**Quantitative food test: protein content of powdered milk**

**Learning Objectives**:

1. To develop practical skills **a, c, l** and begin to demonstrate **competencies 1a and 2b**
2. To construct a calibration curve
3. Use the calibration curve to estimate the concentration of protein in a sample of milk powder.

Casein is a protein found in milk. Neutrase is a bacterial enzyme, a protease, produced by a selected strain of Bacillus amyloliquefaciens. It will break down proteins to peptides. When Neutrase is added to a dilute solution of milk powder, the casein is digested and the solution goes clear.

**Method**

You are provided with the following:

Test tube rack

Test tubes

Beaker of Neutrase™ solution, 40 cm3 of a 1% dilution 5 cm3 Syringe

Marker pen

Stopclock/ stopwatch

Milk powder solutions at concentrations of 1%, 2%, 3%, 4% and 5% protein, 10 cm3 per working group, in beakers with 10 cm3 syringes

Milk powder solutions at two other concentrations within this range, known to staff but not revealed to students, labelled X and Y

Enzyme solution, such as Neutrase™, at a 1% dilution, 40 cm3 per working group (**Note 2**)

**Health & Safety and Technical notes**

Neutrase™ is a protease – so wear eye protection when handling and wash splashes off the skin immediately.

**Procedure**

SAFETY: Wear eye protection when handling the Neutrase™ solution. Powdered enzymes can cause irritation by inhalation and sensitisation – so work in a fume cupboard if making up solutions from powder, and clear up spills of solutions immediately.

**Investigation**

**a** Set out 7 test tubes and label them 1%, 2%, 3%, 4%, 5%, X and Y.

**b** To each tube, add 10 cm3 of the appropriate milk powder solution.

**c** Add 5 cm3 of enzyme solution to the first tube (1%). Squirting it in can help to make the mixing rapid, but may result in splashing. Shake to mix thoroughly, and start the stopclock.

**d** Record the time it takes for the solution to become clear. Mark a cross on a piece of white paper, view the cross through the mixture, and record the time it takes for the cross to become visible.

**e** Repeat with the other concentrations of milk powder, both known and unknown.

**f** Identify any really anomalous results, and repeat if time. If repeating, use clean glassware, as it is difficult to rinse the enzyme fully from the tubes. If clean glassware is not available, add the enzyme to the tube first and start the clock on adding the milk.

**g** Add each group’s results to the class set and discuss.

**h** Plot two graphs of time taken for the solution to clear against concentration for the known concentrations. On one graph, plot your own results, and on the other plot the class average as calculated after discussion.

**i** Use these calibration curves to estimate the concentration of protein in solutions X and Y.

**j** Collate estimates from each group to a set of class data, and discuss the variations.

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|  | Apparatus and techniques |
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| AT a | use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH) |
| Competencies |
| 1. Follows written procedures | a. Correctly follows instructions to carry out experimental techniques or procedures. |
| 2. Applies investigative approaches and methods when using instruments and equipment | b. Carries out techniques or procedures methodically, in sequence and in combination, identifying practical issues and making adjustments when necessary.  |

This is a simple protocol allowing students to construct a calibration curve from which to estimate the concentration of protein in a sample of milk powder. It allows discussion of accuracy, error and reliability. It also allows discussion of the difference between quantitative and qualitative tests, and the commercial applications of analytical techniques like this.

**Lesson organisation**

The basic technique is relatively simple. You can adjust the quantity of enzyme used to ensure that no solution takes more than two minutes to clear. In this version, each group has 5 standard solutions and 2 unknowns to test, so the practical element should take no more than 20 minutes. If you have only one colorimeter, select one group to work with that and compare their results to those of other groups. Let each group acquire a set of results, and then collate the class data to a spreadsheet visible to all.

**Teaching notes**

This practical is an opportunity for a quantitative test of a foodstuff – to compare with more familiar qualitative tests. Although it is relatively simple, it offers scope for discussion of accuracy, error and reliability. It also offers an example of a situation where using a specialised piece of equipment (a colorimeter) would establish the end-point of the reaction more easily.

The spreadsheet [Quantitative assay of milk powders – sample results (31 KB)](http://www.nuffieldfoundation.org/sites/default/files/PB_uantitative-assay-of-milk-powders-sample-results.xls) [2] shows the results obtained by a class of BTEC students working on this protocol in spring 2009. It provides quite a range of results! This is because of variation in techniques of mixing the two solutions together, and of deciding the endpoint. Some groups agitated the mixture steadily during the course of the reaction; others left it alone in the test tube rack.

From each row, anomalous results have been removed. Results were classified as anomalous if they did not follow the trend of taking more time to digest and clarify with greater concentration of protein. The class average result is shown on the graph on the second sheet of the spreadsheet in a heavier dark blue.

Reading from the class average calibration curve, the concentrations of the two mystery solutions of protein are X = 1%, Y= 4%. This is accurate for X, but not for Y which was, in reality, a 2.5% solution.

All the groups produced a set of results showing the trend: more protein takes longer to clear. The students agreed that it is difficult to mix the enzyme and milk solutions quickly, difficult to work out exactly when to start the clock, and difficult to decide the end-point of the reaction. All these elements of the procedure introduce experimental errors which are worth discussion.

An 'error' in this situation is not the same thing as a 'mistake', but the inconsistencies introduce variations into the results across the group. After some practice, any individual practitioner will work out a routine giv9ng their own personal data set an internal consistency. However, another individual working with the same materials could get a different set of results. If each practitioner is consistent within their own procedure, the results will show a 'systematic error' – with one set consistently different from the other by the same amount (in this case, the same number of seconds). If one practitioner is consistent and the other is inconsistent, the results will show a 'random error', with an unpredictable variation between the two data sets. This is why it is important for people working in laboratories to repeat practical procedures until the results are consistent, and why technicians should prepare their own calibration curves whenever possible, rather than rely on a standard data set.

The difficulty of deciding the endpoint also introduces a degree of inaccuracy within the results of each working group. The students became more confident about declaring the end point as the practical progressed, so they knew that they had probably called 'Stop' at a slightly different point each time.

Despite these variations, the students could see that the practical is broadly reliable. Given time to repeat the procedure and develop a consistent technique, with a definite measure of endpoint, they could see that the procedure could give repeatable results.

The basic method could be modified to become an investigation of the enzyme activity – using different concentrations of enzyme, or observing the activity at different temperatures, or observing the effect of prior heat treatment of the enzyme at different temperatures. Temperatures from 40 °C to 50 °C will denature the enzyme and reduce its activity.

[Read our standard health & safety guidance](http://www.nuffieldfoundation.org/node/1634/) [1]

**1** Marvel® seems to be the best powdered milk to use for this procedure, but some other brands may work. Most other powdered milks contain more fat which confuses the result, and/or whiteners or other proteins which are not broken down. Use the data on the packet about the percentage of protein to calculate how much powder to use to make your standard solutions. Set out each beaker of milk solution with 3 or 4 syringes (10 cm3) to make dispensing milk speedy and accurate.

**2** Carry out a trial run to check that the concentration and quantity of enzyme you are using digests the most concentrated milk solution in about 2 minutes. Neutrase™ is recommended, but trypsin and pancreatin are reported to work for this practical too. When handling proteases, wear eye protection, wash any splashes off the skin, and wipe up any spills immediately.