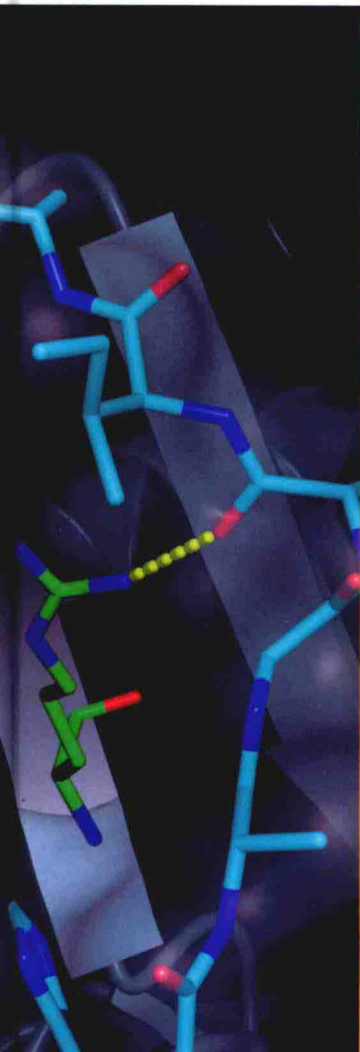
Enzyme Technology in Food Processing



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Preface

The enzyme Catalase has found limited use in one particular area of cheese production. Hydrogen peroxide is a potent oxidizer and toxic to cells. It is used instead of pasteurization, when making certain cheeses such as Swiss, in order to preserve natural milk enzymes that are beneficial to the end product and flavour development of the cheese. These enzymes would be destroyed by the high heat of pasteurization. However, residues of hydrogen peroxide in the milk will inhibit the bacterial cultures that are required for the actual cheese production, so all traces of it must be removed. Catalase enzymes are typically obtained from bovine livers or microbial sources, and are added to convert the hydrogen peroxide to water and molecular oxygen. Lipases are used to break down milk fats and give characteristic flavours to cheeses. Stronger flavoured cheeses, for example, the italian cheese, Romano, are prepared using lipases. The flavour comes from the free fatty acids produced when milk fats are hydrolyzed. Animal lipases are obtained from kid, calf and lamb, while microbial lipase is derived by fermentation with the fungal species *Mucor meihei*. Although microbial lipases are available for cheese-making, they are less specific in what fats they hydrolyze, while the animal enzymes are more partial to short and medium-length fats. Hydrolysis of the shorter fats is preferred because it results in the desirable taste of many cheeses. Hydrolysis of the longer chain fatty acids can result in either soapiness, or no flavour at all.

For decades, enzymes such as malt and fungal alpha-amylases have been used in bread-making. Rapid advances in biotechnology have made a number of exciting new enzymes available for the baking industry. The importance of enzymes is likely to increase as consumers demand more natural products free of chemical additives. For example, enzymes can be used to replace potassium bromate, a chemical additive that has been banned in a number of countries. The dough for bread, rolls, buns and similar products consists of flour, water, yeast, salt and possibly other ingredients such as sugar and fat. Flour consists of gluten, starch, non-starch polysaccharides, lipids and trace amounts of minerals. As soon as the dough is made, the yeast starts to work on the fermentable sugars, transforming them into alcohol and carbon dioxide, which makes the dough rise.

The book has been written keeping in mind for the graduate and post graduate level bio-sciences and interdisciplinary courses.

—Ranvijay Singh

Contents

Preface *vii*

1. **Fruit Processing** **1**
The Fruit Cell Wall • Fruit and Vegetable Processing • General Properties of Fruit and Vegetables; Chemical Composition and Nutritional Aspects; Structural Features • Chemical Composition • Activities of Living Systems • Deterioration Factors and their Control • Chemical Changes • Biological Changes • Methods of Reducing Deterioration • General Procedures for Fruit and Vegetable Preservation • Preservation by Reduction of Water Content: Drying/Dehydration and Concentration • Chemical Preservation • Preservation with Sugar • Heat Preservation/Heat Processing

2. **Enzymes in Sugar Industries** **78**
Glucose from Cellulose • Glucose Oxidase and Catalase in the Food Industry • Yeast in Biological Research • Yeast in Commercial Applications • Breaks Down Strong Glucose Chains • Enzymes in Fat, Oil, Flavour and Fragrance Industries • Vanillin • Trypsin

3. **Enzymes in Milk and Cheese Industries** **95**
Enzymes in Baking Industry • Dough Conditioning • Enzymes in Fruit Juices and Beverages Industry • Uses of Enzymes in Fruit Juice Wine Brewing Distilling Industries • Enzymes in Meat Industries • Bromelain • Actinidain

4. **Enzymes in Brewing Industry** **109**
Ingredients • Bottle Fermentation • Cask Conditioning • Barrel Aging

5. **Technological Principles of Pasteurization** **129**
Food Irradiation • Global Developments • Consensus on Wholesomeness • International Trade • Auxiliary Raw Materials • Sweeteners • Food Acids • Packaging Materials • Protection of Food by Packaging Materials • Fruit Specific Preservation

Technologies • Harvesting and Preprocessing • Technology of Semi-processed Fruit Products • Fruit Sugar Preserves Technology; Jams, Jellies, Marmalade, Fruit Paste • Banana and Plantain Processing Technologies • Product Stability and Spoilage Problems • Preparation Methods for Fresh Bananas and Plantains • Mango and Guava Processing Technologies • Guava Processing Technologies • Vegetable Specific Processing Technologies • Quality Control/Quality Assurance and International Trade • Good Manufacturing Practices (GMP); Hygiene Requirements

6. Microbiology of the Milk **215**
Lactose Intolerance • Additives and Flavoring • Packaging • Spoilage and Fermented Milk Products • Microorganisms in Milk • Microbiology of Raw Milk • Milk-borne Diseases • Milk Allergy • Pasteurization and Sterilization of Milk • Concentrated Milks • Dried Milks and Dry Whey Products • Major Products Made from Whey in New Zealand

7. Microbial Control by New Nonthermal Methods **245**
Microbiological Concerns • Control Measures • Labeling • Microbial Genomics: A New Tool to Increase Food Quality and Safety

Bibliography 267

Index 271

Chapter 1

Fruit Processing

Enzymes are an integral component of modern fruit juice manufacturing and are highly suitable for optimising processes. Their main purposes are:

- increase extraction of juice from raw material
- increase processing efficiency (pressing, solid settling or removal)
- generate a final product that is clear and visually attractive.

Pomme Fruits

Depending on the chosen processing technology, different enzymes are used for mash treatment or extraction as well as for maceration. Juice depectinisation and starch degradation are important criteria for manufacturing concentrates.

The use of enzymes in fruit juice and cider processing: The juices from a wide variety of fruits such as apples, pears, mango and berries can be extracted to produce natural beverages. The processes used vary considerably depending on the type of fruit, its age and maturity. In general, extraction of fruit juice involves maceration followed by pressing or decanting, to separate the juice from the solids. Enzymes can play a key role in these processes improving yield, clarity and stability of the juice. Blends of macerating enzymes such as Macer8™ FJ and Pectinase 62L consisting of pectinases in combination with other carbohydrate degrading enzymes are useful in breaking open the fruit tissues to release more juice. Enzymatic maceration can result in extraction of >95% of the soluble solids from the fruit. Enzymes are also used to help clarify and stabilize the juice by degrading soluble pectins and starches that would otherwise cause haze.Biocatalysts provide a comprehensive range of enzymes to aid the production of fruit juice and cider.

The Fruit Cell Wall

The most important characteristic affecting the extraction of juice is the fruit cell wall, which provides the structure and rigidity of the pulp.

The composition of the cell wall can vary significantly for different types of fruit, but mainly consists of pectin, hemicellulose, cellulose, lignin and other components. The fruit cell wall has a highly complex structure. It is composed of bundles of crystalline cellulose microfibres embedded in an aqueous gel of hemicellulose and pectin. The xylan chains of the hemicellulose are cross-linked by phenolic acids (usually ferulic acid) and also linked to the arabinogalactan side chains of the pectin.

Fruit and vegetables are particularly rich in pectin which forms the major structural polysaccharide component of the fruit lamella and cell wall. It is composed of lengths of polygalacturonic acid (PGA) and rhamanogalactouronan 1 (RG1) with arabinan and galactan side chains.

Pectins are often described as containing 'smooth' regions of linear PGA and 'hairy' regions of RG1 with multiple side chains. The linear chains of polygalacturonic acid (smooth regions) are largely esterified with methyl or acetyl groups. The extent of methylation affects the susceptibility of the pectin to pectinases and is characteristic of different types of fruit.

Apples, for example, usually have a very high pectin methylation.

Enzymes Used in Fruit Processing

Because fruit cell walls have such a complex structure of interwoven polymers, no single enzyme will breakdown cell walls. Complete liquefaction of a cell wall is likely to require a complex cocktail of carbohydrase enzymes. In fact, there is no single enzyme that will completely breakdown complex pectins.

Commercial 'pectinases' are usually mixtures of enzymes such as polygalacturonase and pectin lyase. Biocatalysts range of macerating enzymes, for fruit juice processing, has been carefully tailored to contain the correct ratio of the enzymatic activities required for specific applications. The optimal activity of these enzymes is pH 4 - 5 (the natural pH of the fruit) and temperatures below 50°C. These are ideal conditions for fruit processing.

Polygalacturonase (PG)

Responsible for the random hydrolysis of alpha-1,4- glycosidic linkages between galacturonic acid residues. Depolymerise low esterified pectin (endo- and exo- enzymes) Pectin lyase (PL) - (EC 4.2.2.10), Cleaves the pectin, by an elimination reaction releasing oligosaccharides with non-reducing terminal alpha-1,4- linked galacturonic acid residues, without the necessity of pectin methyl esterase action Pectin methylesterase (PE) - (EC 3.2.1.11), Releases methanol from the pectyl methyl esters, a necessary stage before the polygalacturonase can act fully (the increase in the methanol content of such treated juice is generally less than the natural concentrations and poses no health risk).

Xylanase (Hemicellulase)

a mixture of hydrolytic enzymes including xylan endo-1,3-beta-xylosidase, (EC 3.2.1.32) and xylan 1,4-â-xylosidase, (EC 3.2.1.37), which degrade hemicellulose.

Arabonases (ARA)

Hydrolyse arabinans (EC 3.2.1.55).

Ferulic Acid Esterase (FAE)

Cuts ferulic acid and other phenolic linkages between the xylan chains opening the structure to further degradation by xylanases.

Cellulase

Breaks down cellulose (EC 3.2.1.4).

Amylases

Breaks down starch (EC 3.2.1.1).

Washing and Mechanical Maceration

The fruit is first washed to remove external surface dirt and topical chemical residues. It is then chopped and/or crushed to mechanically break open the tissues. This should be completed as quickly as possible to avoid browning reactions.

Enzymatic Maceration

Treatment of the mashed fruit with macerating enzymes such as Macer8™ FJ or Pectinase 62L further breaks down the fruit pulp resulting in increased yields of juice, reduced viscosity and improved run-off. The enzymes can be added in the crusher/pulper and may be diluted in water to assist homogenous distribution through the pulp.

The dose, time and temperature should be determined in trials depending on the degree of maceration required and the type of fruit. It is not usually necessary to adjust the pH of the pulp as Biocatalysts enzymes are designed to work at the natural pH of the fruit. Liquefaction may be achieved, although the enzymes are equally beneficial in yield improvement for juice extraction and viscosity reduction. Enzymatic maceration can also result in subtle but generally beneficial changes in the flavour and, in the case of cider-making, shorter fermentation times as valuable components are released.

Separation of Juice and Solids

The juice can be separated from the remaining solids in a press or in a decanter.

Secondary Extraction

To achieve the maximum utilization of the raw material, a secondary extraction using further enzymes may be carried out. The solids are reslurried with condensate, and after an appropriate residence time, extracted again. Using belt presses, yields of 92% of soluble solids are possible. A decanter instead of a belt press may possibly be an interesting alternative.

Clarification

One of the major problems encountered in the preparation of fruit juices and wine is cloudiness due primarily to the presence of pectins. One litre of juice with a dry matter content of 13% can contain 2-5g of pectin. The pectin can be associated with other plant polymers and the cell debris. The cloudiness that these cause is difficult to remove except by enzymic depectinization. After pressing, the juice is transferred to a stirred holding tank.

Pectinases such as Macer8™ FJ, Pectinase 62 L or Pectinase 444L can be added to the juice and incubated typically at 40°C-50°C. The dose should be determined in trials depending on the degree of depectinisation desired and the type of fruit used. It is not usually necessary to adjust the pH as Biocatalysts enzymes are designed to work at the natural pH of the fruit. The reaction is usually allowed to continue until a negative pectin (alcohol) test is obtained. The arabinase activity of preparations such as Pectinase 444L are particularly useful if the fruit contains high levels of arabans to prevent them forming a haze. As immature fruit can also contain high levels of starch it may be beneficial to also add a fungal amylase such as Depol 220L to prevent formation of haze in the pasteurisation stage. Enzymatic treatment has the additional benefits of reducing the solution viscosity. Insoluble plant material is easily removed by filtration, or settling and decantation, once the stabilising effect of the pectins on the colloidal haze has been removed.

Fining Agents (Bentonite)

The activity of pectinases are reduced by bentonite and it is recommended that enzyme activity is allowed to complete before fining agents are added.

Concentration/Pasteurization

The clarified fruit can then be concentrated, for example, by ultrafiltration and heat-treated (pasteurised) to kill any microorganisms that might be present. This heat treatment also helps improve the overall clarity of apple juice. Before being placed in the appropriate container (such as bottles), the juice may be further filtered and given an additional heat treatment to ensure safety.

Biocatalysts Enzymes for Juice and Cider Cellulase 13L/P;

- Suitable for use when complete cellulose hydrolysis is required e.g. fruit liquefaction
- Reduces viscosity
- Available as a powder or liquid
- Side activities of xylanase and glucanase.

Cellulase 13P is an enzyme preparation for the degradation of cellulose and other viscosity forming polysaccharides. Added to the crusher/pulper in combination with pectinases it can aid the complete liquefaction of fruits.

Macer8™ FJ;

- Gives improved performance in fruit juice extraction processes
- Successfully used with cranberries, strawberries and grapes
- Effective depectinisation due to high PG & PL
- Works at acidic pH (3.0 - 5.0)
- Low methanol production due to the low pectin esterase activity.

Macer8™ FJ is ideally suited for the production of high quality apple and pear juices. It contains a balanced mix of pectinases designed to provide controlled maceration and depectinisation of fruit, without generating undesirable levels of methanol. The controlled disintegration of the fruit results in improved juice yield and full flavour recovery.

Pectinase 444 L;

- Used to increase breakdown of cell wall components in processing
- Results in increased extraction of apple juice and decrease in waste
- Hydrolyses structural pectin to release shorter chain molecules which are more soluble and less fibrous
- High arabinase activity helps clarification and prevents haze
- Low PE ensures minimal methanol production
- Active down to pH 2.5 and from 10 to 60°C.

Pectinase 444L is a unique pectinase designed to provide economical depectinization of fruit juices. It can be added either to the pulp or juice to provide a controlled disintegration and rapid reduction in viscosity. It promotes improved juice yield, full flavour recovery and rapid clarification of the juice. It has an optimised PG to PL ratio and high maceration index. The high arabinase activity helps prevent arabinan haze formation and the low PE activity ensures only minimal levels of methanol are produced.

Depol 220 L;

- High strength fungal amylase
- Used to remove starch from apple juice
- Required when early season apples are used
- Eliminates the haze that can form on pasteurisation
- Increases stability of the product
- Active at low pH (3.0 - 6.0).

Depol 220L contains a high strength fungal alpha amylase used to prevent the formation of haze in juice made from early season apples. Although ripe fruit contains very little starch, immature fruit can contain up to 2% by weight of starch granules.

Following pasteurisation, this can produce a haze which is difficult to remove. Depol 220L can be added routinely at the clarification stage as a precaution against starch haze. It is active in the acidic conditions of apple juice. The additional sugars released sweeten the juice and can act as fermentable substrates in cider making.

Pectinase 62 L;

- Breakdown of structural pectins
- For use in white wine production
- Improves yield of grape must
- Enhances flavour by releasing desirable components from cell wall material
- High PG/PL ratio
- Arabanase activity.

Pectinase 62 L is a mixed pectinase preparation with a high PG to PL ratio and a moderate maceration index.

It also contains arabinase activity. It is used to improve the yield from pressed fruit pulps by breaking down pectins and can be used to clarify juice. It demonstrates excellent stability at low pH and is particularly suited for processing high-acidity fruit.

Glucose Oxidase G789L;

- Removal of oxygen from soft drinks
- Desugaring.

Glucose Oxidase G789L can be used to extend the shelf life of soft drinks by scavenging oxygen. The enzyme catalyses the oxidation of glucose with oxygen to form gluconate and hydrogen peroxide.

Exotic Fruit

Exotic fruit such as mangoes contain a high proportion of recalcitrant tissues which are not readily degraded by general fruit processing enzymes. These may require enzymes more typically associated with vegetable processing such as Depol™ 40L (D040L) and Depol™ 670L (D670L) for effective juice extraction.

Fruit and Vegetable Processing

In developing countries agriculture is the mainstay of the economy. As such, it should be no surprise that agricultural industries and related activities can account for a considerable proportion of their output. Of the various types of activities that can be termed as agriculturally based, fruit and vegetable processing are among the most important. Both established and planned fruit and vegetable processing projects aim at solving a very clearly identified development problem.

This is that due to insufficient demand, weak infrastructure, poor transportation and perishable nature of the crops, the grower sustains substantial losses. During the post-harvest glut, the loss is considerable and often some of the produce has to be fed to animals or allowed to rot.

Even established fruit and vegetable canning factories or small/medium scale processing centres suffer huge loss due to erratic supplies. The grower may like to sell his produce in the open market directly to the consumer, or the produce may not be of high enough quality to process even though it might be good enough for the table. This means that processing capacities will be seriously underexploited.

The main objective of fruit and vegetable processing is to supply wholesome, safe, nutritious and acceptable food to consumers throughout the year.

Fruit and vegetable processing projects also aim to replace imported products like squash, yams, tomato sauces, pickles, etc., besides earning foreign exchange by exporting finished or semi-processed products.

The fruit and vegetable processing activities have been set up, or have to be established in developing countries for one or other of the following reasons:

- diversification of the economy, in order to reduce present dependence on one export commodity;
- government industrialisation policy;
- reduction of imports and meeting export demands;
- stimulate agricultural production by obtaining marketable products;
- generate both rural and urban employment;
- reduce fruit and vegetable losses;
- improve farmers' nutrition by allowing them to consume their own processed fruit and vegetables during the off-season;
- generate new sources of income for farmers/artisans;
- develop new value-added products.

Importance of Fruit and Vegetables in World Agriculture

Fruit and vegetables represent an important part of world agriculture production; some figures are seen in Table.

Table 1 : Fruit and Vegetable World Production, 1991

Crop (Fruit)	*Production, 1000 T*	
	Total World	*Dev.ping all*
Appies	39404	14847
Apricots	2224	1147
Avocados	2036	1757
Bananas	47660	46753
Citrus fruits NES	1622	1231
Cantaloupes and other melons	12182	8733
Dates	3192	3146
Grapes	57188	14257
Grapefruit and pomelo	4655	2073
Lemons and limes	6786	4457
Mangoes	16127	16075
Oranges	55308	40325
Peaches and nectarines	8682	2684
Pears	9359	4431
Papayas	4265	4205
Plantains	26847	26847
Plums	5651	1806
Pineapples	10076	9183
Raisins	1041	470
Tangerines, mandarines, clementines	8951	4379
Watermelons	28943	19038
Currants	536009	
Raspberries	369087	
Strawberries	2469117	342009
Beans, green	3213	1702
Cabbages	36649	15569
Cauliflower	5258	2269
Carrots	13511	4545
Chilies + peppers, green	9145	6440
Cucumbers and gherkins	13619	7931

Eggplants	5797	4608
Garlic	3102	2446
Onions, dry	27977	17128
Peas, green	4856	1038
Pumpkins, squash, gourds	7933	6245

What Fruit and Vegetables can be Processed?

Practically any fruit and vegetable can be processed, but some important factors which determine whether it is worthwhile are:

a. the demand for a particular fruit or vegetable in the processed form;

b. the quality of the raw material, i.e. whether it can withstand processing;

c. regular supplies of the raw material.

For example, a particular variety of fruit which may be excellent to eat fresh is not necessarily good for processing. Processing requires frequent handling, high temperature and pressure.

Many of the ordinary table varieties of tomatoes, for instance, are not suitable for making paste or other processed products. A particular mango or pineapple may be very tasty eaten fresh, but when it goes to the processing centre it may fail to stand up to the processing requirements due to variations in its quality, size, maturity, variety and so on.

Even when a variety can be processed, it is not suitable unless large and regular supplies are made available. An important processing centre or a factory cannot be planned just to rely on seasonal gluts; although it can take care of the gluts it will not run economically unless regular supplies are guaranteed.

To operate a fruit and vegetable processing centre efficiently it is of utmost importance to pre-organise growth, collection and transport of suitable raw material, either on the nucleus farm basis or using outgrowers.

Processing Planning

The secret of a well planned fruit and vegetable processing centre is that it must be designed to operate for as many months of the year as possible. This means the facilities, the buildings, the material handling and the equipment itself must be inter-linked and coordinated