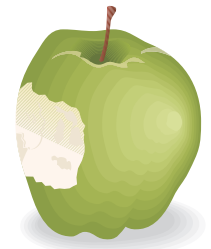


enzymes in fruit juice production

Jam-makers have valued it for hundreds of years, wine-makers endeavour to destroy it and food technologists are variously trying to block or improve the enzymes that attack it. After cellulose, pectin is one of the most abundant carbohydrates on Earth. Yet despite its importance in the production of food and drink, pectin and the enzymes that break it down seldom receive the attention they deserve. This article describes the rôle of pectin, pectinases and other enzymes in fruit juice production.



Structure of fruit

The structure of plant cells and the way they are built into tissues is complex. Only a simplified description will be given here, including sufficient detail for the reader to appreciate the action of enzymes in fruit juice production.

Fruits are comprised mostly of parenchyma cells. These are relatively simple 'general purpose' plant cells, with thin walls made of two layers. The outermost of these, the primary cell wall, is made from cellulose fibres called microfibrils surrounded by a matrix of pectins, hemicelluloses and proteins. The inner, or secondary, cell wall is similar but contains less pectin (Figure 1).

Between the cellular 'bricks' is a 'mortar' of pectin. Complete removal of this binding layer causes the tissue to fall apart. This is what has happened to fruits or vegetables which have gone soft. In such cases digestive enzymes, which have broken down the long chains of pectin molecules, have been produced by microbes such as the 'soft-rot' bacterium *Erwinia carotovora*.

Cellulose is susceptible to enzymatic attack too, especially in the primary cell wall where its

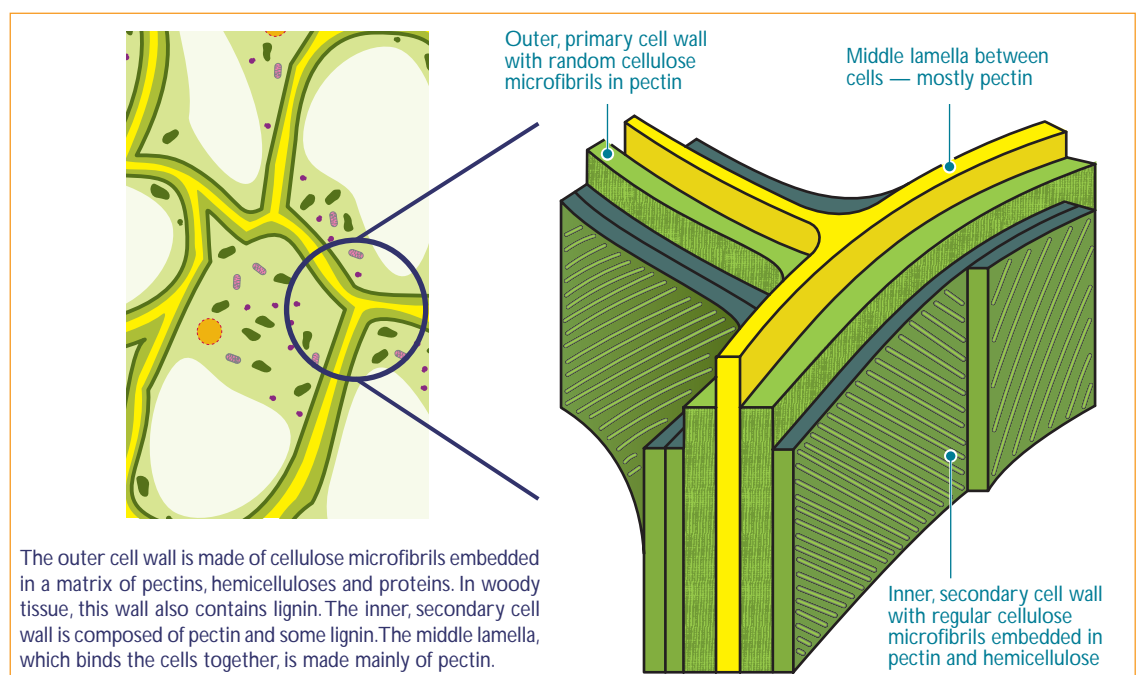
microfibrils are arranged at random rather than in the stronger regular pattern seen in the secondary wall. Degradation of the cellulose microfibrils leads to breakdown of the cell walls and their eventual disintegration. Table 1 shows the proportions of various materials found in the cell walls of some important fruit crops.

Changes to fruit during ripening

There are two main stages in the formation of fruit. Soon after fertilisation the cells of the ovary (and in fruit like apples, those of the surrounding tissue as well) divide rapidly. In later weeks the young fruit swells as both these cells and the spaces between them enlarge. As the fruit ripens it becomes progressively softer.

Much research has been done to discover exactly which enzymes are responsible for these and other changes in maturing fruit. One hope is that by monitoring the levels of these enzymes it might be possible for growers to harvest their crops at just the right time or even to select varieties with desirable characteristics, such as better storage properties or greater yields and improved quality of juice.

► **Figure 1**
The general structure of plant cell walls.



The outer cell wall is made of cellulose microfibrils embedded in a matrix of pectins, hemicelluloses and proteins. In woody tissue, this wall also contains lignin. The inner, secondary cell wall is composed of pectin and some lignin. The middle lamella, which binds the cells together, is made mainly of pectin.

For many years it was (not unreasonably) assumed that the softening associated with ripening was caused by cellulases acting on the cell walls. For apples at least this has been shown not to be so, although cellulases do play a part in the ripening of softer fruits such as peaches and tomatoes.

The other candidate for change causing fruit to soften is pectin, the inter-cellular 'cement'. In unripe fruit, the pectin is bound to cellulose microfibrils in the cell walls. Such pectin is insoluble and the liquid within the cells remains fluid, conferring rigidity on them (by pushing on the cell wall). During ripening, the pectin is altered by naturally-occurring enzymes in the fruit. These 'alterations' may involve the breakdown of the pectin chains or of side chains attached to the units from which they are made (see diagram of pectin structure on page 9). In either case, the result is that the pectin becomes more soluble and its grip on the surrounding cell walls is loosened, so that the plant tissue softens.

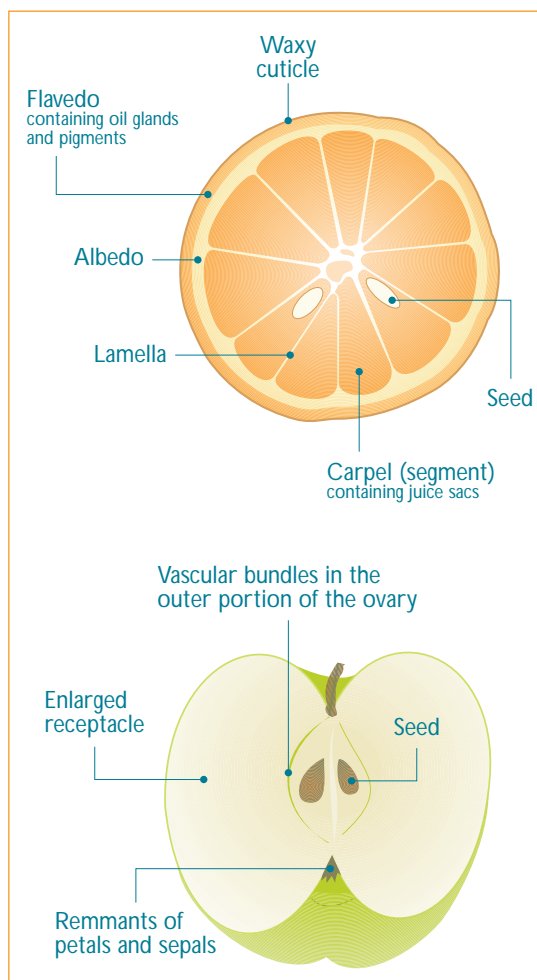
Intuitively, this suggests that it should be easier to press the juice from ripe fruits than unripe ones. Surprisingly, the reverse is true. One consequence of the partial breakdown of insoluble pectin is that it becomes soluble in water. Some of the pectin molecules are released into the juice, so that it becomes more viscous, and therefore more difficult to squeeze from the fruit during processing. Important colour and flavour compounds may also be retained within the fruit so that juice pressed from it is of inferior quality. Furthermore, the juice may be difficult to clarify and filter due to suspended pectin particles. In the fruit juice industry pectinases, obtained from the fungus *Aspergillus*, are used to help overcome these problems.

Use of pectinases and other enzymes in fruit juice production

Home winemakers will be familiar with pectinase or pectolytic enzyme. Pectinases were some of the first enzymes to be used at home and they were utilised in the commercial preparation of wines and fruit juices during the 1930s. Before this juices had been prepared by mechanical means — simply by pressing fruit and filtering the liquid which emerged. Even so the early use of pectinase to clarify apple juice was developed by trial and error. Only in the 1960s did the chemical structure of plant tissues become apparent, and with this knowledge food technologists began to use a greater range of enzymes more effectively.

Enzymatic juice extraction from apples was introduced 30 years ago and now more than 5 million tons of apples are processed into juice annually

	Percentage of fresh mass of cell wall			
	Pectin	Hemicellulose	Cellulose	Glycoprotein
Cherry	0.51	0.06	0.17	0.32
Pineapple	0.21	0.35	0.27	0.12
Mango	1.02	0.23	0.59	0.32
Apple	0.54	0.34	0.70	0.15
Pear	0.42	0.22	0.40	0.12



▲ **Table 1**

The major components of the cell walls of some important fruits.

From Voragen and van den Broek, 1991.

◀ **Figure 2**

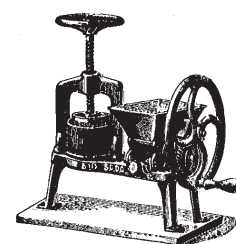
The structure of citrus fruit and apples.

The bulk of both of these fruits is formed from unspecialised tissue known as parenchyma, which holds most of the juice.

throughout the world. Some 4 000 million litres of orange juice and over 450 million litres of grapefruit juice are also produced each year.

Pectinases and other enzymes are an essential part of fruit juice technology (see Table 2, page 7). They are used to help extract, clarify and modify juices from many crops including berries, stone and citrus fruits, grapes, apples, pears and even vegetables. Where a cloudy juice or 'nectar' is preferred (for example, with oranges, pineapples or apricots) there is no need to clarify the liquid, though enzymes *are* used here to enhance extraction or perform other modifications.

The methods used for apple juice production are the same as those for many other fruits, and therefore serve as a useful example. Citrus fruits present special requirements of their own, so these are dealt with separately. →



▲ An apple crusher and juice press, for home use, circa 1890.



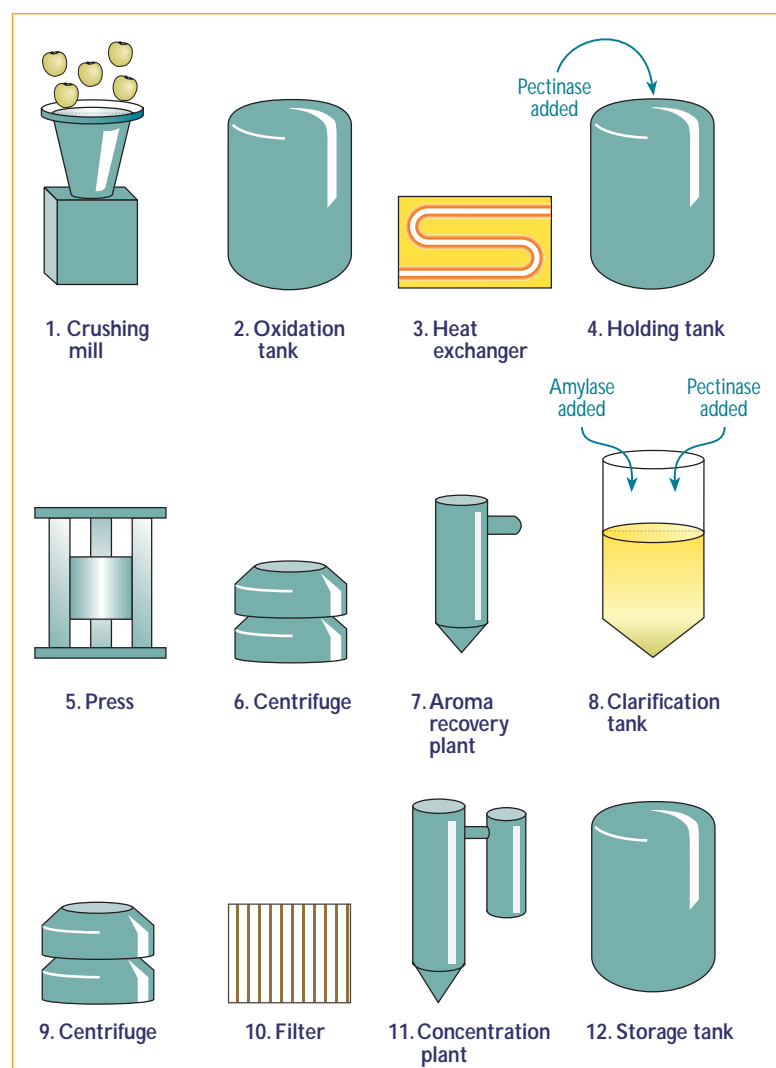
Apple juice production

Several types of apple juice are available commercially: the most familiar are hazy unfiltered and unclarified juice (usually a premium product); and a clear, filtered, amber-coloured juice. Both can be prepared using enzymes — the main steps involved are shown in Figure 3.

Pre-press treatment

After the apples have been washed and sorted, they are crushed in a mill. Peels and cores from apple slice or sauce production may also be used together with whole apples. Although pectinases are often added at this stage, better results are achieved if the apple pulp is first stirred in a holding tank for 15–20 minutes so that enzyme inhibitors (polyphenols) are oxidised (by naturally-occurring polyphenol oxidase in the fruit). The pulp is then heated to an appropriate temperature before enzyme treatment. For apples, 30 °C is the optimal temperature, whereas stone fruits and berries generally require higher temperatures — around 50 °C. This compares with 60–65 °C required if pectinase is not used (in which case the juice is liberated by plasmolysis of the plant cells).

▼ **Figure 3**
The main stages in the large-scale commercial production of apple juice.



Prepress treatment with pectinases takes anything from 15 minutes to 2 hours depending upon the exact nature of the enzyme and how much is used, the reaction temperature and the variety of apple chosen. Some apple varieties such as *Golden Delicious* are very difficult to break down.

During incubation, the pectinase degrades soluble pectin in the pulp, making the juice flow more freely. The enzyme also helps to break down *insoluble* pectin, which hampers juice extraction in two ways. First, if they are left, these slimy pectin particles become saturated with juice which is then difficult to extract from the pulp and second, they block drainage channels in the pulp through which the juice must drain. Enzyme treatment is considered to be complete once the viscosity of the juice has returned to its original level or less. (Remember, the degradation of insoluble pectin causes the juice to thicken at the start.) It is important that the pulp is not broken down too much as it would then be difficult to press.

Pressing

Pressing is done using continuous filters or rotary presses. Juice yields may be increased by up to 20%, depending upon the age and variety of apple used and whether pre-oxidation was employed. Enzyme treatment is particularly effective with mature apples and those from cold storage. Significant increases in yield are not usually achieved from fresh, early season apples.

Clarification

If a cloudy product is required, the juice is pasteurised immediately after pressing to denature any residual enzymes. Centrifugation then removes large pieces of debris, leaving most of the small particles in suspension. For a clear juice these suspended particles have to be removed. It may seem simple merely to filter them out, but unfortunately some soluble pectin remains in the juice, making it too viscous to filter quickly. Treatment with pectinase is the accepted way of removing unwanted pectin.

Depectinisation has two effects: it degrades the viscous soluble pectins and it also causes the aggregation of 'cloud' particles. Figure 4 shows how this happens. Pectin forms a protective coat around proteins in suspension. In an acidic environment (apple juice typically has a pH of 3.5) pectin molecules carry a negative charge. This causes them to repel one another. Pectinase degrades this pectin and exposes part of the positively-charged protein beneath. The electrostatic repulsion between cloud particles is thereby reduced so that they clump together.

These larger particles will eventually settle out but to improve the process flocculating agents (finings)

Clarification of fruit juices

e.g., apple juice and lemon juice: depectinised juices can also be concentrated without gelling and developing turbidity.

Enzyme treatment of pulp

of soft fruit, red grapes, citrus and apples, for better release of juice (and coloured material); enzyme treatment of pulp of olives, palm fruit and coconut flesh to increase oil yield.

Maceration of fruit and vegetables (disintegration by cell separation)

to obtain bases for thick fruit 'nectars' and baby foods

Liquefaction of fruit and vegetables

to obtain products with increased soluble solids content (pectinases and cellulases combined).

Special applications to citrus fruits

preparation of clouding agents from citrus peel; cleaning of peels for use in candying and marmalade production; recovery of oil from citrus peel; depectinising citrus pulp wash.

Cellulases

The addition of cellulases during extraction at 50 °C improves the release of pigments from the skins of fruit. This is particularly useful for treating blackcurrants and red grapes. Cellulases are sometimes used at the time of the initial pectinase addition to totally liquefy the plant tissue, so that juice can be filtered straight from the pulp without any need for pressing.

Arabanase

The polysaccharide araban (a polymer of the pentose sugar, arabinose) may cause a haze in fruit juice a few weeks after it has been concentrated. Although commercial pectinase preparations often contain arabanase, certain fruits (like pears) are rich in araban and may require the addition of extra arabanase.

Glucose oxidase

Glucose oxidase catalyses the breakdown of glucose. This reaction uses molecular oxygen and generates hydrogen peroxide. Glucose oxidase (coupled with catalase to remove the hydrogen peroxide) is used to remove the oxygen from the air above bottled drinks, reducing the non-enzymatic browning (due to oxidation) which might otherwise occur.

◀◀ Table 2

Uses of pectinases in the production of fruit juices.

After Rombouts and Pilnik, 1978.

◀ Table 3

Other enzymes that are often used in the production of fruit juices.

After Rombouts and Pilnik, 1978.

such as gelatin, tannin or bentonite (a type of clay) can be added. Some fining agents adsorb the enzyme onto their surface, so it is important not to add them before the enzyme has done its job. Yeasts and other microbes which may have contaminated the juice are also precipitated by finings. What is left is a translucent but by no means clear juice. A second centrifugation and subsequent filtration are needed to give the clear juice that many people prefer.

Starch haze

Another potential contributor to the haziness of juice is starch. This is particularly so if unripe or eating apples have been used (eating apples are often harvested early to ensure they stay crisp in storage). Unripe apples may contain up to 15% starch. Although the first centrifugation (before the juice reaches the clarification tank) removes most of the starch, about 5% usually remains. This can be broken down using an amylase (strictly speaking, an amyloglucosidase) active at the pH of apple juice, added at the same time as the pectinase.

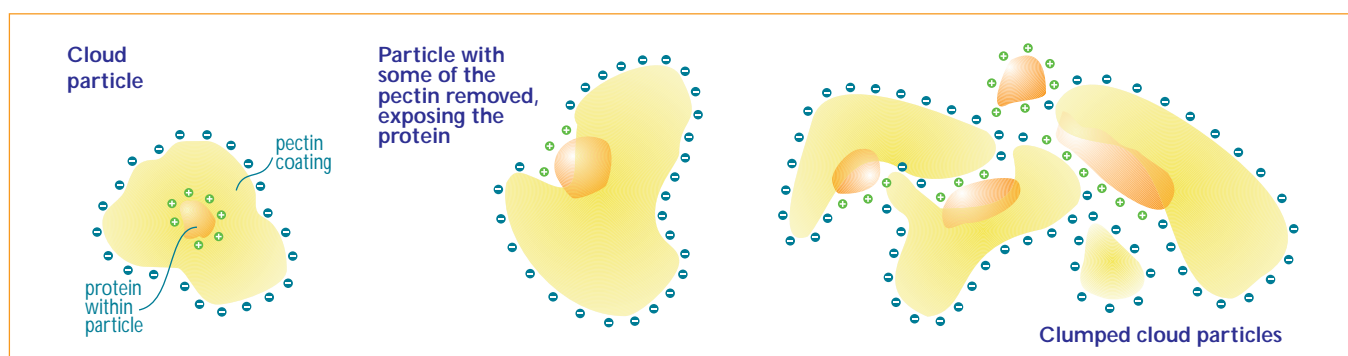
For most juice processing both depectinisation and destarching are *essential*. This is because much apple juice is concentrated before storage by evaporating up to 80% of the water. This makes the juice easier to transport and store, and the concentrate's high sugar content acts as a natural preservative. Unfortunately, heat treatment also drives off the juice's pleasant aroma so it is necessary to gently heat the juice and to collect the volatile smell and flavour compounds so that they can be put back again when the juice is reconstituted.

Heating can cause residual pectin or starch in the juice to gel or form a haze — hence the necessity of enzyme treatment. Indeed, it is seldom economically feasible to produce clear apple, blackcurrant or cranberry juices or to concentrate them without the use of enzymes. →

Note: In the USA and several other countries non-alcoholic apple juice is known as 'cider'. Hence Web searches for 'cider' will often unearth useful information about apple juice production.

▼ Figure 4

How the breakdown of pectin leads to the formation clumps of 'cloud' particles which can be precipitated.





Citrus juices

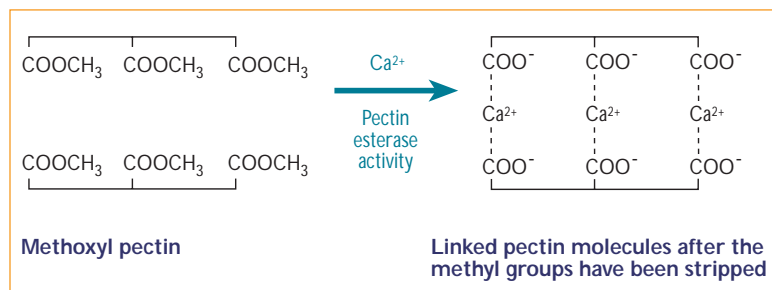
Juice extraction

Anyone who has wielded a lemon squeezer will appreciate how difficult it is to extract citrus juice by pressing alone. Much of the liquid is retained within the fruit bound to pectin. Consequently, pectinases are used in the citrus juice industry to assist the removal of pectin and release of juice from fruit pulp. As with core fruits, this enables the juice to be concentrated without the risk of gelling and helps to reduce the viscosity of the finished concentrate. However, it is usually important that some of the insoluble pectin remains in suspension, giving orange juice, for example, its characteristic cloudy appearance.

Cloud loss and its prevention

The maintenance of a stable cloud in citrus juices presents a considerable challenge to fruit processors. It is possible to use 'artificial' clouding agents, but in many countries their use is prohibited. Ironically, one effective procedure to prevent the loss of pectin uses a pectinase — the very substance that one would expect to break it down. To appreciate this process, it is necessary to understand something of the nature of pectin molecules and the different enzymes that act upon them.

▼ **Figure 5**
Calcium pectate is precipitated when calcium ions cross-link non-methylated pectin.



Two sorts of pectin

Pectin is not just one substance, but a group of polysaccharides with a large molecular structure. Pectin molecules are composed of long chains of galacturonic acid residues (see Figure 6). On each residue is a carboxyl ($-\text{COOH}$) group. Sometimes these groups are modified by the addition of methyl ($-\text{CH}_3$) groups. The result is a methoxyl group ($-\text{COOCH}_3$). Pectin in which half or more of the acid residues have methoxyl groups tagged onto them is called *high methoxyl pectin*; where there are fewer the term is *low methoxyl pectin*.

When fewer than a tenth of the galacturonic acid molecules are methylated, the substance is referred to as pectic acid (or pectate), rather than pectin.

Pectin esterase

Apple pectin is highly methylated. In contrast, pectin from oranges is only partly methylated. This is because orange juice naturally contains large amounts of pectin esterase — an enzyme that strips methoxyl groups from the pectin molecules (see Figure 6). In the presence of calcium ions, insoluble calcium pectate is formed in orange juice, leading to the undesirable precipitation of haze particles (Figure 5). Two methods are widely employed to prevent this cloud loss. One is to denature the pectin esterase by heating the juice to $\sim 90^\circ\text{C}$. Unfortunately this spoils the juice's flavour somewhat. An alternative is to freeze the juice, thereby holding the enzyme in an inactive state. Whole orange juice is often stored like this so that it can be sold out of season, but this expensive procedure is limited to premium products.

Other pectinases provide an answer

By adding a different enzyme to orange juice, an enzyme that rapidly cuts up the pectin chains, it is possible to prevent the formation of calcium-linked precipitate. The enzyme endo-polygalacturonase (Figure 5) has been shown to be especially suitable for this task. This enzyme is found in many commercial pectinase preparations.

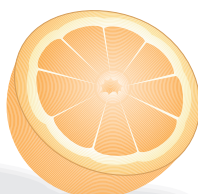
Pectin esterase inhibitors

Another approach is to *inhibit* the pectin esterase. It is possible to do this by adding short pectin chains to the juice. These compete with longer pectin molecules for the attention of the enzyme, slowing the rate at which it can attack them. The chains have to be just the right length (8–15 residues long) so that they do not end up as precipitates themselves.

Making 'artificial' cloud from citrus skins

The white spongy layer (or albedo) immediately beneath the skin of citrus fruits is a rich source of pectin. In citrus-growing areas, therefore, orange or lemon peels are often used in the manufacture of pectin for food processing (apple cores and peels are used instead in the UK). Citrus peels also contain large quantities of useful pigments (carotenoids) and substances which can be used to impart desirable cloudiness to fruit drinks. An enzymatic treatment can be used to prepare such natural clouding agents.

The citrus peel is ground to produce particles roughly 3–5 mm in diameter. These are mixed with an equal volume of water and boiled to soften the tissue. After cooling, pectinases are added. During incubation at 50°C the peel tissue is broken down, forming a cloudy liquid that contains cellulose, pectins, hemicelluloses and cell organelles. Pasteurisation, centrifugation and concentration complete the process.



Clarification of lemon juice

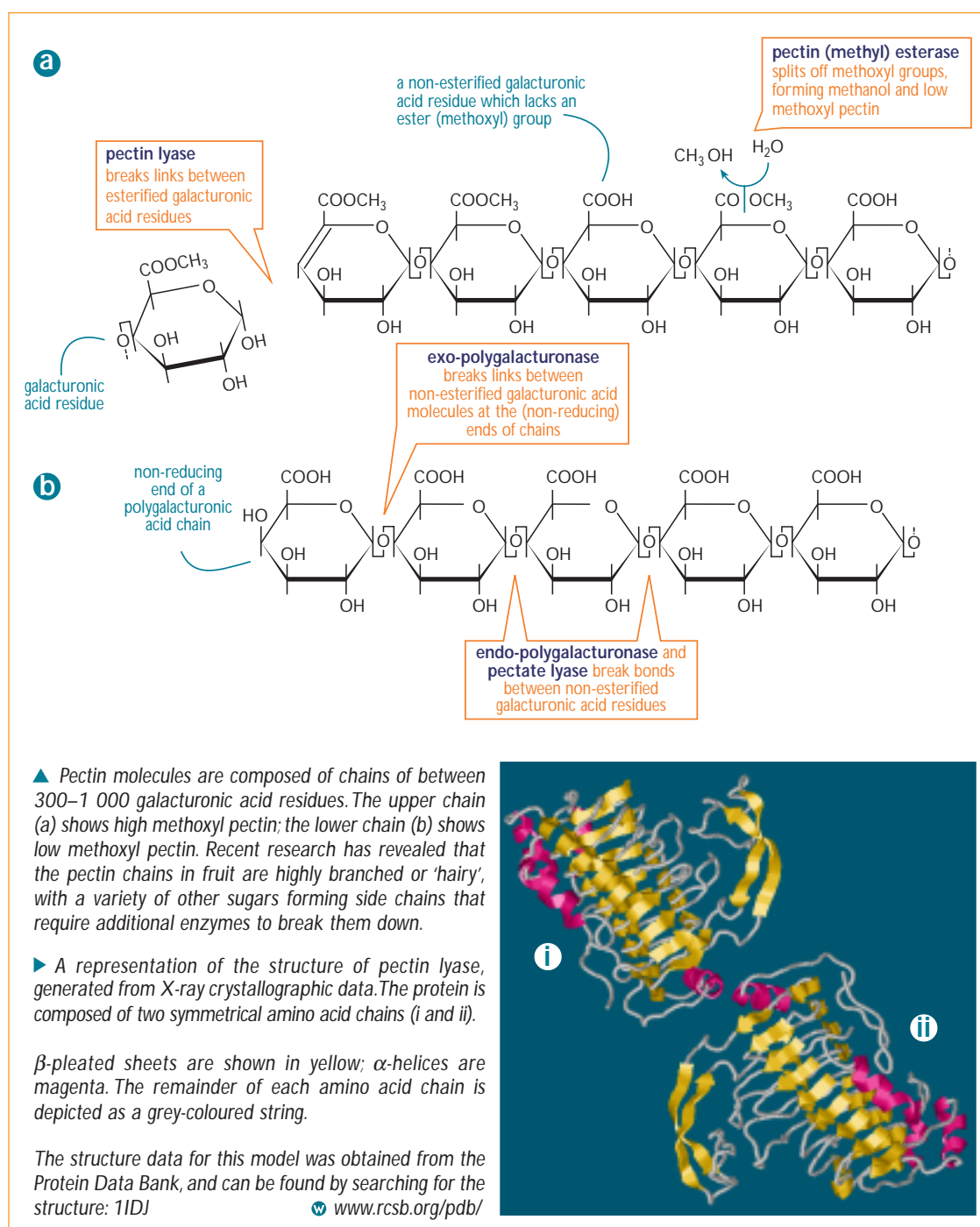
Unlike orange juice, that derived from lemons is often sold as a clear liquid. Traditional methods of clarifying lemon juice rely upon the fruit's natural pectin esterase content. Suspended solids are precipitated as calcium pectate (Figure 5), but this process can take some 4–16 weeks. Preservatives (*e.g.*, *sodium metabisulphite*) must be added to the juice to prevent microbial spoilage during this period.

Fungal enzymes are now available which exhibit pectolytic activity even in very acidic juices such as lemon (pH 2.2–2.8). Their use enables clarification to be achieved in 6 hours without the need for preservatives.

Bitter compounds in citrus fruits

The bitter flavour associated with citrus fruits such as grapefruit or navel oranges is caused by two distinct groups of compounds: flavonoids and limonoids. The enzymatic degradation of both types of compound has been extensively studied with a view to producing sweeter-tasting citrus juices. As we shall see below, this research, aimed at producing tastier fruit juice, has had several unexpected medical spin-offs.

One of the main bitter-tasting flavonoids is called naringin. It is found in all parts of the fruit, but especially in the albedo just beneath the skin. Grapefruit processors try to select fruits with a low naringin content, and often blend juices →



◀ **Figure 6**
The action of four of the major enzymes found in pectinase preparations.

obtained from different grapefruit varieties to obtain the desired degree of bitterness. It is important to leave some naringin because this compound enhances our perception of taste by stimulating the taste buds; hence grapefruit juice is often consumed before a meal.

Immobilised naringinase

During the early 1960s the enzymatic hydrolysis of naringin as a means of controlling bitterness was studied in the USA and Japan. Naringinase preparations obtained from fungi were shown to break down naringin first to the less bitter compound prunin, then to non-bitter naringenin.

The correct degree of breakdown was achieved by controlling the flow rate over a column of immobilised naringinase, but the process was not developed commercially. Resins that absorb the naringin (which are more stable than the immobilised enzyme) proved to be cheaper and more reliable. However, unlike a highly-specific enzyme, such resins also absorb other *desirable* compounds, so this was not the end of the naringinase story.

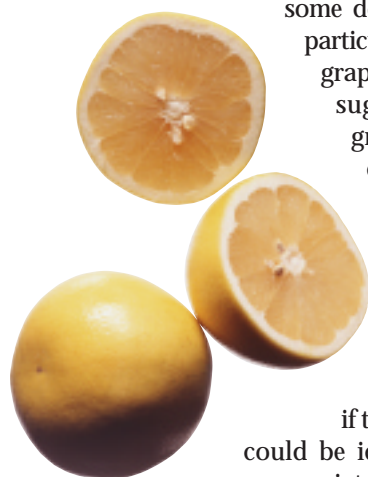
In 1997 researchers at Cornell University in the USA developed a novel type of 'active' packaging which was able to improve the flavour of juice during storage. Typically, fresh grapefruit juice in the refrigerated sections of supermarkets is packaged in cardboard containers lined with a waterproof polymer. The Cornell food technologists used a thin cellulose-acetate film on the inside of the box, in place of the normal polymer lining. This layer contained immobilised naringinase (actually a mixture of two enzymes). When the juice came into contact with the film, its bitter taste was reduced due to the activity of the enzyme preparation.

▼ Grapefruit contain a bitter compound, naringin, which can be removed by using a naringinase enzyme preparation.

Drug interactions

Naringin also came to public attention in 1991 when it was suspected of boosting the action of certain, specific types of medicine. This has led some doctors to recommend that people on particular medications should avoid drinking grapefruit juice. More recent research has suggested that another compound in grapefruit may be responsible for the effect, rather than the naringin itself.

Nevertheless, this surprising finding has led to speculation that it might be possible to reduce the doses of some expensive drugs (such as *Cyclosporin*, which is used to prevent the rejection of transplanted organs) if the active component in grapefruit juice could be identified, purified and administered appropriately alongside the medicine.



Limonoid bitterness

Unlike naringin, limonin is not found in intact fruits. However, freshly-squeezed citrus juice can turn bitter after only a few hours as limonin is formed by natural chemical reactions (so-called 'delayed bitterness'). This reaction is enhanced when the fresh fruit juice is pasteurised.

In the late 1980s, it was discovered that oranges and other citrus fruits contain an enzyme called limonin glucosyltransferase (LGT). LGT converts the bitter limonins into tasteless compounds called limonoid glucosides. Unfortunately, the necessary enzyme is only present in small quantities for a short time during the ripening of the fruit.

In 1998, Japanese scientists determined the genetic sequence of LGT. With their American co-workers, they hope to use this information to help produce genetically-modified orange trees that bear fruit with more limonoid glucosides. That would mean citrus juice that wouldn't turn bitter.

Another potential benefit of limonoid glucosides is that they may help prevent tumour formation. Several studies have shown that these compounds inhibit the growth of tumour cells (including human cancer cells) in experimental conditions. In 1998 a Japanese research organisation test-marketed a mandarin orange juice, *LG1000*, supplemented to contain five times the normal level of limonoid glucosides (1 000 ppm, hence the name).



Future developments

Many different enzymes can be used to process fruit juices, bringing a range of potential benefits to producers, retailers and consumers. The enzymes used to date have generally been crude preparations but our greater knowledge of the substances they act on has stimulated the demand for purer enzyme preparations with specific, well-defined and predictable properties.

While improvements in enzyme purification processes are important, the focus has now turned to DNA technology. The availability of a wider range of pure enzymes from genetically-modified, food-grade organisms will lead to new applications for such enzymes. However, while the genes encoding several potentially useful enzymes have been identified, much has to be done before laboratory research yields commercial products that gain regulatory approval and general acceptance for food use.

