PRACTICAL ACTION
Technology challenging poverty

PAPAIN PRODUCTION

technical brief

Introduction

Papain is a common enzyme is obtained from the green papaya (pawpaw) fruit. Enzymes are proteins that can increase the rate of biological changes such as the ripening of fruit. At the end of an enzyme catalyzed reaction the enzyme itself is unchanged and is able to react again.

Enzymes are generally recognised by the ending *-ase*. This either indicates the nature of the substance affected by the enzyme - carbohydrase acts on carbohydrate material and proteases acts on proteins, for example -, or to indicate the nature of the reaction – such as transferases, which catalyse the transfer of atoms or groups of atoms within a substance.

Enzymes occur naturally in foods. Many traditional food processing technologies involve their use. With today's advanced understanding of food science, these enzymes can be extracted, concentrated and added to foods during processing. An example is meat tenderisers. Table 1 describes some of the more traditional technologies, and the enzymes involved.

Traditional food processing technologies	Enzymes used	To catalyze 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	Polymerization of colourless phenolic compounds to brown coloured compounds	Give desirable colour and flavour to tea infusions

Table 1: Traditional food processing using enzymes

One important group of enzymes is the **proteases**. These enzymes catalyse the breakdown of proteins. Examples of the application of proteases in the food processing industry are the chill-proofing of beer (ensuring that fine precipitates – or haziness - does not occur in the beer when it is cooled), tenderisation of meat, the production of dough for pizzas, and the production of batter for waffles and wafers. The most common protease is papain.

There are a number of special difficulties in setting up small-scale papain production. Thorough research is needed before attempting to start up a programme. The following factors must be considered:

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1) Papain is potentially dangerous: prolonged contact will damage the skin of workers' hands. In some cases it may cause allergic reactions.
2) The market for papain in Europe is shrinking as alternative chemicals are found. Papain may be banned from some foods - such as beer - in the near future.
3) Low grade (sun-dried) papain has a much smaller market than higher quality spray-dried papain (see below). A thorough market survey should be made to ensure that the product can be sold.
4) Plantations of papaya trees are needed for economical production. Merely collecting latex from the fruits of 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 most commonly used in the food processing applications mentioned above. Other important proteases ***ficin***, obtained from figs, and ***bromelain,*** which is obtained from pineapple.

In addition to its relevance to the food industry, papain is also used in the pharmaceutical industry for medicines, such as in preparation of vaccines, or for the treatment of hard skin. Papain also has veterinary applications such as in the de-worming of cattle, and is used in the tanning of leather, in the paper and adhesive industries, and in sewage disposal. Medical research uses papain for plastic surgery on cleft palates.

Methods of collection and extraction
Papain is obtained by cutting the skin of the unripe - but almost mature - papaya before collecting and drying the latex which flows from the cuts. The fruit should be tapped some time during the morning (a period of high humidity). The lower the humidity levels, the lower the flow of latex.

Two or three vertical cuts (except the first cut, see below) 1-2mm deep are 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, lowering quality.

Fruits should be tapped at intervals of about 4-7 days. 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 liquid; it is then scraped into a polythene-lined box with a close fitting lid. The box should then be stored in the shade. Using a close fitting lid and keeping the box in the shade reduce the reactions which cause loss of enzyme activity.

Foreign matter such as dirt and insects should be kept out of the collected latex. Any secreted latex adhering to the fruit should be carefully scraped off and put into 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: it will cause burning. Neither should the latex come into contact with heavy metals such
as iron, copper or brass, as it causes discolouration and loss of activity. Pots, knives and spoons should only be used if made from plastic or stainless steel. Fresh latex does not keep well and should therefore be dried to below 5% moisture as soon as possible. This gives it a dry and crumbly texture.

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

Drying papaya latex

The method of drying is the main factor that determines the final quality of papain. Various grades have been used since the enzyme has become an international commodity. Up until the mid-1950s, Sri Lankan papain dominated the market, three grades were known:
1 - fine white powder;
2 - white oven-dried crumb, and
3 - dark sun-dried crumb.

Until the 1970s there were two grades:
1 - first or high grade oven-dried papain in powder or crumb form (usually creamy white in colour);
2 - second or low grade sun dried brown papain in crumb form.

As a result of new processing techniques, papain has been reclassified into three groups since 1970:
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 of product, as there is considerable loss of enzyme activity and the papain can easily turn brown. In many countries, however, sun-drying is still the most common processing technique. 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 outdoor stoves. They are typically about one metre high and made of mud or clay bricks. Drying times vary, but an approximate guide is 4-5 hours at a temperature of 35-40°C. Drying is complete when the latex is crumbly and no longer sticky. A better quality product is obtained if the latex is sieved before drying. The dried product should be kept in air-tight, light-proof containers (eg. sealed clay pots or metal cans) and stored in a cool place. Metal containers should be lined with polythene.

Spray-drying

Not possible at small-scale because considerable investment in equipment is required. However, it is feasible to buy spray-dried papain for the small-scale processing of foods. Spray-dried papain has a higher level of enzyme activity than other types of papain and is totally soluble in water. Extreme care must be taken when handling this form of papain: it can cause allergies and emphysema if inhaled. For this reason, spray-dried papain is often encapsulated in a gelatine coat.

Enzyme activity

Whether papain is to be exploited commercially for an export market or for local food industry use, it is important to be able to determine the level of enzyme activity, a process known as assaying.
This can be carried out by, say, the National Standards office. Papain is used to hydrolyse (or break down) proteins. Therefore, assays to measure papain activity are based on measuring a product of the hydrolysis. There are two main methods of carrying out assays:

Method 1

This method relies on the ability of papain to clot milk. It is a low-cost method but is time consuming. Furthermore, the absence of a standardised method of finding the clotting point, combined with variations in the milk powder used, can introduce errors.

A papain sample is prepared by dissolving a known weight of papain in a known volume of acetic acid solution. This is then added to a fixed amount of milk, which is prepared by dissolving a known weight of milk powder in a known volume of water, warmed to 30°C in a water bath. The contents are thoroughly mixed and then observed until the first signs of clotting – the formation of lumps - are detected. The length of time from when the papain was added to the milk until clotting began is recorded.

The experiment is repeated using different known amounts of papain solutions. The varying 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 samples 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. In order to introduce a measure of standardisation, the amount of milk can be fixed at a 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 in order 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.

Method 2

The second method is based on the science of light absorption, or **absorptiometry**. This technique measures the amount of radiation (or 'colour' of light) absorbed by a chemical solution. It is known, for example, that a yellow-coloured solution absorbs blue light. (Blue is the complementary colour to yellow). The greater the concentration of yellow in the solution, the more blue light is absorbed. This is a useful technique because the resulting products of some chemical reactions are coloured. The more intense the colour, the more concentrated the resulting product. Therefore, by shining the

relevant complementary 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 this second method of determining the amount of activity of papain, a known amount of the enzyme 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 adding a strong acid. The resulting product of the reaction is 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 then be compared 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 Democratic Republic of Congo (formerly Zaire), Tanzania, Uganda and Sri Lanka. Most spray-dried papain comes from DRC.

Main importers are USA, 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 chill-proofing beer and lager. However, an increasing trend in additive-free beers - initiated by other European countries - is taking effect in Britain, whose market for papain is therefore declining. Papain is, however, used in the tenderising of meat and in production of meat tenderising powders.

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