PAPAIN PRODUCTION

Introduction

Enzymes are proteins that can increase the rate of biological changes such as the ripening of fruit. At the end of an enzyme catalysed reaction the enzyme itself is unchanged and is able to react again.

Enzymes can be generally recognised by the ending *-ase*. This either indicates the nature of the substance affected by the enzyme (eg carbohydrase acts on carbohydrate material and proteases acts on proteins) or to indicate the nature of the reaction eg transferases catalyse the transfer of atoms or groups of atoms within a substance.

Enzymes occur naturally in foods and many traditional food processing technologies involve the use of enzymes. Today, with more advanced knowledge of food science these enzymes can be extracted, concentrated and added to foods during processing (e.g. meat tenderisers). Table 1 describes some of the traditional technologies and the enzymes involved. It is worthwhile noting that only relatively recently has a detailed understanding of the enzyme-catalysed reactions involved in these food processing technologies become known.

Traditional food processing technologies	Enzymes used	To catalyse reaction	Reason
Breadmaking	Amalase in flour Maltase in yeast Zymase in yeast	Starch-maltose, maltose-glucose, glucose-carbon dioxide & ethanol	Produce sugars for yeast action and carbon dioxide to aerate bread
Cheese production	Rennin in rennet	Coagulation of milk protein	To help form curd
Alcoholic drink	Amylases, maltases and zymase in raw materials	Starch-maltose, maltose-glucose, glucose-carbon dioxide & ethanol	Produce sugars for yeast action and CO^2 for 'texture'
Tea and coffee	Oxidases in leaf and bean	Polymerisation of colourless phenlic compounds to brown coloured compounds	Give desirable colour and flavour to tea infusions

Table 1:

One important group of enzymes are called proteases. These are enzymes which catalyse the breakdown of proteins. Chillproofing of beer, see Table 1, tenderisation of meat and the production of dough for pizzas and batters for waffles and wafers are applications of proteases in the food processing industry. The most common of these proteases is papain.

A word of caution - there are a number of special difficulties in setting up small-scale papain production and very thorough research is needed before attempting to start such a programme. Specifically these are as follows:

- Papain is potentially dangerous - prolonged contact will damage the skin of workers' hands and in some cases it may cause allergic reactions.
- The market for papain in Europe is getting smaller as alternatives are found and it may be banned from some foods (eg beers) in the near future.
- Low grade (sun dried) papain has a much smaller market than higher quality spray dried

papain (see below). Therefore a thorough market survey should be made to ensure that the product can be sold.

- Plantations of papaya trees are needed for economical production-collection from a few trees in home gardens is not a viable activity.

Papain: source and uses

Papain is in the dried latex obtained from the papaya fruit (Carica papaya L). It is the protease which is most commonly used for the food processing applications mentioned above. However, other proteases which are important are ficin which is obtained from figs and bromelain which is tained from pineapple.

Papain is used in the pharmaceutical industry, in medicine as well as in the food processing industry (eg in the preparation of vaccines and for the treatment of hard skin). It also has veterinary applications such as deworming of cattle. Papain is also used in the tanning of leather and has applications in the paper and adhesive industries as well as in sewage disposal. Medical research includes plastic surgery on cleft palates using papain.

Methods of collection and extraction

Papain is obtained by cutting the skin of the unripe but almost mature papaya and then collecting and drying the latex which flows from the cuts. Tapping of the fruit should start early in the morning and finish by mid-late morning (ie during periods of high humidity). At low humidity the flow of latex is low.

Two or three vertical cuts (except the first cut, see below) 1-2mm deep are then made, meeting at the base of the fruit. The incisions are made using a stainless steel razor blade set into a piece of rubber attached to a long stick. The blade should not protrude more than about 2mm as cuts deeper than 2mm risk juices and starch from the fruit pulp mixing with the latex which lowers the quality.

Fruits should be tapped at intervals of about 4-7 days and for the first tapping it is usually sufficient to make only one cut. On subsequent tappings the two or three cuts are spaced between earlier ones (as explained above).

After about 4-6 minutes the flow of latex ceases. A dish is used to collect the latex and the latex is then scraped into a polythene lined box with a close fitting lid; such a box should be stored in the shade. The use of a close fitting lid and keeping the box in the shade are both important because they reduce the reactions which cause the loss of enzyme activity. Foreign matter such as dirt and insects in the latex should be avoided. Latex adhering to the fruit should be carefully scraped off and transferred to the collecting box. However, dried latex should not be mixed with fresh latex as this lowers the quality.

When handling fresh latex, care should be taken to ensure that it does not come into contact with skin as it will cause burning. Neither should it come into contact with heavy metals such as iron, copper or brass as this causes discolouration and loss of activity. Pots, knives and spoons should not be used unless they are made from plastic or stainless steel. Fresh latex does not keep well and should be dried to below 5% moisture (when it will have a dry and crumbly texture) as soon as possible.

After two or three months the fruits are ripe and should be removed from the tree. The ripe fruits are edible but have very little sale value because of their scarred appearance. However, the skin of the green ripe papaya does contain about 10% pectin (dry weight) and the fruits could be processed to extract this.

Drying of papaya latex

The method of drying is the main factor that determines the final quality of papain. There have been various grades used since papain became an international commodity. Up to the mid 1950s when papain from Sri Lanka dominated the market three grades were known:1 - fine white powder, 2 -white oven-dried crumb, and 3 - dark sun-dried crumb. Up to the 1970s there were two grades: 1 - first or high grade oven-dried papain in powder or crumb form usually creamy white in colour, and 2 - second or low grade sun dried brown papain in crumb form. Since 1970 as a result of new processing techniques papain has been re-classified into three groups: 1 - crude papain - ranging from first grade white down to second grade brown. 2 - crude papain in flake or powder form - sometimes referred to as semi-refined. 3 - spray dried crude papain -in powder form, referred to as refined papain.

Sun drying
Sun drying gives the lowest quality product as there is considerable loss of enzyme activity and the papain can easily turn brown. However, in many countries sun drying is still the most common processing technique for papain. The latex is simply spread on trays and left in the sun to dry.

Oven drying
Papain driers can be of simple construction. In Sri Lanka they are generally simple out-door stoves (about one metre high) made out of mud or clay bricks. Drying times vary but an approximate guide is 4-5 hours at a temperature of about 35-40°C. Drying is complete when the latex is crumbly and not sticky. A better quality product is obtained if the latex is sieved before drying. The dried product should be stored in air-tight and light-proof containers (eg sealed clay pots or metal cans) and kept in a cool place. Metal containers should be lined with polythene.

Spray drying
This is not possible at small-scale. A considerable investment in equipment (eg £10,000) is required. However, spray dried papain may be bought for the small-scale processing of foods.

Spray dried papain has a higher enzyme activity than other papains and is totally soluble in water. Extreme care must be taken when handling this form of papain because it can cause allergies and emphysema if inhaled. For this reason spray dried papain is often encapsulated in a gelatine coat.

Enzyme activity
If papain is to be exploited commercially for an export market or local food industry use, it is important to be able to determine the enzyme activity. The method is known as assaying. The assaying could be carried out by, for example, the National Standards office.

Papain is used to hydrolyse (or breakdown) proteins. Therefore assays to measure papain activity are based on measuring a product of the hydrolysis. There are two main assay methods. The first relies on the ability of papain to clot milk. It is a low cost method but is time consuming. Also the lack of a standard method to find the clotting point and variations in the milk powder used can introduce errors.

In this method a known amount of papain sample (made by dissolving a known weight of papain in a known volume of a solution of acetic acid) is added to a fixed amount of milk (made by dissolving a known weight of milk powder in a known volume of water) which has been warmed to 30°C in a water bath.

The contents are thoroughly mixed and then observed until the first signs of clotting (formation of lumps) are detected. The time taken to reach this stage, from when the papain was added to the milk, is recorded. The experiment is then repeated using different known amounts of papain solutions. The different amounts of papain sample used should give a range of clotting times between 60 and 300 seconds for optimum results.
The activity of the papain sample is then calculated by plotting a graph, finding the time taken to clot milk at an infinite concentration of papain and then using that value in a formula to calculate the activity.

To introduce a measure of standardisation the amount of milk can be fixed at a certain known concentration. This is done by reacting a known concentration of high grade papain with the milk. The concentration of milk powder solution can then be adjusted to obtain the desired clotting time under fixed reaction conditions. The 'activity of pure papain' at this known amount of milk can then be calculated. Testing the sample papain under the same reaction conditions and same (known) amount of milk will then give an activity relative to the pure papain.

The second method is based on the science of the absorption of light known as absorptiometry. This is the analytical technique for measuring the amount of radiation (or 'colour' of light) absorbed by a chemical solution.

It is known that, for example, a yellow-coloured solution will absorb blue light. (Blue is the 'complement colour' to yellow). The greater the concentration of yellow in the solution the more the absorption of blue light. This is a useful discovery because certain products of chemical reactions are coloured. The more intense the colour, the greater the concentration of product. Therefore by shining the relevant complement colour through the sample liquid the amount of light absorbed can be related to the concentration of product.

Not all 'colours' (or radiations of light) are visible to the human eye. The technique used when the 'colours' extend beyond the visible spectrum is known as spectrophotometry and the instrument used is called a spectrophotometer.

In the second method to determine the activity of a papain sample, a known amount of papain sample is mixed with a fixed amount of casein (the protein found in milk). The reaction is allowed to proceed for 60 minutes at 40°C. After this time, the reaction is stopped by the addition of a strong acid.

The product of the reaction is known as tyrosine which is known to absorb ultra-violet light (invisible to the human eye). The solutions containing the tyrosine are prepared for analysis using the spectrophotometer. The amount of ultra-violet light absorbed by the solution can be related to the number of tyrosine units produced by the papain sample. Hence the greater this number, the greater the activity of the papain sample.

World trade in papain

The principal producers of crude papain are Zaire, Tanzania, Uganda and Sri Lanka. Most of the spray dried papain comes from Zaire.

The principal importing countries are the United States, Japan, United Kingdom, Belgium and France. Almost all the best quality papain goes to the United States.

Crude papain is used, in Britain, in the brewing industry for chillproofing beer and lager. However, the increasing trend for additive free beers initiated by other European countries is taking effect in Britain and so this market for papain is declining. Another use for papain is in the meat industry for the tenderisation of meat and the production of meat tenderising powders.