# Enzyme preparation for experiments.

Posted by Anonymous on Thu, 2015-05-07 12:08

Enzyme preparation for experiments: Could you provide guidance in the correct preparation of enzymes such as trypsin, pepsin and amylase for enzyme digestion practical activities? Furthermore, can you make suggestions for the investigation on the effects of pH changes on enzyme activity, please?

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No votes yet

**Year Level:**
Senior Secondary
**Laboratory Technicians:**
Laboratory Technicians

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# Enzyme preparation for experiments.

Submitted by sat on 12 May 2015

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Firstly, please read and be familiar with the “General Safety Precautions when using Enzymes” below prior to preparing enzyme solutions. Enzymes in powder form are considered hazardous substances.[1,2,3] However, in dilute aqueous concentrations, they are a low hazard.

It is important to understand that enzymes, when dissolved into solution, are much less stable

than in powder form and lose their activity quickly. Therefore, it is best to prepare only what is required, and only just before use. Enzymes in powder form should be stored in the fridge (4° C) unless otherwise specified. Diluted solutions can be stored in the fridge but should be used within an hour or two of preparation and be kept on ice during an experiment.

## General safety precautions when handling enzymes:

Safe handling of enzyme preparations can be accomplished through proper work practices, engineering controls, and use of personal protective equipment.

Note: Enzymes are biologically active proteins. It is advised to avoid inhalation of enzyme dust or aerosols, which can lead to sensitisation and allergic reactions. Enzymes may cause asthma and are irritating to the eyes, respiratory system, mucous membranes and skin. Always wear safety glasses and gloves. When working with powdered enzymes, wear a dust mask or work in a fume cupboard, that is not turned on, to minimise exposure to any dust. Always use practices that do not generate dust or aerosols.

Minor spills should be cleaned up immediately, without generating dust. Place waste into a labelled container for disposal via a waste contractor. Do not discharge waste into the sewer or waterways.

Science ASSIST recommends you conduct a site-specific risk assessment to assess and control the risks. You will need to determine how to safely prepare, handle and dispose of the solution. We have developed a Risk Assessment template for schools to use, see Risk Assessment Template and we have detailed information regarding enzymes in the Chemical Management Handbook for Australian Schools - Edition 3.

Enzymes are usually made up as a percentage concentration. A 0.5% to 1% w/v solution is generally suitable for enzyme digestion practicals carried out in schools. It is always best to use the lowest concentration and smallest amount possible. The optimum reaction conditions can be different for each enzyme. It should also be noted that both trypsin and amylase work optimally around a neutral pH, whilst pepsin requires a pH of 1.5–2 to be active. The protocol for the procedure that you are following should indicate the type and amount of acid required to acidify a reaction using pepsin. It may mean that an acid, in many cases hydrochloric acid, is added to the reaction, or that pepsin is made up in a dilute acidic solution instead of water.

## How enzymes work:

Enzymes are proteins that are catalysts of chemical reactions. Catalysts increase the speed of the chemical reaction but do not form part of the final product. Enzymes act on substrates to make products in a chemical reaction, and they are highly specific to the reactions they catalyse (the lock and key model).

It is always advisable to check the enzyme reaction is working as required and adjust to the conditions and concentrations if needed before any practical class. Enzyme activity is affected by concentration, temperature, pH, substrate concentration and can be affected by the age of the reagents.

## Digestive enzymes:

**Amylase** is found in saliva in the mouth (salivary amylase) and in the pancreatic juice in the pancreas (pancreatic amylase). It is an enzyme that breaks down starch into sugar. Amylase operates optimally at a pH of 6.7 to 7.0 and at 37°C.

**Pepsin** is the main gastric enzyme that digests proteins into their component peptides and amino acids. Pepsin is secreted in the stomach and operates optimally in an acidic environment around pH 1.5—2.

**Trypsin** is a pancreatic enzyme secreted in the small intestine. It digests proteins into their component peptides and amino acids. Trypsin operates optimally at a neutral or slightly alkaline environment of pH 7–9.

Protein + trypsin à amino acids

## Preparation of digestive enzymes

Here is a recipe that is suitable to prepare 100 mL of a 0.5% w/v solution of trypsin, pepsin or amylase using distilled water.

Wear PPE: safety glasses, gloves, laboratory coat, face mask or work in a fume cupboard that is not turned on to minimise exposure to dust or aerosols. If working outside a fume cupboard, make sure you are in a draft-free area.

- Weigh out 0.5 g of the enzyme.
- Add to 80 mL of distilled water at room temperature in a beaker.
- Stir gently to dissolve.
- Adjust to a final volume of 100 mL.
- Store at 4°C (fridge) for a short period of time or on ice during use.
- Do not heat or allow the solution to froth, as this will denature the enzyme.

## Investigating the effects of pH change on enzyme activity.

Using pineapple in a jelly is a good way to observe the effects of an enzyme on the setting of a jelly.

**Enzyme activity** is dependent on variables such as temperature and pH. Enzymes are generally effective within a small pH range, depending on the specific enzyme. Altering the pH to be outside the working range of an enzyme can lead to changes in the intermolecular bonds, shape and effectiveness of the enzyme[4].

**Commercial jelly** is made of gelatine, water, sugar and food colouring[5]. Gelatine is produced from the protein collagen, which is the principal constituent of connective tissues and bones in vertebrate animals[6] and is the key component for allowing jelly to set. Jelly will firm and set when prepared following the instructions on the package. The addition of an acid or a base to

the jelly during preparation may alter its ability to set.

**Fresh, uncooked pineapple** contains bromelain, a proteolytic enzyme which breaks down gelatine destroying its gelling ability. However, tinned pineapple will allow the jelly to set, as the bromelain is denatured due to the heat used in the canning process. Bromelain activity is optimal at pH 5.5 to 8[7].

**Altering the pH of a jelly** can affect any enzymes present, as well as the ability of the jelly to set, so it is important to consider this in the design of an activity. The following suggested method treats the pineapple prior to use.

## Method for investigating the effect of pH on the setting of gelatine.

The following method is adapted from Beware the Biology.[8] A site-specific risk assessment should be undertaken before proceeding with this activity.

Equipment:

- 10 disposal plastic cups
- 1 M hydrochloric acid
- 1 M sodium hydroxide
- gelatine—made up as per package for 100 mL
- fresh pineapple juice
- pH strips (0–14)
- water

Method:

- Prepare fresh pineapple juice by running pineapple through a juice extractor or slice and squeeze by hand.
- Label 5 cups for pineapple juice—pH 3J, pH 5J, pH 7J, pH 9J, pH 11J
- Label 5 cups for control—pH 3C, pH 5C, pH 7C, pH 9C, pH 11C
- Place 3 mL fresh pineapple juice in each cup labelled ‘J’.
- Adjust the pH accordingly with 1M hydrochloric acid or 1M sodium hydroxide. Check the pH with a pH strip.
- Place 3 mL water in each cup labelled ‘C’.
- Adjust the pH accordingly with 1M hydrochloric acid or 1M sodium hydroxide. Check the pH with a pH strip.
- Pour 10 mL gelatine into each cup. Mix thoroughly.
- Leave for 30–60 minutes before checking the setting of each cup.

The addition of 1 M hydrochloric acid or 1 M sodium hydroxide will alter the pH of the pineapple juice. Students should see the gelatine NOT set within the optimal range of pH 5.5-8.0 for the bromelain enzyme when fresh pineapple juice is used because the enzyme is active for those samples.

To prepare acid and base solutions, see Science ASSIST SOP Diluting concentrated hydrochloric acid and Preparing sodium hydroxide solutions.

## References


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[3] Chem-Supply. (2019, November). *Trypsin*, Safety Data Sheet. Search https://shop.chemsupply.com.au/ to source the latest Safety Data Sheet via the product information page.

[4] Royal Society of Biology. (2013- 2022). *Chemistry for Biologists (Enzymes).* Retrieved from Royal Society of Biology: https://www.rsb.org.uk/education/teaching-resources/secondary-schools/chemistry-for-biologists

[5] Brinson, L.C. (2022, May 20). *What exactly is Jell-O made from?* Retrieved from How Stuff Works: https://recipes.howstuffworks.com/j-ello.htm

[6] Gelatin Manufacturers Institute of America. (2012). *Technical data - The Gelatin Handbook*. Retrieved from Gelatin Manufacturers Institute of America: http://www.gelatin-gmia.com/technical-data.html

[7] Curenature.com. (2022, April). *Pineapple enzyme – Bromelain.* Retrieved (11 January 2023) from Curenature.com: https://web.archive.org/web/20200122132446/http:/www.curenature.com/2013/05/pineapple-enzyme-bromelain.html

[8] Beware the Biology. (2014, February 18). *The Effects of pH on the enzyme Bromelain (lab report).* Retrieved from Beware the Biology blog: http://bewarethebiology.blogspot.com.au/2014/02/the-effects-of-ph-on-enzyme-bromelain.html

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