Casein Concentration of Mammalian Milk

Aim: To compare the casein concentration of mammalian milk

Background

Milk is a rich source of protein. The major protein constituents are whey and casein; in bovine milk, casein proteins (α, β, γ, and κ) account for 80% of milk proteins. The casein proteins are found as self-assembled particles called “micelles”. This practical activity allows learners to investigate the mass of casein protein in mammalian milk. Prior to this practical work, a bioinformatics approach to learning more about this protein group is beneficial.

This protocol involves 4 main stages:

1. Precipitation of casein from milk using its isoelectric point – to isolate casein (this will still have fat molecules adhered to the protein)
2. Centrifugation of the precipitated protein (which will help remove salts, some fat and other proteins from casein)
3. Resuspension in an ethanol/ethyl ether mix (to dissolve fat)
4. Vacuum filtration to remove excess liquid (to isolate the fat from the casein protein)

STAGE 1: Precipitation of casein from milk

Materials (per pair)

10 cm^3 syringe	Universal of pH4 buffer
Milk (cow, sheep, goat) - 40°C	Universal of pH7 buffer
100 cm^3 beaker	Wash bottle of distilled water
Clamp stand	Discard tub
Pasco pH sensor	Magnetic flea
Magnetic stirrer	Glacial ethanoic acid (1:20 dilution)
1cm^3 plastic pipette	Water bath at 40° C

Method

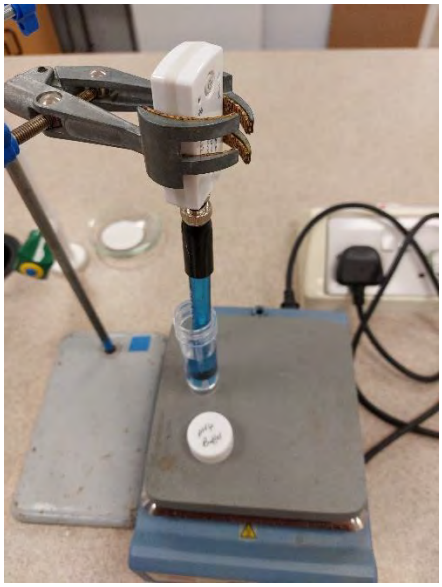
1. Pre-incubate a container of milk at 40°C in a waterbath.

2. Mount the pH sensor in the clamp stand and follow the manufacturer’s instructions to calibrate at pH4 and pH7.

3. Using the syringe, add 20ml milk to the beaker.

4. Add a magnetic flea to the beaker and use the magnetic stirrer to continuously stir the contents.

5. Lower the pH sensor into the milk and monitor the pH of the solution.



6. Using a 1ml plastic pipette, add ethanoic acid (dropwise) to the solution until pH 4.6 is achieved (this is the IEP of casein). At this point, precipitated casein should be observed.

STAGE 2: Centrifugation of milk suspension

Materials (per pair):

- Centrifuge
- 2x centrifuge tubes (in a rack)
- 10 cm^3 syringe (from Stage 1)
- Beaker of milk containing precipitated casein (from Stage 1)
- 3 cm^3 plastic pipette
- Discard tub

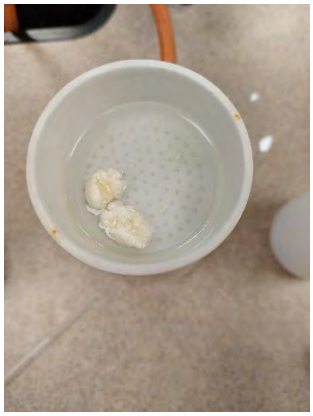
Method

1. Use the 10 cm^3 syringe to divide the suspension (from Stage 1) between 2 centrifuge tubes. The tubes must be balanced.

2. Centrifuge at 6000 rpm for 5 minutes.

3. Use a plastic pipette to carefully remove and discard the supernatant.

The pellet will be transferred to a Buchner funnel for vacuum filtration, but fat molecules need to be removed. The pellet must be washed with a 50:50 mix of ethyl ether/ethanol (see Stage 3).



<u>STAGE 3: Washing the pellet to remove fats</u>

Materials (per pair):

- 3 cm^3 Plastic pipette
- 50:50 mix of ethyl ether and ethanol

Method

1. Use a plastic pipette to resuspend the pellet with ethyl ether / ethanol mix. The pellet will not fully resuspend but use the smallest volume possible to give

the centrifuge tube and pellet a thorough wash with the ethyl ether / ethanol mix. This will dissolve the fat particles.

STAGE 4: Vacuum filtration to dry the casein protein

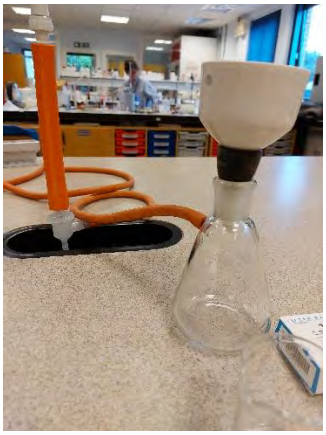
Materials (per pair)

- 50mm Grade 1 Whatman filter paper
- Buchner funnel
- Cork ring
- 1-armed 250 cm^3 conical flask
- Vacuum filtration tubing
- Access to a tap and sink
- Balance
- 50:50 mix of ethyl ether and ethanol
- Forceps
- Watch glass
- Incubator at 40°C.
- 10 cm^3 syringe

Method

1. Measure and record the mass of a 50mm piece of Whatman 1 filter paper.

2. Set up the Buchner funnel for vacuum filtration and place the filter paper in the funnel.



3. Transfer the partially resuspended contents of the centrifuge tubes into the Buchner funnel.

4. Use the 50:50 ethyl ether:ethanol mix to transfer any remaining washings from the centrifuge tubes to the filter paper.

5. Run the vacuum filtration to dry the protein.

6. Use the syringe to transfer 10 cm^3 of ethyl ether:ethanol mix to the Buchner funnel for a final wash.

7. Once all the liquid has filtered through into the conical flask, use forceps to transfer the filter paper to the watch glass.

8. Place the watch glass in an oven/incubator at 40°C overnight.

9. The next day, record the mass of the dried filter paper + casein. Use a balance to 2 or 3 decimal places. Calculate the mass of casein extracted from the 20 cm^3 of milk. Record the mass of casein per litre of milk.

Results – the following results have been obtained at SSERC

Milk type	Mass of filter paper only (g)	Mass of filter paper + dried casein (g)	Mass of casein in 20cm^3 milk (g)	Mass of casein in per litre of milk (g / L)
Cow 1	0.22	0.89	0.67	34
Cow 2	0.22	1.00	0.78	39
Cow 3	0.22	0.82	0.6	30
Cow 4	0.22	0.99	0.77	39

This experimentally derived value is in line with the published value of 30 g casein/L bovine milk (Davoodi, S. H. (2016), Vincent, D. (2016)).

References

Davoodi, S. H., *et al.* (2016), Health-related aspects of milk proteins, Iran Journal Pharmacology Research, 15(3): 573-591.

Vincent, D., *et al*. (2016), Quantitation and identification of intact major milk proteins for High-throughput LC-ESI-Q-TOF MS analyses, available here: https://doi.org/10.1371/journal.pone.0163471