL sucrose solution into the 4 test tubes and gently place in respective water baths.
2. **Stir yeast suspension** and extract 10mL with syringe. Add 10mL of yeast suspension to each test tube with sucrose solutions. Keep the test tube resting in the water bath.
3. Attach gas collecting apparatus to test tube and fit under cylinder. Make sure the bent-up glass tip is correctly under the measuring cylinder – but do not let the cylinder rest directly

on it, or it may break. **Note: attach to test tube first, before putting tip under cylinder – this makes it less likely that the tip will break off.*

4. Wait and measure amount of gas produced against time (every minute or so). Ensure the water bath temperature is maintained. **Note: some of the froth of the yeast suspension may move through the gas collecting tubes. This does not matter.*
5. Record your data carefully for 36 minutes. When complete, calculate that rate of reaction with the following:

$$Rate\ of\ Gas\ Production = \frac{\text{volume of gas } (mL)}{\text{time } (sec)}$$

Variables:

Independent Variable – Temperature of Yeast

5,20,40,55^{o}C will be used.

Dependent Variable – Rate of anaerobic respiration (*mL/min*)

This will be calculated by measuring volume of CO_2 produced (*mL*) by yeast in 36 minutes, then dividing result by 36.

Controlled Variables:

Volume & concentration of yeast suspension (between temperatures) – Different amounts of yeast undergoing anaerobic respiration will affect rates of yeast fermentation. Therefore, volume of yeast suspension must be constant between temperatures to ensure reliable results; 10mL was used for each.

Time CO_2 was measured – Volume of CO_2 produced is used to calculate rate of anaerobic respiration, therefore time this was measured must be constant for each temperature to ensure reliable results. CO_2 was measured every two minutes for 36 minutes for each.

Volume & concentration of sucrose solution – Sucrose is broken down by yeast in fermentation to produce energy. Increasing sucrose will increase the rate of yeast fermentation, therefore volume and concentration of sucrose must be constant between temperatures to ensure reliable results; 10% concentration and 20mL was used for each.

Uncontrolled Variables:

Volume of water in water baths – Water was constantly added to baths to regulate temperature, causing amount of water in baths to differ between each temperature. This may affect the rate of yeast fermentation as baths facilitated the heating of yeast, thus temperatures may be inaccurate.

Concentration of yeast (between groups) – Varying amounts of yeast remained in packets between groups, causing concentration of yeast suspension to differ between groups. Different quantity of yeast undergoing anaerobic respiration will affect the rate of yeast fermentation.

pH – pH was unknown and unregulated. pH can impact structure of enzymes if it differs significantly from optimum pH, impacting enzyme activity in yeast, affecting the rate of fermentation.

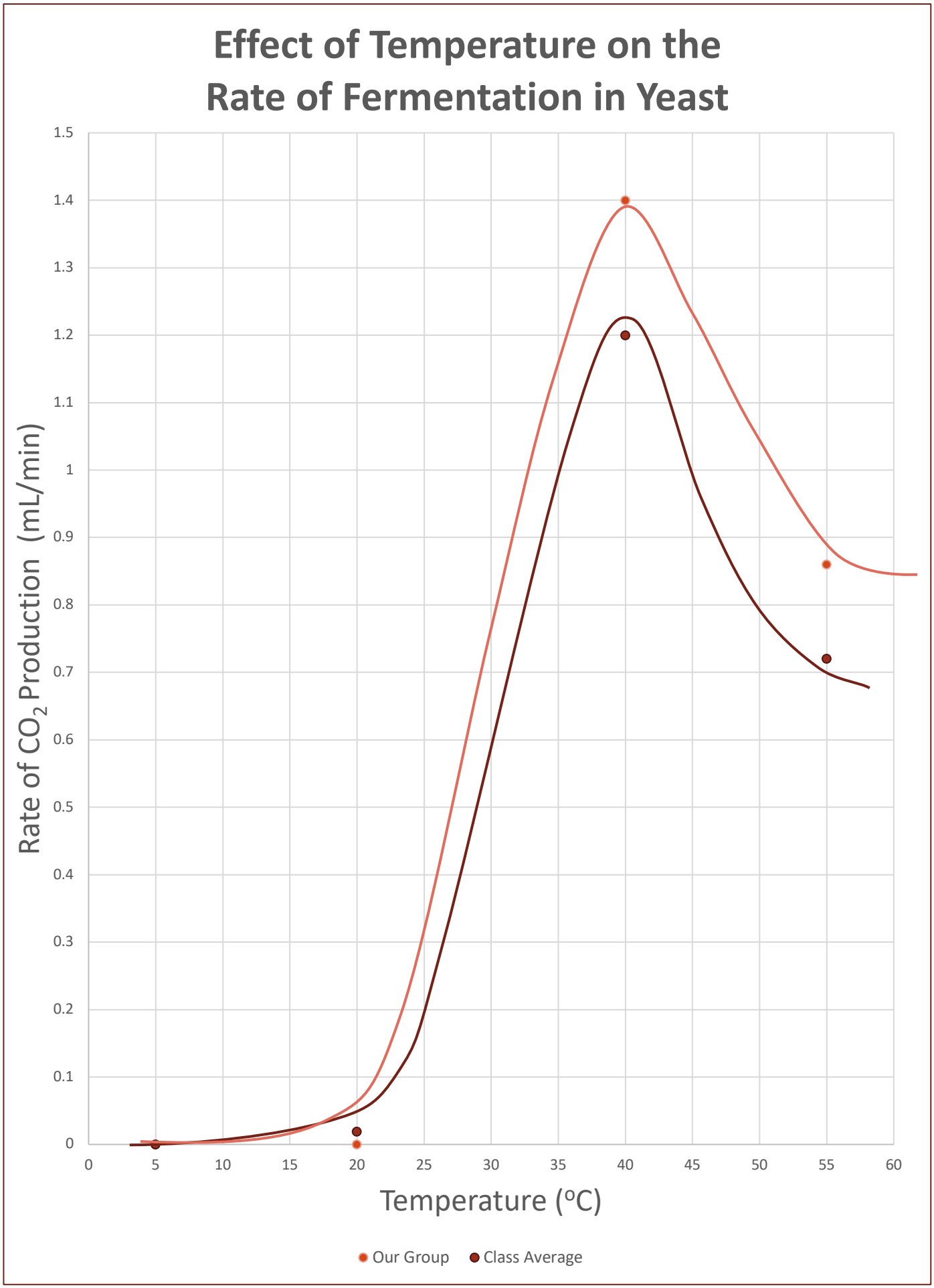
Safety Audit:

Concern	Hazard	Management
Boiling water from kettle	Severe burns may occur.	Care must be taken when handling the kettle. If burns occur, hold damaged area under cold water for 20 minutes. If burns are severe, seek medical attention.
Glassware	May break into sharp fragments and cause cuts.	Care must be taken when handling glassware. If glassware breaks, monitor at all times to ensure it does not cause injuries, and clean up appropriately.

Results:

TABLE 1: Groups & Class Record of Results – Effect of Temperature on Rate of Yeast Fermentation				
	Total Volume of CO_2 Collected over 36 Min (mL)			
Temperature (°C)	**Personal Group (Group 1)**	**Group 2**	**Group 3**	**Class Average**
5	0.0	0.0	0.0	0.0
20	0.0	2.0	0.0	0.7
40	52.0	46.0	32.0	43.3
55	31.0	29.0	18.0	26.0

TABLE 2: Groups & Class Record of Results – Effect of Temperature on Rate of Yeast Fermentation				
	Rate of Yeast Fermentation (Volume of gas/time) (mL/min)			
Temperature (°C)	**Personal Group (Group 1)**	**Group 2**	**Group 3**	**Class Average**
5	0.00	0.00	0.00	0.00
20	0.00	0.056	0.00	0.019
40	1.40	1.28	0.89	1.20
55	0.86	0.81	0.50	0.72


Effect of Temperature on the Rate of Fermentation in Yeast
Rate of CO_2 Production (mL/min)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Temperature (°C)
Our Group
Class Average

Discussion & Analysis:

Analysis:

Results showed the rate of yeast fermentation hardly increased between 5°C and 20°C. Class average showed a 0.019mL/min increase between these temperatures, with only one group recording any increase; generally, the rate of fermentation remained at 0mL/min. This miniscule increase is represented by the low gradient of trend curves. This may indicate yeast were not active at these temperatures. The slight increase achieved by one group may be caused by errors in temperature regulation or differing concentrations of yeast. Furthermore, results reveal a sharp increase between 20°C and 40°C. Class average rate of fermentation was 0.019mL/min at 20°C, and 1.20mL/min at 40°C, with 40°C achieving optimum rate of fermentation. This increase is represented by the steep gradient of trend curves on the graph. This supports the hypothesis, which states rate of fermentation would increase sharply with temperature, with 40°C achieving optimum. The trend is anticipated as kinetic energy of yeast and their enzymes is in proportion with temperature. Kinetic energy controls movement of enzymes and other molecules catalysing respiration, consequently impacting the rate of fermentation. At low temperatures, kinetic energy is low, causing only a slight increase in rate of fermentation; as heat increases, kinetic energy would begin to rapidly increase, as does the rate of fermentation.

Furthermore, results reveal the rate of yeast fermentation dropped once temperature exceeded 40°C. Class average rate of fermentation at 55°C was 0.72mL/min, which is a 0.48mL/min difference from 40°C. This decrease is represented by the sharp decline in trend curves from approximately 40°C. This decline could be attributed to high temperatures causing enzymes catalysing stages of yeast fermentation to denature, altering their structure, preventing the formation of enzyme-substrate complexes.

Group results are mostly reliable when comparing to the class average. They follow the same general trend, as represented by trend curves on the graph. Between class and group averages recorded, there is no difference for 5°C and 0.019mL/min for 20°C; these are insubstantial differences, indicating general reliability of group results. However, the difference is 0.20mL/min for 40°C and 0.14 for 55°C, which is slightly more significant, indicating slight unreliability in that area. This may be due to random error occurring when regulating temperature of water baths. Maintaining these high temperatures was difficult, as they dropped quickly in the cool environment, potentially slightly impacting the rate of fermentation.

Evaluation:

Reliability:

Data is mostly **reliable**, as the procedure was followed fairly precisely, and differences between personal group and class average results were insignificant. However, there is a major difference in mL of CO_2 gas produced between different groups, particularly from group 3, who were noted to have made several errors; for 40°C, production of CO_2 for this group differed from personal results by 20mL. This reveals partial **unreliability** of results, and thus class average. A major source of uncertainty contributing to differing results between groups is the uncontrolled factor of concentration of yeast between groups. Not all yeast was extracted from packets; the extent of this would cause concentrations to differ between groups. Additionally, Group 3 diluted water more than other groups, changing their overall yeast concentration. This would cause an inconsistency between rates of yeast fermentation, partly reducing **reliability**.

Further sources of uncertainty can be identified, partially impacting **reliability** of personal group results. One of these is fluctuating temperatures of water baths; although precaution was taken to regulate temperature, temperatures dropped sporadically. At one instance, the 55°C bath dropped 14-16°C. As the independent variable, temperature impacts rate of yeast fermentation by controlling kinetic energy of yeast; fluctuation in temperature will thus cause partial unreliability of results. Additionally, the CO_2 collecting apparatus introduced potential error, as varying amounts of gas may escape at certain intervals, reducing volume of CO_2 collected, causing results to be inconsistent and partially **unreliable**. This also may have occurred when sealing test tubes, as they were not sealed immediately. Despite all care taken, occurrence of errors remains feasible; therefore, data has a degree of uncertainty concerning its **reliability**. To reduce uncertainty and verify results, the experiment could be repeated.

Accuracy:

True results for the experiment are unknown, therefore it is difficult to assess **accuracy**. When using the class average as an indicator of accuracy, personal results appear mostly **accurate** as there is little difference between values. However, major differences between different group results cause class average to be partly unreliable, and thus cannot be considered a true representation of **accuracy**. Additionally, systematic errors, such as the impreciseness of measuring devices, due to poor calibration, would cause results to consistently differ from 'true' values, as many measurements are required throughout the experiment. Therefore, results are deemed partly inaccurate.

Validity:

Results are only partly **valid**, partly due to small sample size and range of independent variables. The experiment was only conducted once for each group, and only four temperatures were investigated, partially limiting the generalisation of conclusions from results. Validity could be improved by increasing sample size and independent variable range.

However, results are relatively **valid** as general trends of the data reflect the expected impact of temperature on the rate of yeast fermentation, and therefore general conclusions can be drawn. As well as this, live yeast and appropriate conditions for fermentation were utilised, causing yeast fermentation to occur. Additionally, measuring CO_2 gas, as a product of the reaction, was a suitable method for investigating rates of fermentation, partly increasing the experiment's validity.

Conclusion:

Overall, the aim of the experiment was mostly met as effects of temperature on rates of yeast fermentation was observed. It was determined rate of fermentation increased between 20°C and 40°C, with 40°C achieving the optimum rate. Once temperature exceeded 40°C, a sharp decline in rate of fermentation was observed. Limitations impacting overall reliability, accuracy and validity of results include uncontrolled concentration of yeast between groups; errors concerning temperature regulation and CO_2 measuring apparatus; use of imprecise measuring devices; limited sample size and independent variable range. Despite these, data is predominantly reliable as personal and class average results are consistent, and trends observed are in conjunction with biological understanding.

Word Count: 1,495

References

Fine Dining Lovers. (2012, 7 6). *How To Make Yeast And Use It At Home: The Science Of Yeast*. Retrieved from Finedining Lovers: https://www.finediningLovers.com/article/how-make-yeast-and-use-it-home-science-yeast