HWA CHONG INSTITUTION
JC2 Preliminary Examinations
Higher 2

CANDIDATE NAME		**CT GROUP**	**21S7 __**
CENTRE NUMBER		**INDEX NUMBER**	

BIOLOGY **9744/04**

Paper 4 Practical **31 August 2022**

No additional materials are required. **2 hours 30 minutes**

INSTRUCTIONS TO CANDIDATES

There are **three** questions to this paper. Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page.

Answer **all** questions in the spaces provided on the question paper.

Shift
Laboratory

INFORMATION FOR CANDIDATES

The use of an approved scientific calculator is expected, where appropriate.

You may lose marks if you do not show your working or if you do not use appropriate units.

The number of marks is given in brackets [ ] at the end of each question or part question.

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
1	**/ 20**
2	**/ 15**
3	**/ 20**
Total	**/ 55**

This document consists of **17** printed pages.

QUESTION 1

Cheese-making is a process that concentrates protein, fat and other nutrients from milk. This process involves coagulating the casein protein in milk. Coagulation of milk occurs when the protein and lipid molecules in milk aggregate together forming a solid substance that is separated from the liquid component.

Coagulation of milk can be caused by acidification of milk, the action of an enzyme (such as rennin), or a combination of the two. It has been found that the usage of fruit extracts as a commercially-produced milk coagulant can increase the flavour and texture of the final cheese.

You are required to:

- make and record observations on the coagulation of milk by acid and rennin,
- investigate the relative effectiveness of acid and rennin in milk coagulation by measuring the time taken for milk coagulation to occur,
- determine whether a fruit extract that is often used to make commercial coagulants contain acids, rennin, or both, as its active ingredient.

You are provided with:

- one slice of pineapple **P**, in a plastic bag,
- diluted hydrochloric acid solution, in a container labelled **H**,
- diluted rennin enzyme, in a container labelled **E**,
- milk, in a container labelled **M**.

Diluted hydrochloric acid H is corrosive. Suitable eye protection and gloves should be worn. If H comes into contact with your skin, wash off immediately under tap water. Care should be taken when using hot water.

Proceed as follows.

The coagulation of **M** by **H** can be observed using the following steps:

1 Add 5.0 cm^3 of **M** to a test-tube.

2 Add 1.0 cm^3 of **H** to the **M** in the same test-tube. Gently push the plunger of the syringe so that **H** runs slowly down the side of the test-tube to the bottom. This is shown in Fig. 1.1 (a).

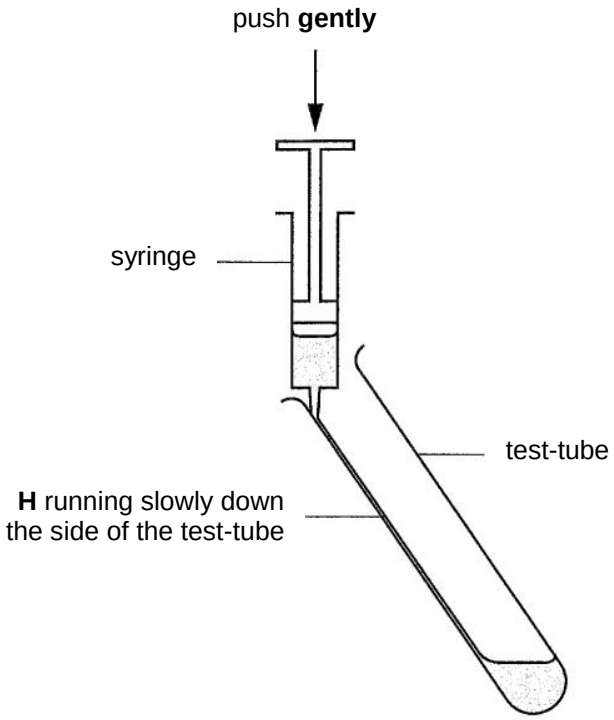

Fig. 1.1(a)

3 Tilt the tube and rotate it slowly as shown in Fig. 1.1(b). Do **not** shake the tube.

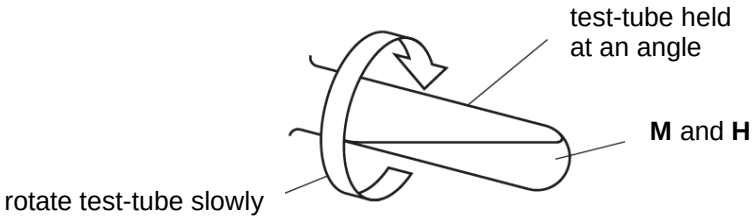

Fig. 1.1 (b)

(a) (i) Record your observations of the sides of the test-tube.

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..... [1]

4 Repeat the procedure in steps **1** to **3**, using a new sample of **M**, clean glass apparatus and **E** instead of **H** to observe the coagulation of **M**.

(ii) State how your observations from addition of **E** differ from your answer in **(a)(i)**.

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..... [1]

(iii) Based on your observations in **(ii)**, state whether **H** or **E** is more effective as a milk coagulant.

.....[1]

(iv) Confidence in the results of this investigation may be limited by lack of replication and sources of error.

Describe **two** other significant sources of error in this procedure.

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.....[2]

(v) Suggest how you could make **two** improvements to the procedure so that a more accurate estimate of the relative effectiveness on coagulation could be obtained.

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.....[2]

(b) In some commercially produced milk coagulants, fruit extracts are used. Pineapple contains numerous enzymes as well as acid that could act as coagulants. A student wished to investigate the relative effectiveness of using pineapple extract, **PE**, to coagulate milk, as compared to **H** and **E**.

5 Cut the piece of pineapple **P** into smaller pieces on a white tile.

6 Use the mortar and pestle to crush and grind the pineapple pieces to obtain a mash.

7 Label a large plastic vial '**PE**'.

8 Filter the mash through the sieve into the large plastic vial labelled **PE**.

*Discard the soiled gloves. You may use a new pair when answering Question 2. While you are collecting filtrate **PE**, continue to read through this Question Paper up to step **9** and complete **(b)(ii)**.*

(i) Use the information provided to plan a method that you can use to determine if **PE** coagulates milk by its acidity or by the presence of coagulating enzymes such as rennin. The time taken for coagulation can be recorded, up to a maximum of 120 seconds.

You will carry out this method in step **9**.

You do **not** need to:

- plan to carry out repeats and replicates
- include details of how to test the samples for coagulation
- include details of how the pineapple extract can be obtained.

In your plan, you must use:

- milk, **M**
- pineapple extract, **PE**
- thermometer and boiling water bath.

You may select from any of the other apparatus and materials provided.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- include details to ensure that the results are as accurate and repeatable as possible
- use the correct technical and scientific terms
- only make use of the apparatus and materials provided.

[5]

Before proceeding further, use the container labelled **hot water** to collect approximately 400 cm^3 of hot water from where it is provided in the laboratory.

The container labelled **tap water** can be filled as necessary from the tap.

9 Carry out your method described in **(b)(i)** to determine if the coagulative properties of pineapple is due to acidity, enzyme or both.

(ii) Use the space provided to record your results in a suitable format.

[3]

(iii) Based on your results, state and explain whether the coagulative properties of pineapple is due to acidity, enzymes or both.

[4]

(iv) State an assumption that needs to be made in order for your results to be valid.

[1]

[Total: 20]

QUESTION 2

Another way to make cheese is through the use of a starter culture containing bacteria and yeast, mixed with a substrate. Different substrates can be used, such as glucose and maltose.

The chemical tetrazolium chloride (TTC) can be used in investigating the rate of respiration in living cells. The intensity of the pink colour is proportional to the rate of respiration of the cell.

It was hypothesised that the higher rate of respiration of the yeast and bacteria in the starter culture corresponds to a higher rate of cheese formation.

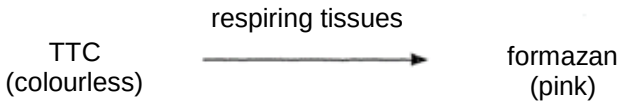

Fig. 2.1

In order to investigate the effect of the sugars, glucose and maltose, on the rate of respiration, you are required to use TTC to estimate the rate of respiration.

You are provided with:

- starter culture of yeast and bacteria, in a plastic vial labelled **Y**
- two unknown sugars of the same concentration, in plastic vials labelled **C1**, **C2**
- TTC solution, in a plastic vial labelled **T**
- colour chart showing the different intensity of formazan.

(a) (i) State **and** explain a hypothesis that could be used to predict the results of testing samples **C1** and **C2**.

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..... [2]

Proceed as follows.

1 Prepare two test-tubes by labelling them **C1** and **C2**.

2 Put 1.0 cm^3 of sample **Y** into the test-tube labelled **C1**.

3 Put 1.0 cm^3 of sample **C1** into the same test-tube.

4 Put 1.0 cm^3 of **T** into the same test-tube.

5 Start the stopwatch. Allow the mixture to incubate at room temperature for 5 minutes.

6 After 5 minutes, use the colour chart to record in **(a)(ii)**, the intensity of colour of the mixture in the test tubes labelled **C1**.

7 Repeat steps **2** to **6** using **C2** instead of **C1**.

(ii) Record your results in the space below.

[2]

(iii) Identify and explain whether **C1** or **C2** is more likely to be maltose.

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..... [2]

(b) A student wanted to investigate the effect of starter culture composition on effectiveness of cheese-making. The student used two separate starter cultures in the experiment:

- starter culture **B**, containing bacteria only
- starter culture **Y**, containing yeast and bacteria.

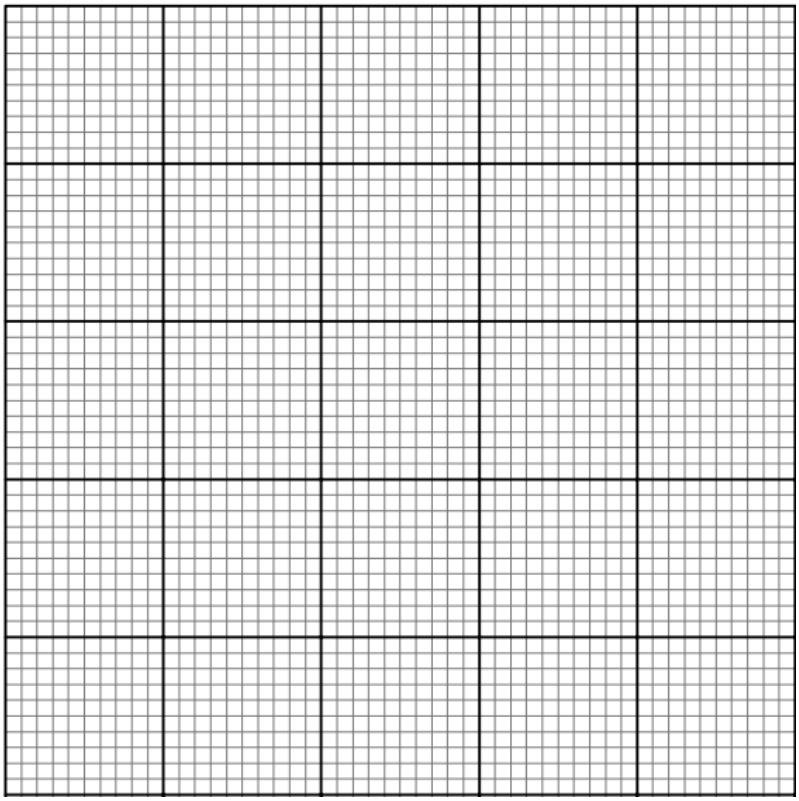
Instead of using TTC to approximate the rate of respiration, he measured the rate of respiration using a respirometer. The student used the same mixture of sugars as substrates and obtained two replicates of data. All other conditions in the experiment, other than the starter culture used, were kept constant.

The table shows the results obtained by the student.

Table 2.1

contents of starter culture	rate of respiration / a.u.	
	replicate 1 (**R1**)	replicate 2 (**R2**)
bacteria (**B**)	5.3	4.9
yeast and bacteria (**Y**)	8.0	7.6

(i) Use the grid provided to display the results in Table 2.1.



[4]

The same experiment was repeated by measuring the rate of respiration for 15 samples of starter culture **B** and 15 samples of starter culture **Y**.

The results are shown in Table 2.2.

Table 2.2

	rate of respiration / a.u.	
	starter culture **B**	starter culture **Y**
mean ($\bar{x}$)	5.0	7.5
standard deviation (s)	0.4	0.8

(ii) State what the standard deviation, s, shows about the results of this investigation.

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..... [1]

(iii) A t-test can be used to determine whether there is any significant difference between the rates of respiration of starter cultures **B** and **Y**.

Calculate the value of *t* and the number of degrees of freedom, using these formulae:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

key to symbols
s = standard deviation $\bar{x}$ = mean n = sample size (number of observations) v = degrees of freedom

Show your working.

value of *t* = ……...

number of degrees of freedom = …….....
[2]

(iv) The critical value of *t* at the 5% level of significance is 2.05.

Explain whether the results of this t-test can help the student determine which starter culture is better for cheese making.

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…… [2]
[Total: 15]

QUESTION 3

During this question, you will require access to a microscope and slide **L1**.

(a)(i) Fig. 3.1 is a photomicrograph of a stained transverse section through a leaf of a pineapple plant.

You are not expected to be familiar with this specimen.

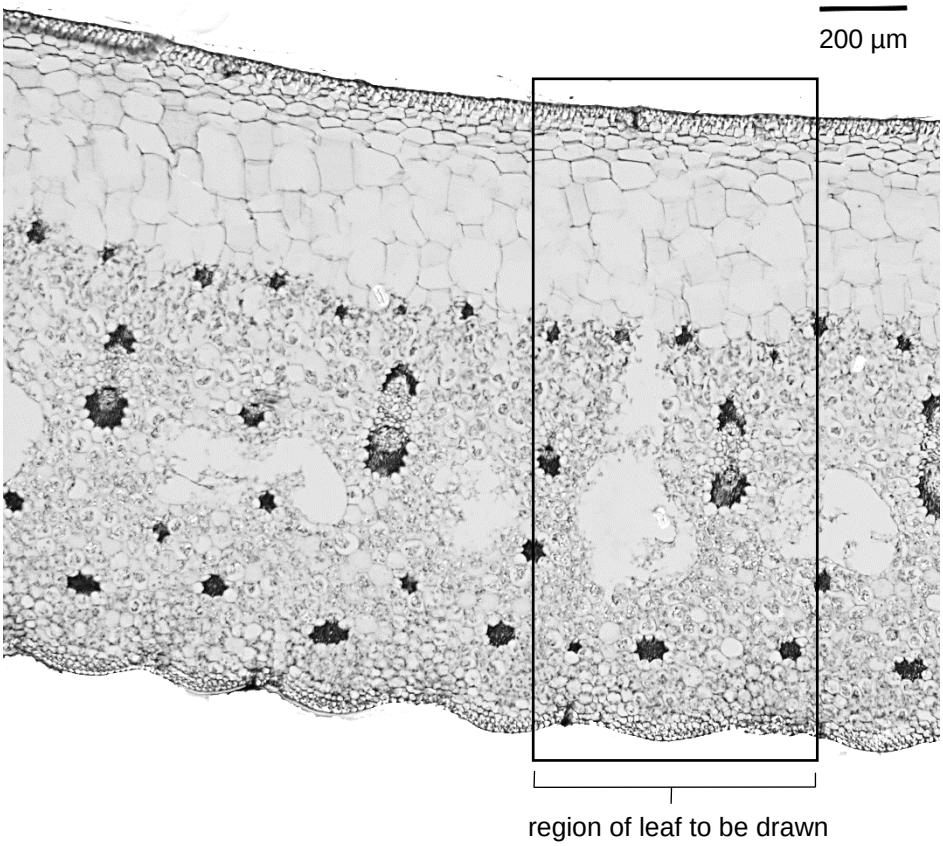

Fig. 3.1

Draw a plan diagram of the region of the leaf indicated between the lines on Fig. 3.1.

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of different tissues.

No cells should be drawn.

Labels are **not** required.

[4]

(ii) You can assume that the actual length of the bar in Fig. 3.1 is 200 μm.

Use this information to calculate the magnification of your drawing in **(a)(i)**.

Show all the steps in your calculation, including the appropriate units.

magnification = x…… [2]

(b)(i) **L1** is a microscope slide of a stained transverse section through a leaf of another plant, *Nerium sp* plant. The epidermal cells form the outer cell layer of the leaf, as shown in Fig. 3.2.

You are not expected to be familiar with this specimen.

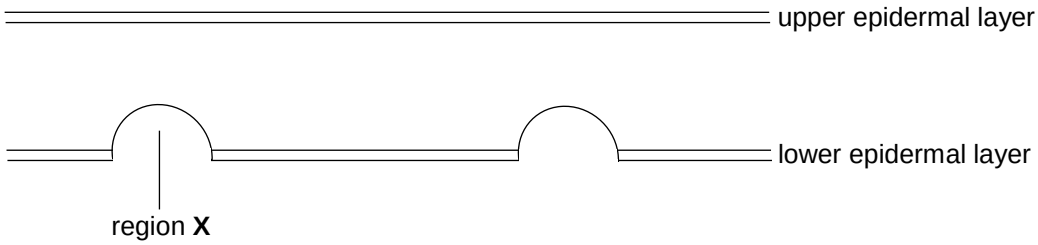

Fig. 3.2

Select a group of two palisade mesophyll cells and three cells above it.

Each cell of the group must touch at least one of the other cells.

Make a large drawing of this group of **five** cells.

[4]

(ii) There are numerous hair-like projections in region **X** of Fig. 3.2. Using the eyepiece graticule fitted in the eyepiece lens of your microscope, and the stage micrometer, find the actual length, in μm, of a single hair-like projection.

Show all the steps in your calculation, including the appropriate units.

length of a hair-like projection = ……μm [2]

(iii) Suggest a possible function of these hair-like projections.

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…… [1]

(iv) Identify **two** observable differences between the leaf in Fig 3.1 and the leaf on **L1**.

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…… [2]

(c) The use of pineapple in cheese production form bitter by-products. One factor that could influence the amount of bitter by-products formed is the ripeness of the pineapples. The sugar-acid ratio, which reflects the relative contents of sugars and acids in pineapples, changes during the different stages of ripening.

Table 3.1 shows the sugar-acid ratio of two varieties of pineapples, Smooth Cayenne and MD2, at various time points.

Table 3.1

stages of ripening	Smooth Cayenne	MD2
unripe	19.8	46.8
half ripe	24.0	64.2
full ripe	27.8	90.0
mean	23.6	67.2

(i) Explain the effect of ripening on the sugar-acid ratio of pineapples.

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..... [2]

(ii) State one variable which would need to be controlled to obtain reliable results in this investigation.

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..... [1]

(iii) The researcher wishes to conclude that the mean sugar-acid ratio of MD2 appears to be higher than that of Smooth Cayenne at every stage of ripening.

Suggest why this conclusion may not be valid.

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..... [2]

[Total: 20]