SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Scientific Inquiry

Year 11-12

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Scotch College - Senior

Oliphant Science Awards
Scientific Inquiry

Subject: Biochemistry

Topic:
Impact of flavourings on the curding process of milk in cheesemaking (g)

Research Question:
“To what extent can banana flavouring be added to the milk mixture for mozzarella cheesemaking while still producing a sufficient mass of at least 100g of cheese curds?”

Joshua Kirsten and Alexander Lee, Year 11

Scotch College Adelaide

Word Count: 1884

INTRODUCTION

Background information

Milk curds used in cheeses are semisolid structures, formed through the coagulation of milk [Amaro-Hernández et al. 2020]. These curds are primarily made of micelles, fat globules, and whey [Amaro-Hernández et al. 2020]. During this process, citric acid or similar is used to lower the pH of the milk, denaturing enzymes, specifically caesin in the milk so they can properly respond to rennet to form curds [Kincaid 2024c]. Next, rennet is added to the milk mixture. Rennet is used to coagulate the denatured casein to form curds [Garcia 2016]. The curds are produced as the micelles (structure where casein is found) is destabilised by the coagulation of casein. This causes the micelles to aggregate and form curds.

Rennet is an assortment of enzymes, necessary for the coagulation of curds during the cheesemaking process [Kincaid 2024b]. Currently, three types of rennet are used: animal, plant-based, and microbial, with the latter being the most commonly used [Kincaid 2024a]. Rennet is produced from fermentation, where specific microorganisms convert sugars into enzymes, the primary enzymes being chymosin and pepsin [Peck 2024]. The rennet enzymes are then harvested and purified so they can be safely used for dairy production. To allow for the optimal conditions for rennet, during the curding process, the mixture is heated to approximately 37˚C to allow the optimal temperature for the rennet enzyme to function, increasing the reaction rate and mass of curd produced [Peck 2024].

Food flavourings are often made using liquid stevia as a solvent [Nirvana Health Products 2025]. Stevia is a sugar substitute made from the leaves of *Stevia rebaudiana*, found usually in liquid form [Mitchell & Ellis 2023]. Stevia is primarily composed of the compounds stevioside and rebaudioside A which give stevia it's sweet taste [Gardana et al. 2003]. Stevioside is known to act as an enzyme inhibitor to digestive enzymes, such as α-glucosidase and α-amylase [Kamal et al. 2022]. This is through the binding to an allosteric site, which changes the shape of the active site, preventing the enzyme from binding to its substrates [Sapkota 2023].

Significance + Relevance of experiment:

A rising trend in younger consumers over the better half of the last decade has been peculiar food combinations, ranging from adding spice to normally savoury foods, or citrus to sweet desserts [Neo, 2023]. Currently, different flavoured cheeses do not exist. Hence, this report acts as a resource for how stevia food flavourings, with banana flavouring chosen in particular, affect the curd formation of cheese, to determine if there is any effect, so that this information may be considered if others chose to produce flavoured cheeses.

Research Question

"To what extent can banana flavouring be added to the milk mixture for mozzarella cheesemaking while still producing a sufficient mass of at least 100g of cheese curds?"

Why this method

This method is a recipe for creating mozzarella. The simplicity of the cheese, given its lack of other factors such as fungus, allow for the validity of the experiment to be increased. The recipe was chosen as it is available on Cheeselinks, suggesting curd formation using this method is common [Cheeselinks 2025]. The method was also chosen given its speed, as the time availability to conduct the experiment was low.

METHODOLOGY

Aim:
To determine how the amount of banana flavouring added into a batch of mozzarella cheese affects the mass of the curds produced.

Hypothesis:
As the amount of banana flavouring added increases, the mass of curds produced will decrease.

Independent Variable:
Amount of banana flavouring added to the mozzarella batch, manipulated by adding different amounts, 0ml, 2ml, 4ml, and 6ml.

Dependant Variable:
Mass of cheese curds produced from the batch of mozzarella (g), measured by weighing the curds on a scale after the curding process has been completed.

Factors to be held constant:
To ensure validity of the experiment, all factors other than the independent variable must be controlled to ensure the independent variable is the only factor affecting the data.

Factors to be Held Constant	Effect on Dependant Variable	Method of Control
Amount of citric acid	Increasing the amount of citric acid will decrease the pH of the milk, increasing the rate of proteins denaturing, increasing the mass of curd produced, and vice versa [Totanes 2023].	Measure the mass of citric acid using electronic scales to ensure the mass is the same for each test.
Time of drainage	Increasing the time of draining will increase the liquid loss of the cheese, decreasing the mass of the curds when they are weighed.	Time the curds draining to ensure they drain for the same duration for each test.
Amount of rennet	Increasing the amount of rennet will increase enzyme availability [LibreTexts n.d.]. This will increase the rate of protein breakdown, increasing the mass of curd produced, and vice versa.	Measure the volume of rennet using a syringe to ensure the same volume is used for each test.

Table 1: Table of Controlled Variables

Uncontrolled Variables:
Despite controlled variables, certain factors cannot be controlled for various reasons, which can only be acknowledged when performing the experiment.

Uncontrollable Factors	Effect on Dependant Variable	Why it cannot be controlled
Concentration of proteins in milk	If a higher concentration of proteins is present in the milk, a higher mass of curd will be produced, as more protein will be present to become curds, and vice versa [LibreTexts n.d.].	Different cows have different gene expression. Therefore, it is impossible to ensure the milk has the same amount of protein other than using one cow.
Agitation of milk	Higher amounts of agitation will increase the number of collisions with enzymes and hence the rate of reaction, and vice versa [Akhlaghi & Najafpour-Darzi 2020].	Agitation cannot be accurately measured by a set value, and given agitation is a necessary part to the curding process, the amount of agitation cannot be accurately controlled.

Table 2: Table of Uncontrolled Variables

Apparatus & Materials

Materials

- 12L full cream milk
- 14.88g Citric Acid
- 36mL Banana Flavouring
- 870mL water
- 12mL Rennet

Equipment

- 1x 2L Pot
- 1x 8L Pot
- 1x Pot Lid
- 12x Cheese Cloths
- 1x Heating Element
- 1x Collander
- 1x Curd Knife
- 1x Thermometer
- 1x Electronic Scale

(Appendix A)

Apparatus

Figure 1: Photo of draining composition.

Figure 2: Photo of curding setup.

Method

1. 1/3 of a teaspoon of citric acid (calculated to be 1.24g) was diluted in ¼ cup of water. (Appendix B)
2. The diluted citric acid was added to 1L of milk. (Appendix C)
3. The banana flavouring was added (0mL, 2mL, 4mL or 6mL). (Appendix D)
4. The milk mixture was placed in the 2L pot and heated to 37˚C (measured using thermometer) and after 37˚C was reached, the mixture was removed from the heat. (Appendix E)
5. 1mL of Rennet was diluted in 10mL of water and added to the milk mixture, stirred thoroughly to ensure even distribution. (Appendix F)
6. The curds were left to form for 12 minutes.
7. The curds were cut into 2cm cubes using the curd knife and transferred to a cheese cloth lined colander to drain for 10 minutes over the 8L pot. (Appendix G)
8. The curds were squeezed to remove excess water and were weighed on the electronic scale. (Appendix H and I)
9. The equipment was washed using soap and water.
10. Steps 1-9 were repeated two more times for a sample size of 3 for each test
11. Steps 1-10 were repeated for the different amounts of banana flavouring.

Safety Concerns

Safety Considerations			
Hazard	Likelihood	Seriousness	Control Measure
Stove Burns	Moderate	Moderate	Avoid touching the stovetop to prevent burns.
Curd Knife Lacerations	Moderate	Moderate	Place away from working area when not in use and handle carefully.
Lactose Intolerance/Allergy	Low	Moderate (Intolerance) High (Allergy)	Test to determine if intolerant/allergic to lactose. If so, wear gloves and mask during experiment. Avoid milk consumption.

Table 3: Table of Safety Concerns

Ethical, Environmental, and Social Concerns

Ethical Concern	Explanation	Control measure
No ethical concerns	No ethical concerns	No ethical concerns
Environmental Concern	Explanation	Control measure
Disposal of cheese curds	The irresponsible disposal of the cheese curds could potentially disrupt the ecosystem they are disposed in [Hobson 2024].	Ensure responsible disposal of the cheese curds by using garbage bags to dispose the cheese into a bin.
Social Concern	Explanation	Control measure
Microbial Rennet being a GMO	Microbial Rennet is a gene product, produced through bacterial cultures. This can have ethical concerns, as public debate around GMOs exists [Tsourekas 2023].	Ensure everyone participating in the experiment is not against the usage of GMOs.

Table 4: Table of Ethical, Environmental, and Social Concerns

RESULTS

Raw Data

Amount of banana flavouring (mL)	Mass of Curd produced (g)		
	Trial 1	Trial 2	Trial 3
0	185.50	158.93	164.96
2	151.72	179.67	144.94
4	138.97	138.40	134.68
6	145.59	159.32	134.88

Table 5: Impact of Banana Flavouring on the Mass of Curd produced across 3 trials (Appendix J).

Processed

Amount of banana flavouring (mL)	Average Amount of Curd Produced (g)	Range (g)
0	169.80	26.57
2	158.78	34.73
4	137.35	4.29
6	146.60	24.44

Table 6: Average Amount of Curd Produced and the range of results for each mL of Banana Flavouring.

ANALYSIS

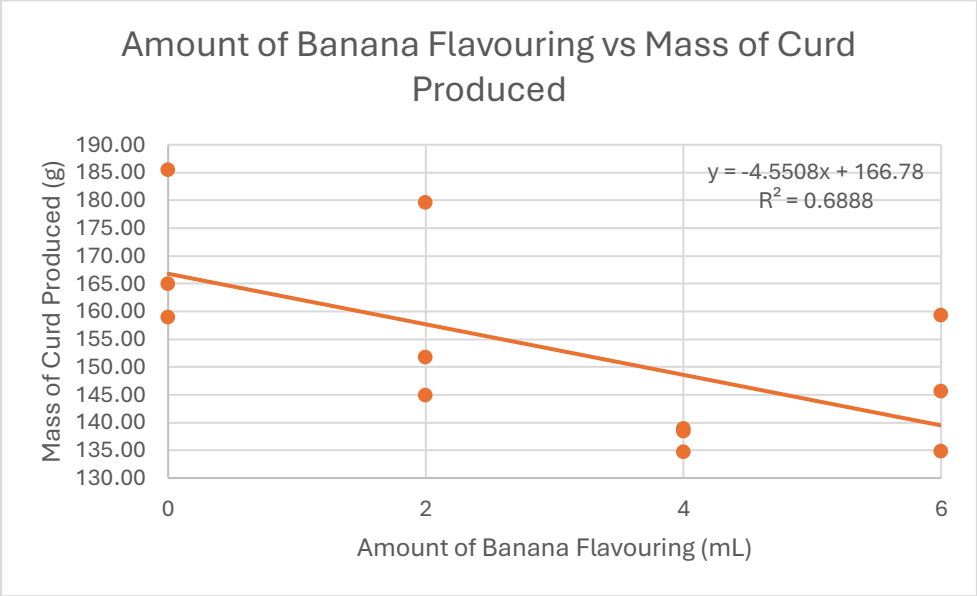

Figure 3: Calibration graph of the Amount of Banana Favouring vs the Mass of Curd Produced using the raw data.

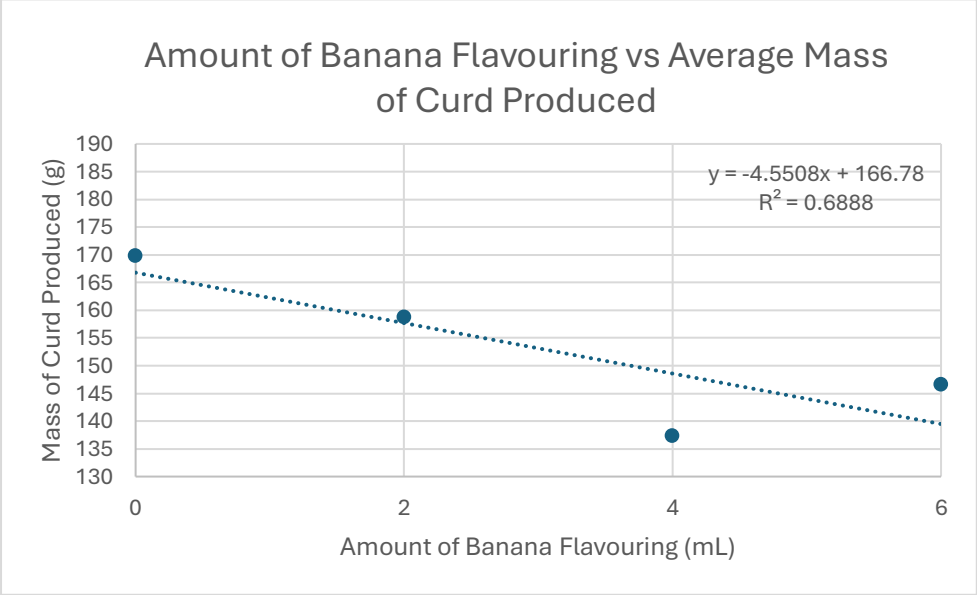

Figure 4: Calibration graph of the Amount of Banana Favouring vs the Mass of Curd Produced using the mean data.

The volume of banana flavouring in the milk during the curding process directly affects the mass of the curds produced. As seen in Figures 3 & 4, the mass of curd produced decreases as the volume of banana flavouring increases. This is potentially due to stevioside inhibiting either chymosin or pepsin. This would occur if stevioside binds to an allosteric site, which would decrease the enzyme availability, hence decreasing the reaction rate.

$$100 = -4.5508x + 166.78$$
$$-66.78 = -4.5508x$$
$$x \approx 14.67\text{mL}$$

Figure 5: Using the line equation to determine the maximum banana flavouring volume that produces 100g or more.

To determine the extent of banana flavouring that must be added to produce at least 100g of curd, the line equation can be used by changing the y value to be equal to 100, as seen in Figure 5. Through this method, it can be determined that approximately 14.67mL of banana flavouring is the limit before the mass of curd produced fall below sufficient levels.

EVALUATION

Precision/Reliability

The precision of this experiment was measured by determining the different ranges of each amount of flavouring; 4mL had the lowest range of 4.29g, with high precision. 2mL had a range of 34.73g, as seen in Table 6 indicating lower precision, indicating random errors affected the experiment. Despite multiple trials and averages mitigating the effect of errors, the results have low reliability given the larger scatter. Furthermore, the r^2 value gives an estimation of reliability, with 1 being the most reliable [Turney 2023]. Given this value is 0.6888, the results are not reliable.

Random Error	Description	Effect on Data	Future Improvements
Parallax error	Parallax error occurs when measuring liquids using pipettes or flasks, as the meniscus of the liquid may be measured from different angles, leading to different volumes of substrates [Helmenstine 2024].	True volume is different to the experimental volume of substrates, which can both increase/decrease the mass of curd produced.	Have a set viewing level using a camera or similar so the meniscus of the liquids will always be measured from the same point.
Heat dissipation	The lid was placed on the 2L pot slightly differently every time, changing the speed of heat dissipation during the curdling process.	Some trials would lose heat faster than others, decreasing the mass of curd produced, as the lower temperature will reduce the kinetic energy of the molecules, decreasing reaction rate during the curding process and vice versa.	Using a pot lid specifically made for the pot used in the experiment would reduce the variation in the pot lid placements, as the lid will fit onto the pot better.
Curd shape	Since the curds have different shapes, the areas of liquid will be different, resulting in the draining speed of the curds being different.	The drainage speed of the curds will be different, increasing/decreasing the final mass as the mass of liquid will be different for the different tests.	Use a curd knife with multiple blades to reduce the variation in the curd shape to have less variation in the drainage speeds [Glengarry Cheesemaking 2025].

Table 7: Table of Random Errors that may have impacted the precision of this experiment.

Accuracy

As there are no true values, given this experiment has not previously been conducted, the accuracy of this experiment cannot be truthfully determined. However, systematic errors may have affected the accuracy of results.

Systematic Error	Description	Effect on Data	Future Improvements
Uncalibrated thermometer	The thermometer used is calibrated for meat, which uses a higher temperature than cheese, leading to inaccuracy in the thermometer.	This causes all tests to be measured at a higher temperature than their true temperature, increasing the denaturing of the enzymes, increasing the mass of curd produced, and vice versa.	Calibrate the thermometer by measuring the temperature of ice (0˚C) and boiling water (100˚C) to ensure the thermometer reads correct values [Queensland Government 2021].
Variation in milk batches	The milk used was bought from different batches, which could have different amounts of protein and specific enzymes than other milk batches.	This would increase the mass of curd produced if more protein and enzymes are present, as more maximum curd can be produced, and vice versa.	Use milk from one batch to ensure the milk has a similar amount of protein and enzymes.

Table 8: Table of Systematic Errors that may have impacted the accuracy of this experiment.

Validity

This experiment provides an answer to the research question, with the specific amount of banana flavouring needed to produce 100g of curds found using the line equation. However, the validity of this inquiry is low due to the large range, which provides evidence that random errors have occurred given the poor r^2 value. This indicates that multiple random variables could have impacted the mass of curd produced, hence invalidating the experiment.

CONCLUSION

From extrapolation of the trendline from Figure _, to produce 100g of curds using the mozzarella curding process, 14.67mL of stevia banana flavouring can be added. However, with limited equipment, this cannot be tested, as a volume of 14.67mL cannot be easily obtained. Furthermore, the low reliability of the experiment suggests if 14.67mL of banana flavouring was to be used, 100g of curd would not be produced, as random error would likely occur, changing the amount of banana flavouring that would give 100g of curd. Furthermore, many limitations are present in this experiment. Due the availability, only stevia banana flavouring was tested, therefore, if a different flavouring was used, different results would likely be produced. Furthermore, pasteurised milk was used instead of unpasteurised. Unpasteurised is preferred for cheesemaking, as the milk is less processed, so if unpasteurised milk was to be used, the results would likely be higher, further affecting the amount of banana flavouring that can be added to produce 100g of curd through the mozzarella curding process.

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APPENDICIES

Appendix A

Photo of pots and lids

Photo of cheese cloths

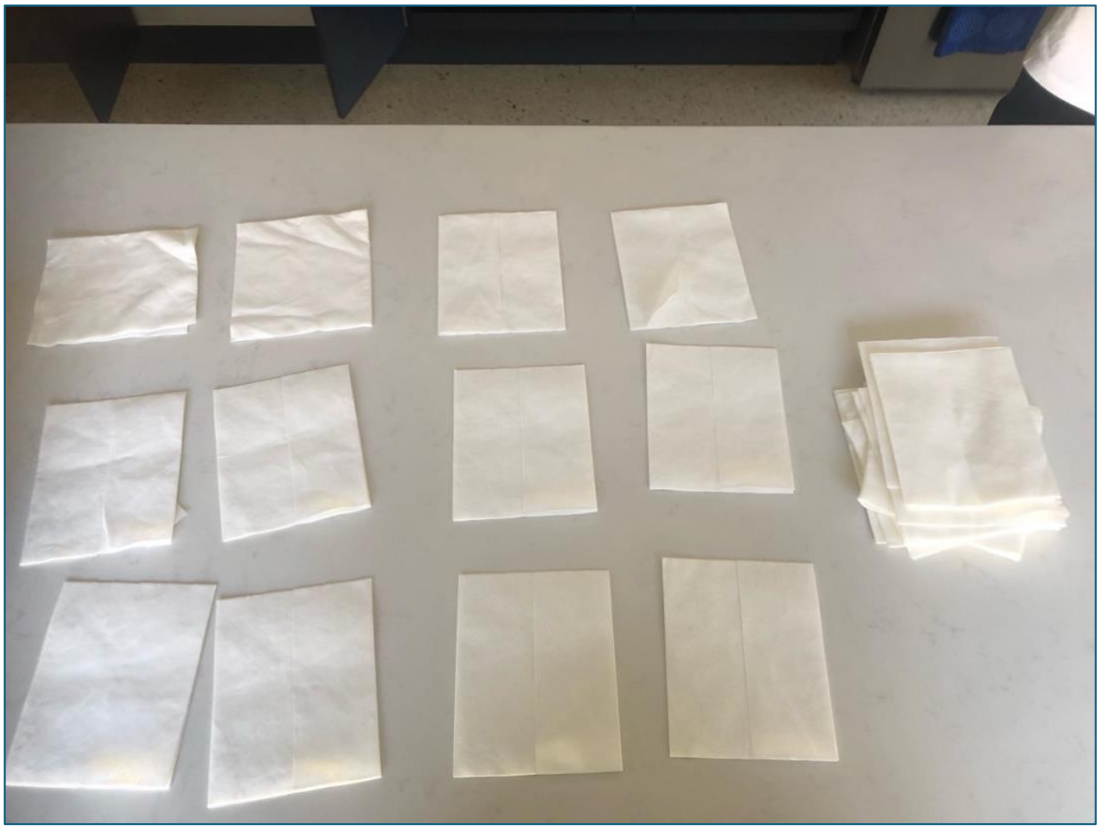


Photo of thermometer

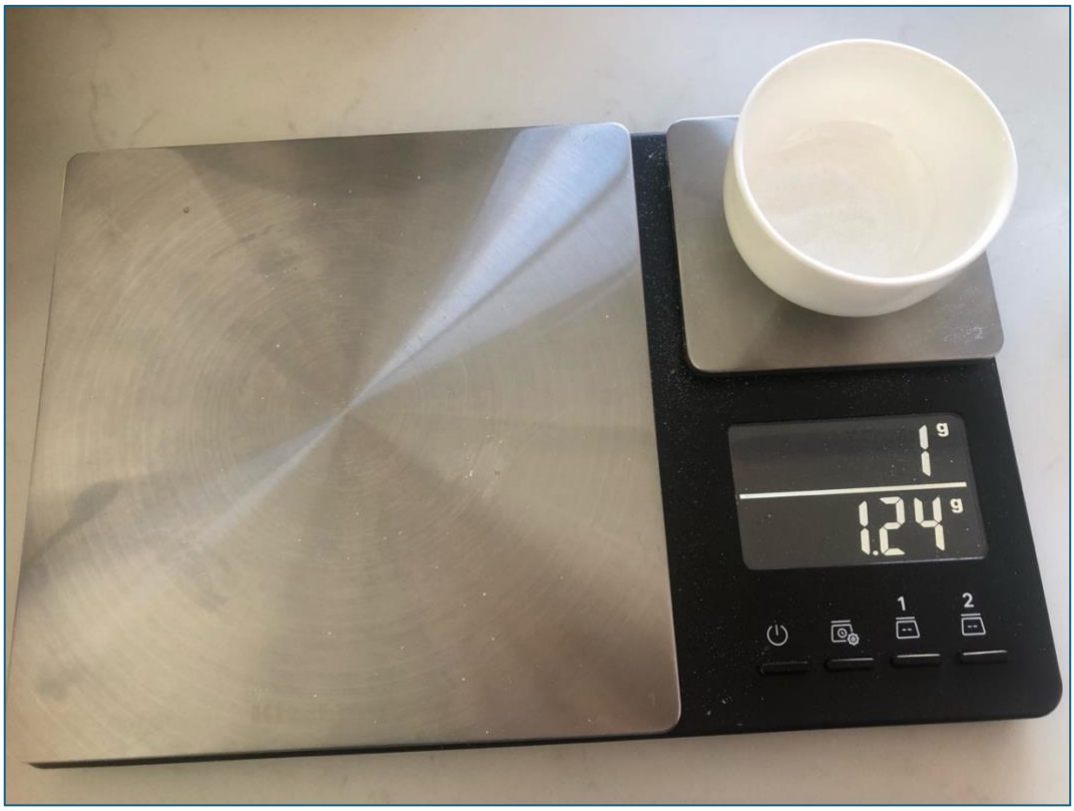
Photo of Rennet

Photo of Banana flavour

Photo of measuring equipment

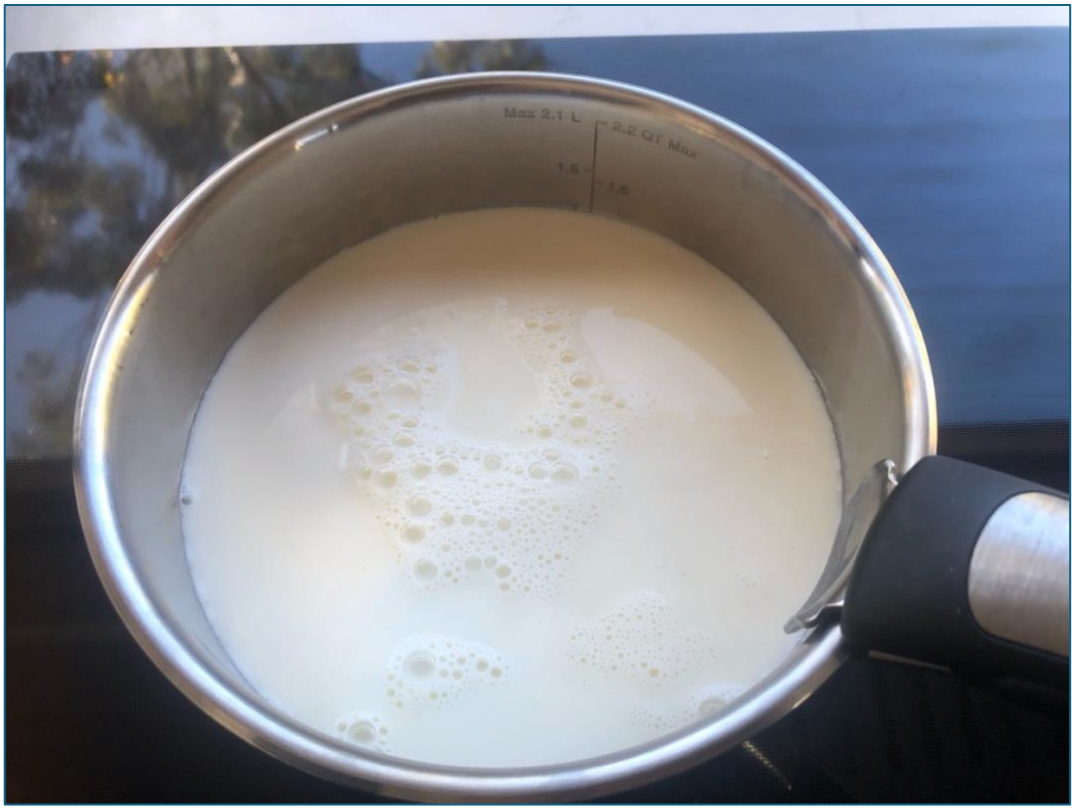
Appendix B

Photo of measurement of citric acid



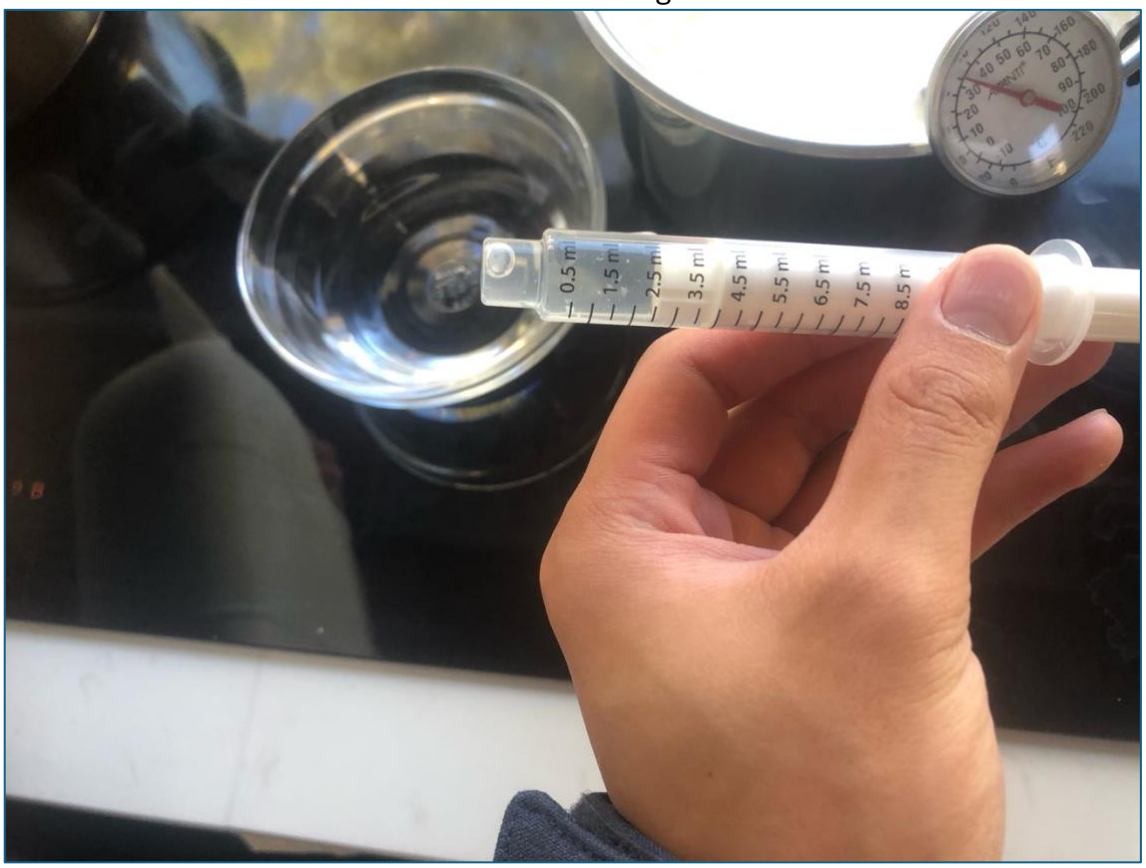
Appendix C

Photo of milk and diluted citric acid mixture



Appendix D

Photo of 4mL measurement of banana flavouring



Appendix E

Photo of milk mixture heating setup

Appendix F

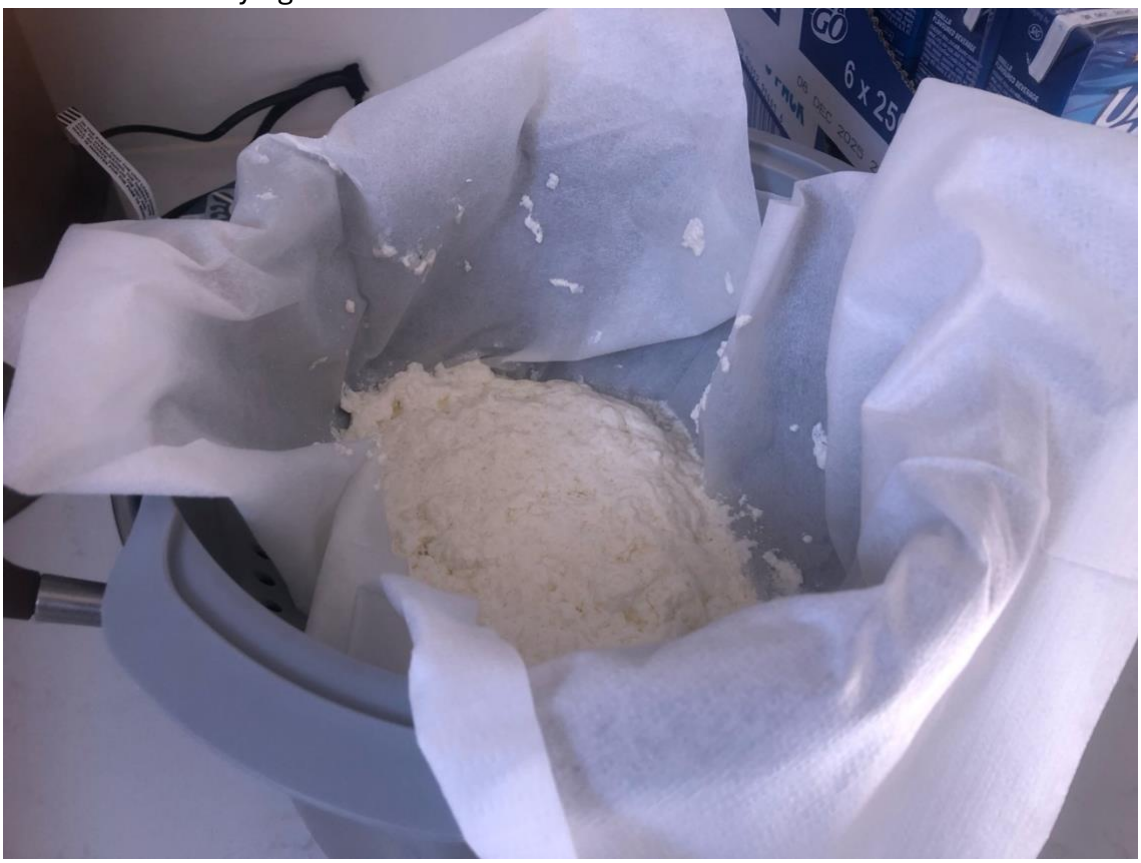
Photo of measurement of 1mL of rennet

Appendix G

Photo of curd knife being used to cut 2cm curds.

Appendix H

Photo of curds drying



Appendix I

Photo of excess water being removed from curds

Appendix J

Photo of curd mass from 0mL trial 1

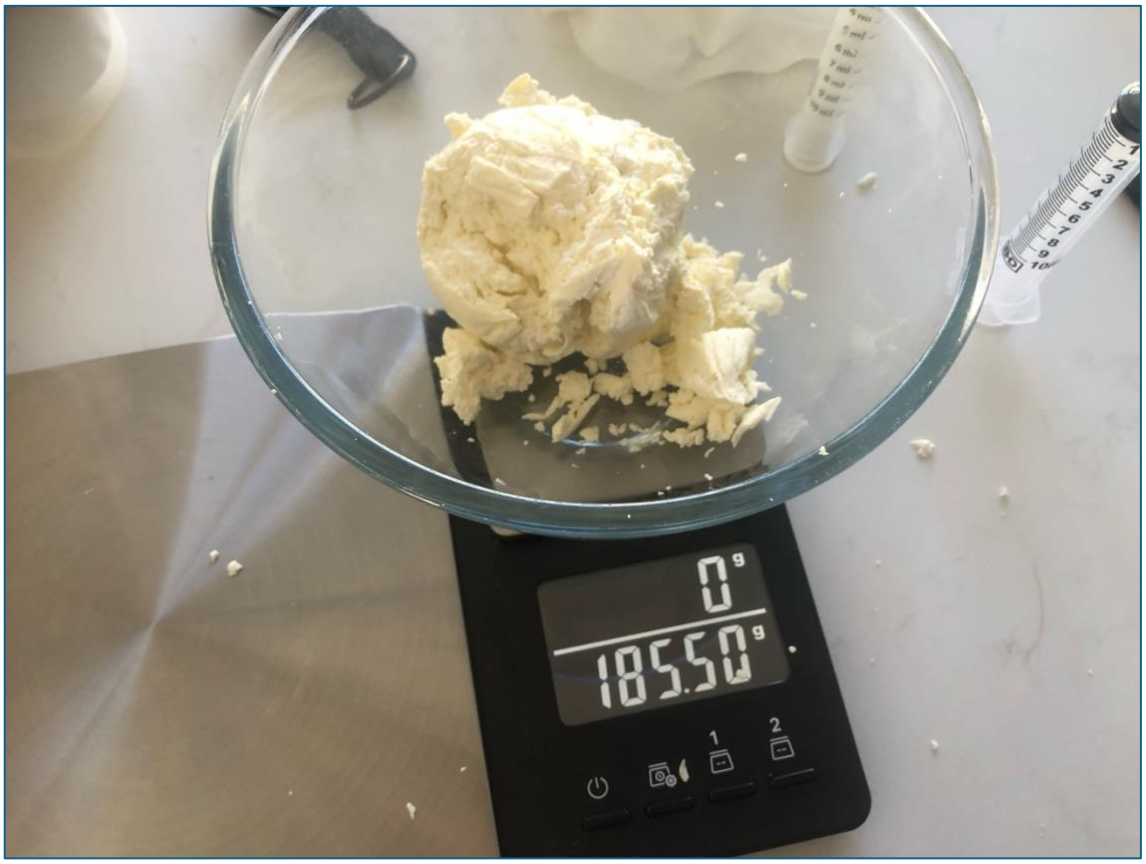


Photo of curd mass from 0mL trial 2

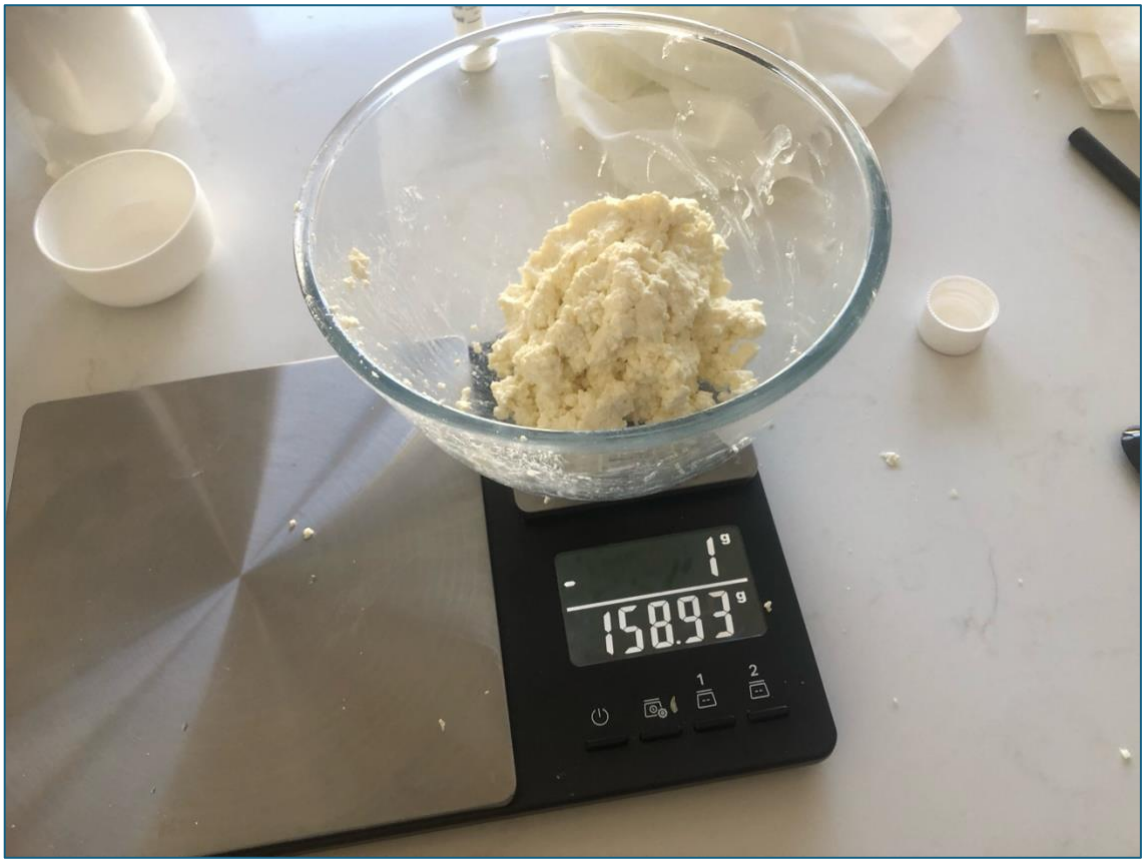


Photo of curd mass from 0mL trial 3

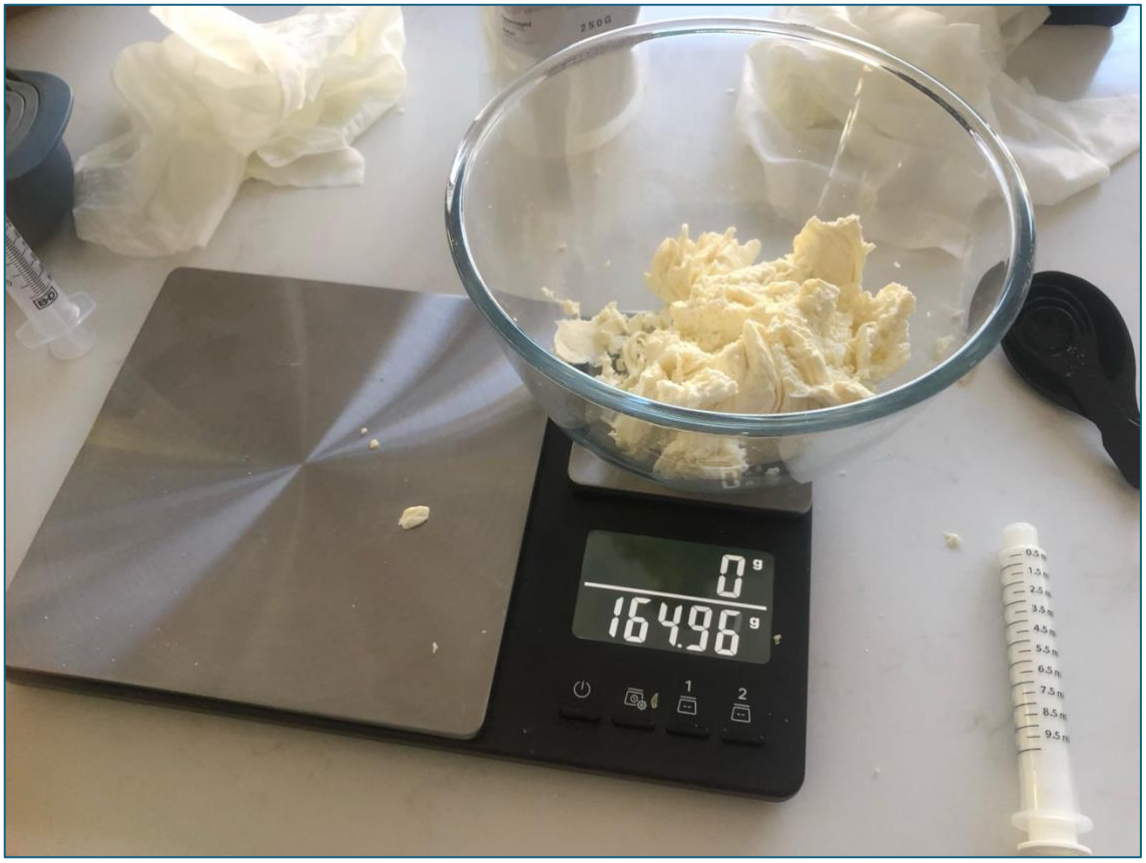


Photo of curd mass from 2mL trial 1

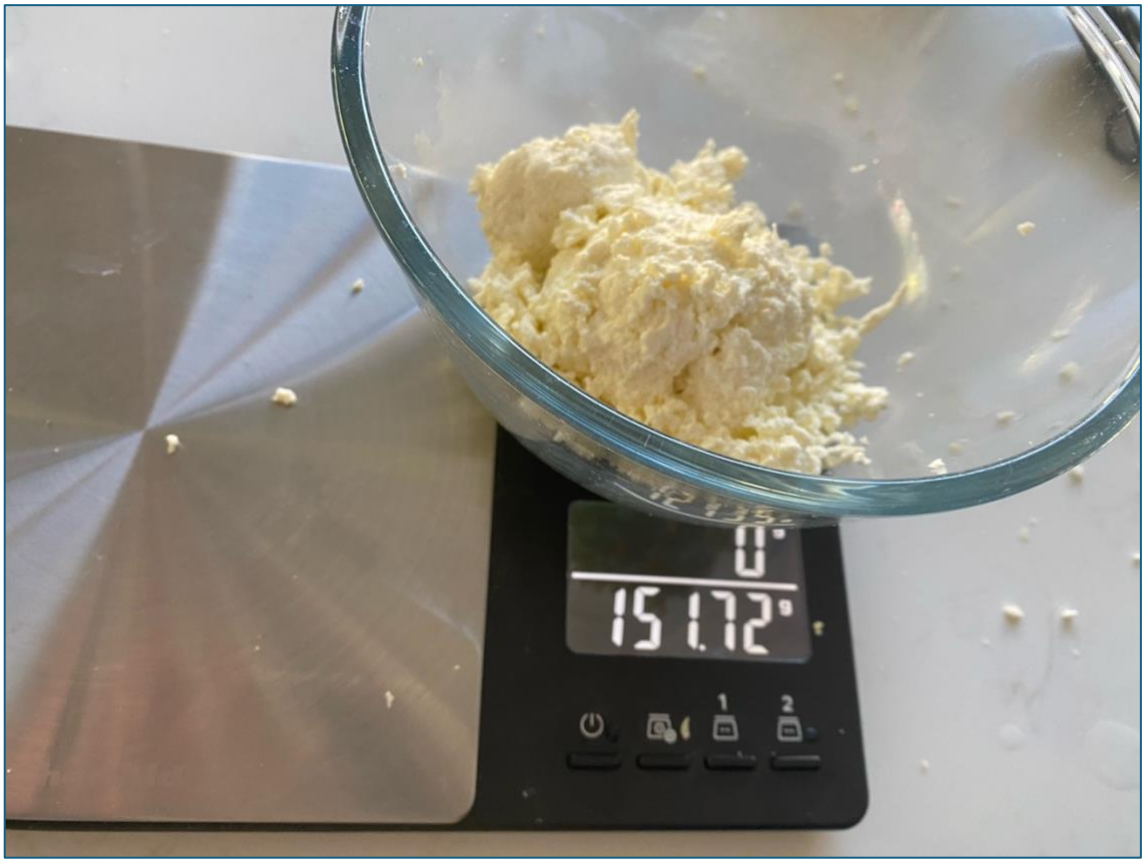


Photo of curd mass from 2mL trial 2

Photo of curd mass from 2mL trial 3

Photo of curd mass from 4mL trial 1

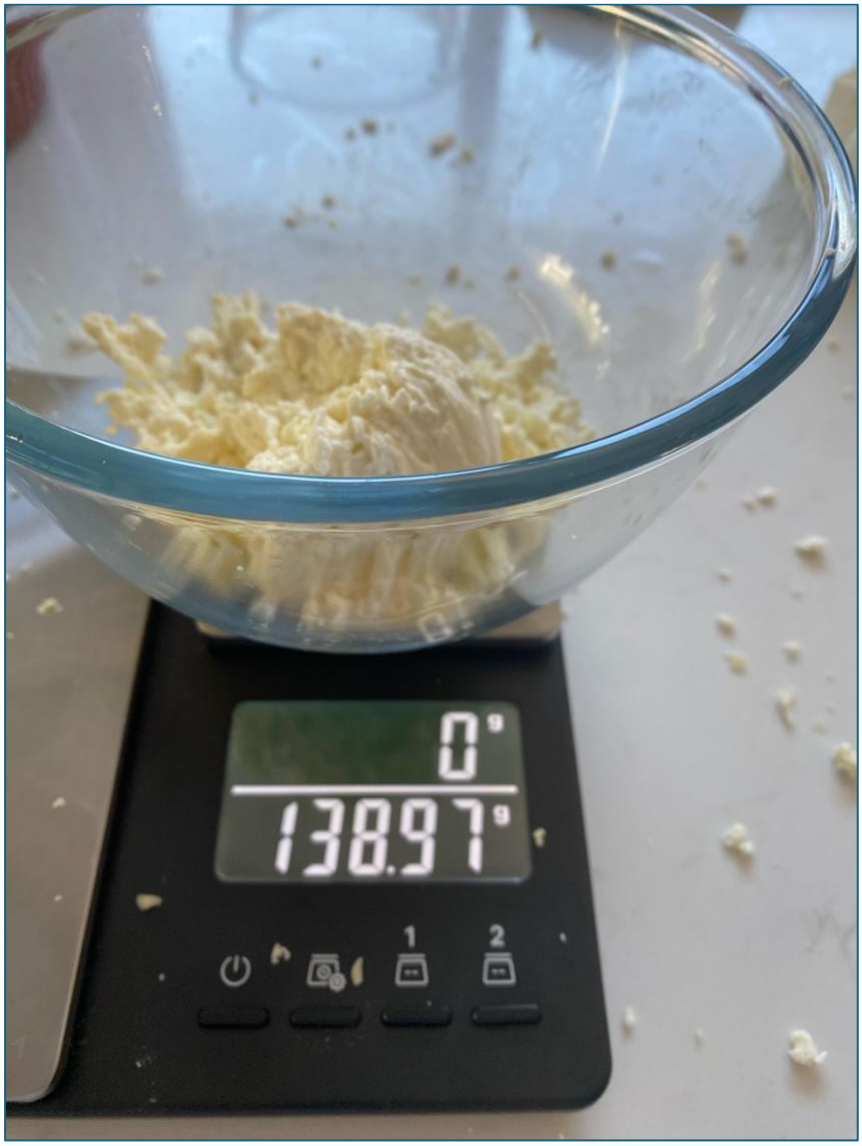


Photo of curd mass from 4mL trial 2

Photo of curd mass from 4mL trial 3

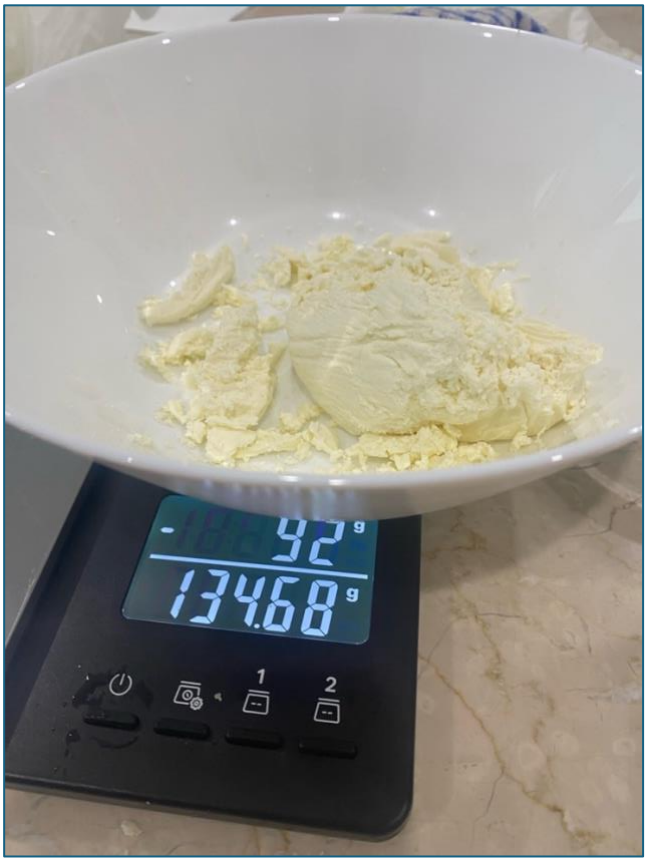


Photo of curd mass from 6mL trial 1

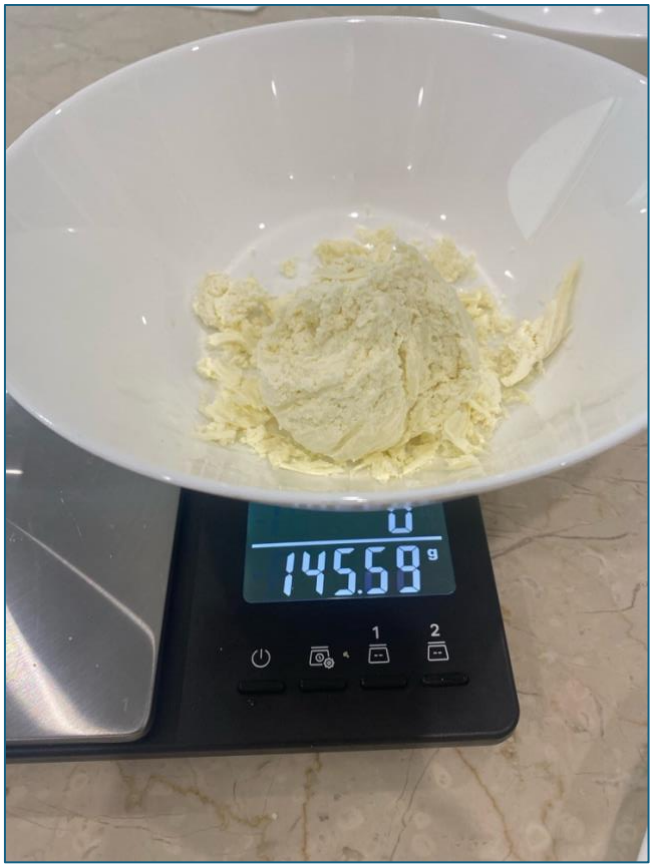


Photo of curd mass from 6mL trial 2

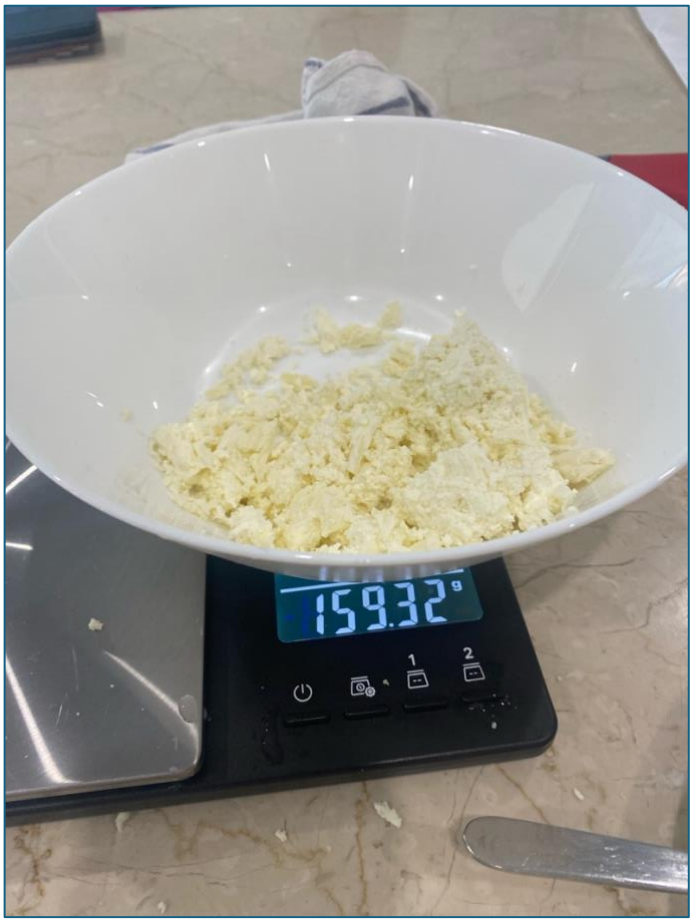
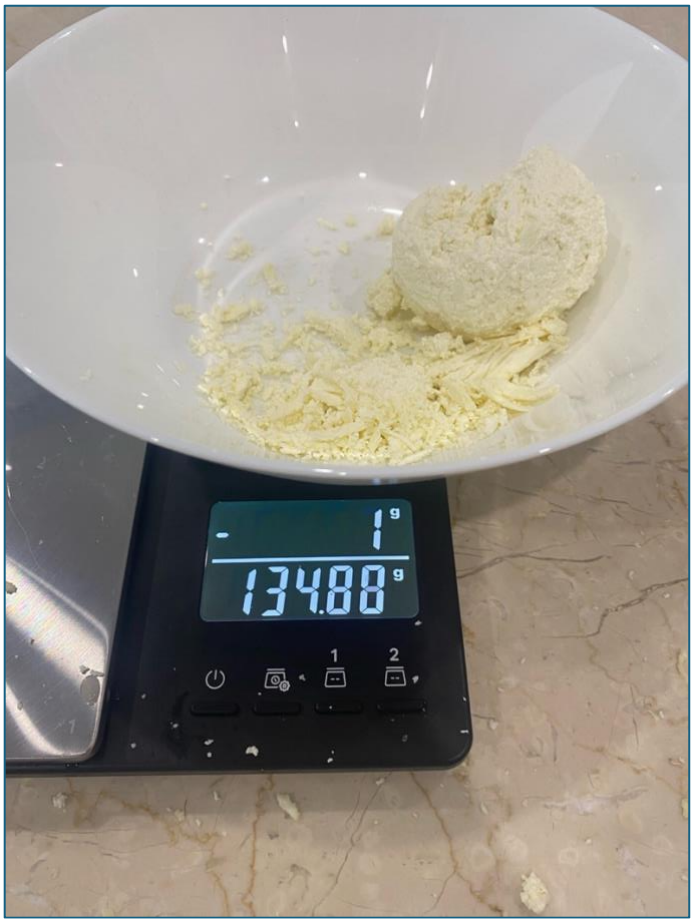


Photo of curd mass from 6mL trial 3



16/3/2024

We came up with the idea of making banana flavoured cheese and testing the effect of banana flavouring on the cheese product. This would use a brie recipe, as it was the most appealing to us. We then bought equipment and made plans to hold a test experiment to see if this was the right option for us.

19/5/2024

We did the experiment for the brie. The brie initially takes a long time to do, as the process requires flipping the hoop over multiple hours, causing us to wake up during the night to do it. Given how long it takes, we only managed to do the control and a couple of banana flavoured cheeses. Mainly for this reason, and the fact that brie uses mould, we likely won't use brie going forward.

20/3/2025

We have agreed to do try this experiment again, however being more valid and using stevia flavouring instead. We'll also do fast mozzarella as we are very time poor with schoolwork, particularly AIF. This will allow us to hopefully do most of the testing in one day.

10/5/2025

We conducted our first round of mozzarella testing. We were able to complete the 0mL tests, 2mL tests, and one 4mL test. The process was similar to making brie but shorter. Instead of needing to wait for the curds to form, the faster process let us complete more tests. The 2mL test curds had a banana smell which was expected, but we didn't expect it to be so strong with only 2mL. We found our expected trend of increasing banana flavouring decreasing the mass of curd to be correct, as the 2 and 4mL tests were lower than the 0mL tests. The 4mL had a very strong banana smell but had less curd and the curds were also weaker.

1/6/2025

We conducted our second round of testing, including the final two 4mL tests and the 6mL tests. The 6mL tests had surprisingly high results, but this could have been because we had done multiple tests and were getting better at the process. The 6mL tests smelled much more of banana than the previous, and the curds were softer and easier to break than the other tests, probably because they had a more incomplete curding process, leading to a weaker structure. The 4mL tests were mostly the same as the other 4mL test and the other tests, with a weaker banana smell than the 6mL tests.

18/6/2025

We've started our write up, making a calibration graph and the results, and doing research to explain the results we got. We are going to try to finish the report soon, but we are more worried about reaching the wordcount rather than reducing it.

22/6/2025

We have finished the report fully. It's well below the wordcount, but we're pretty happy with how its written and especially how it links to biochemistry. Banana cheese started as a dumb idea of ours, so to see it before an actual practical with understandable science behind it was fantastic. This has been really fun doing, so we're so happy its finally done.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: **Joshua Kirsten & Alexander Lee** ID:_____

SCHOOL: **Scotch College Adelaide**

Activity: Give a brief outline of what you are planning to do.

We are planning to test the impact of food flavouring esters on the formation of mozzarella cheese when added to the milk during the curding process.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Heat burns from using a stove	Wearing PPE such as aprons, gloves, enclosed shoes etc.
Food Poisoning	Consuming food may have unanticipated health effects, hence the cheese shall not be ingested.
Bacterial Infection	Infection, sanitize when interacting with bacteria.
Curd Knife laceration	Properly handle the knife and wear PPE.

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): **Joshua Kirsten & Alexander Lee**

SIGNATURE(S): _____

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Owen Osborne

SIGNATURE: _____ DATE: 18/3/25