ENZYMES IN FRUIT PROCESSING / LIQUIFICATION

Subject: Food Technology

By: Rtd. Prof. Ramteke
CFTRI, Mysore

INTRODUCTION

Enzymes are complex globular protein catalysts that accelerate chemical reaction rates by factors of $10^{12} - 10^{20}$ over that of uncatalyzed reactions at temperatures around 37°C. By contrast, industrial catalysts (inorganic substances) are orders of magnitude less effective than enzymes under comparable conditions. For example, the reduction of hydrogen peroxide catalyzed by catalase occurs 10 million times faster than it does when catalyzed by colloidal platinum at 37°C.

The catalytic efficiency of enzymes is very high, whereby one molecule of enzyme can transform as many as 10,000-1,000,000 molecules of substrate per minute. It is this catalytic efficiency of enzymes at low temperature which makes them important to the food scientist. This means that foods can be processed or modified by enzymes at moderate temperature, say 25-50°C, where food products would not otherwise undergo changes at significant rate. It also means, however, that endogenous enzymes are active under these conditions as well, and this can be beneficial or deleterious.

The use of technical enzymes has been an essential part of the entire technology of fruit juice production from the beginning. In the early 1930, the first step were made to process fruit into juice that could be stored for a long time without the danger of alcoholic fermentation or other forms of undesired spoilage. Fruit juices are still considered to be an excellent alternative to alcoholic beverage.

In food manufacture, enzymes are used to improve the specific quality characteristics of food products, to improve storage stability or to provide greater convenience in recipe preparation. Commercial enzymes are used in food industry mainly for fruit juice clarification in beer and wine making, in the starch industry, in dairy and cheese operations.

Presence of active enzymes in food has been recognized and procedures were developed for enzyme inactivation by heat and chemical methods, in order to preserve the stabilityand quality of food during storage.

Further, enzymes because of their tremendous catalytic power and low activation energy are active at subfreezing temperature. Therefore important stimulants of degradativereactions in reference frozen foods.

This episode deals with

Enzyme activity as influenced by environmental conditions

Enzymes in food processing

Sources of enzymes

Pecticand cellulolytic enzymes and

Clarification of fruit juices

Enzyme activity as influenced by environmental conditions

The importance of enzymes to the Food Scientist is often determined by the environment within the food. Control of the environment is necessary to control enzymatic activity during food preservation and processing. In this section, the major factors affecting enzymatic activity are discussed. These factors include pH, temperature, moisture, ionic strength, ionizing radiation, shearing, pressure, and interfacial effects.

pH Effects

Extremes in pH will generally inactivate enzymes. Enzymes usually exhibit maximal activity at a particular pH value, termed the pH optimum. The relationship between pH and activity is illustrated . Most enzymes show maximum activities in the pH range of 4.5-8.0, and maximum activity is usually, but not always, confined to a rather narrow pH range. There are however, enzymes with extreme pH optima, such as pepsin, which has a pH optimum of 1.8.

Control of pH as it relates to enzymatic activity is important to the food scientist. In an industrial process, where enzymatic activity in desirable, the pH should be controlled to maximize that activity. However, the food scientist may wish to prevent or inhibit an enzymic reaction. For example, unwanted phenolase activity can be avoided by reducing the pH of the system well below (<3.0) the optimum pH of 6.5. This is frequently accomplished in fruits by adding natural acidulants, such as citric, malic, or phosphoric acids.

Effects of High-Temperature :

Basically, the food scientist must consider two major apposing factors as the temperature is increased.

- Increasing rate of enzyme reaction
- Increasing rate of enzyme destruction

For enzyme reaction – rate of reaction doubles with 10° C raise in temperature. Temperature coefficient =2 optimal activity =30 to 40°C. After 45°C denaturation occurs. Time – temperature relationship for total inactivation of enzymes is very important.

Low temperature Effects

Although some enzymes are denatured at subfreezing temperatures, most remain quite active after freezing and thawing. In addition, many enzymes exhibit significant activity in partially frozen systems. As the temperature of an enzyme solution is decreased for 0° to about 10° below its freezing point, enzymic activity can either increase or decrease, depending upon the enzyme and the system. A further decline in temperature almost always results in decreased activity.

Enzymes in food processing

Food industry is one of the largest users of industrial enzymes. In general, enzymes are used in the food industry for upgrading quality and byproduct utilization, for preparing synthetic foods, for achieving higher rates and levels of extractions, for improving flavor, and for stabilizing food quality.

The advantages of using enzymes for food processing include:

1. They are natural, nontoxic substances;
2. They catalyze a given reaction without causing unwanted side reactions;
3. They are active under very mild conditions of temperature and pH;
4. They are active at low concentration;
5. The rate of reaction can be controlled by adjusting temperature, pH, and the amount of enzyme employed;

6. They can be inactivated after the reaction has proceeded to the desired extent.

Table 1 : Enzyme of Significance in Food Processing

Enzyme	**Reaction**	**Use or potential use**
Glucose oxidase	β - D - glucose + O_2 → D - glucono - ∂ - lactone + H_2O_2	Remove glucose and oxygen from foods
Catalase	H_2O_2 + H_2O_2 → O_2 + 2 H_2O	Used to remove H_2O_2 from glucose oxidase reaction
Hydrolases 1. α - Amylase 2. β - Amylase 3. Gluco - Amylase	Hydrolyzeα - 1,4 - glucan links in starch 1. Internal random hydrolysis 2. Successive maltose units removed 3. Successive glucose units removed	1. Starch liquefaction 2. Produce maltose in bread fermentations and in high-maltose syrups 3) Produce glucose from starch
Pectic enzyme complex	Hydrolyze pectin to pectic acid, intermediate uronides, galacturonic acid, and 4-deoxy-5-ketogalacturonic acid	Clarify fruit juices and wines; degrade fruit pulp and increase extractability of juices
Hemicellulases	D-Xylans → Xylooligosaccharides+ D - xylose + L - arabinose	Reduce viscosity of coffee concentrates
Naringinase	Naringin → naringenin + rhamnose + glucose	Debitter citrus fruit products

3.0Sources of enzymes

Microbial enzymes for food applications are actually derived from few sources. Table 2 lists the microorganisms generally recognized as safe (GRAS) by the FDA, and the major enzymes associated with them. In addition to the enzymes produced by GRAS microorganisms, enzymes from other microorganisms are sometimes permitted.

Table 2 : Enzymes prepared from GRAS organisms

Organism	**Resulting enzymes**
Bacillus subtilis	Amylase (high temperature) Protease, neutral Protease, alkaline
Aspergillusoryzae,	Amylase, Glucomylase, Protease, Acid protease,
Aspergillusniger	Catalase, Glucose oxidase, Lipase, Anthocyanase, Naringinase, Cellulase, Hemicellulase, Pentosanase,Pectinase
Saccharomyces cerevisiae	Invertase
Saccharomyces fragilis	Lactase

5.0 Pectic enzymes

Pectin is a complex carbohydrate polymer which serves a structural role in plants. The major building blocks of pectin are units of galacturonic acid linked by α-1, 4-glycosidic bonds. Approximately two-thirds of the carboxylic acid groups are esterified with methanol. These are used in clarification of apple juice before membrane filtration by adding them to bulk juice. Food grade pectic enzymes contain mixture of enzymes including pectinase, polygalacturonase, pectin esterase, pectin lyase, cellulase, protease and amylase. Cell walls of each cell are thickened due to deposition of pectin and cellulose on the walls. This prevents the fruit juice to be released and hence remains as pulpy mass. Action of enzymes softens and rupture the cell wall enabling the release of juice. Depending on the type of juice, pectin is either an impediment to clarification (eg. Apple juice has to be clear) or a desirable component to retain (Orange juice must be cloudy).

5.1Pectin MethylEstarase (PME) : This enzyme hydrolyses the esterified methyl groups of the pectin or pectinic acid chains to give methyl alcohol and pectic acid units or chains.

5.2Polygalacturonase (PG) &Polymethylgalacturonase (PMG) : Both enzymes are chain splitting and also called "glycosidases" since they attack on the 1-αglycosidic linkages between two adjacent galacturmic acid units.

Cellulolytic enzymes

These enzymes play an important role in disintegration or softening of the cell wall micro fibrils, celluloseβ, 1-4 glucose units.

Amylases

Enzyme that hydrolyze starch are termed as amylases.

α- amylases : Endo enzyme which hydrolyses α 1-4 glucan linkage. Results in rapid decrease of viscosity

β - amylases

Exo-enzyme, it attacks only the end units of starch chains. It results in increase of sweetness. " Saccharifying" enzyme. Amylases are important in fruit ripening, potato processing and Corn syrup manufacture, etc.

Additions of above enzymes to the mash have following advantages.

Rapid and simple extractions of juice with decreased pressure build up

- Increase in free-run and total yield
- Increase in extraction capacity due to faster filtration rate.
- Fast viscosity reduction

Clarification of fruit juices

Pectin in fruit juice may suspend other materials in a colloidal system for the clarification of fruit juice,Therefore, pectolytic enzymes produced by molds have been long used. For optimizing conditions for enzyme liquefaction of a particular juice, it is important to standardize various conditions such as time of incubation, temperature and enzyme concentration using statistical designs. Based on the juice yield and clarity of the juice the optimum conditions can be deduced.

Banana pulp/ puree clarification

Banana fruit

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Peeling

Number of studies on the clarification of banana pulp/ puree have been carried out .The data on the effect of enzyme concentration, incubation temperature and time on the yield of banana juice shows that, the maximum juice yield (90%) was obtained from the pulp treated with 0.05% of enzyme (Pectinase) after 210, 180, 120 min incubation at RT, 35°C and 45°C.

Similarly, the effect of enzyme concentration on the clarity of the juice has also been studied

At the early stage of enzyme treatment the juice appeared turbid. It was observed that the juice with clarity of 90% showed good appearance. This degree of clarity could be obtained with the enzyme treatment of 0.05% after 180, 150 and 120 min at RT, 35°C and 45°C respectively. This level of clarity was also obtained with enzyme treatment of 0.04% after 240 and 150 min at 35°C and 45°C respectively. The most satisfactory conditions for the preparation of clarified banana juice were incubating the pulp with 0.05% ultrazyme at RT (26-28°C) for 3 hours. Under these conditions more than 87% of the juice yield was obtained with clarity as high as 90%.

Guava pulp/puree clarification

Guava pulp treated with 0.025% of pectic enzyme for 4 hrs at RT yielded about 66% of the juice while the viscosity was about 53%.Clarity of the juice obtained under these conditions was only 45%.However as the enzyme concentration was increased viscosity of the pulp further decreased. The results also indicated the necessity of high concentration of pectic enzyme to obtain high yield of guava juice with satisfactory quality.

The maximum yield of juice obtained was 81% and juice clarity 89.5% after 4 hours incubation at room temperature at an enzyme concentration of 0.75%
Guava pulp was treated with different concentration ofUltrazyme 100 and incubated at 45°C over a period of 4 hours.
The results obtained showed that enzyme treatment at 45°C considerably enhanced the enzyme activity.Higher temperature means higher enzyme activity yielding higher quantities of juice with better clarity.

It was observed that beyond 0.1% enzyme concentration there was no significant increase in juice yield, but clarity values continuously increased attaining its maximum value (94%) at 0.5% enzyme concentration.However even at enzyme concentration of0.1% the juice had good appearance with clarity value of 89%.

Changes in volatile components

Enzyme clarified juices showed very acceptable and characteristic aroma and flavor of the respective juices.However, in case of banana, ester content was decreased to about 3.1% while alcohol content increased by about 6.4% as

compared to control samples.In case of guava the loss in ester was of order 10.8% accompanied by increase in alcohol content of 8.8% as compared with control samples.

Enzymes in apple juice production

Apples are by far the most important fruit for production of clear juice concentrate worldwide.

Juice clarification : The extracted apple juice from the presses are always cloudy due to the presence of a wide range of natural colloidal polysaccharides, cellulosic fragments from skin and pulp and other small particles like protein fragments or polyphenols and tannins with protein. During crushing of the fruit and dejuicing process, these constituents enter the juice in solubilized and unsolublized colloidal form. The most important of these molecular substances is pectin. Hot clarification process is the most frequently used for the production of clear apple juice concentrate worldwide. In this process, the enzyme treatment and subsequent fining are carried out in the juice coming from the aroma recovery where, the juice has been heated up briefly in order to remove aroma and solubilize starch. The aroma has to be removed before concentration and stored separately. Due to quality reason,the dearomatized juice has a temperature of 50-55°C after passing through heat exchanger where it is cooled down by preheating the juice coming from the press. This temperature range is almost ideal for enzyme activity which takes almost 2-3 hrs. for clarification.

Compared to the fining procedures, ultrafiltration is a relatively new technology in fruit juice industry. In respect to the enzyme application, both the fining process and ultrafiltration process require complete enzymatic breakdown of pectin and starch or other polysaccharides. The practical application of enzymes for apple juice clarification is fairly simple. Liquid enzymes, which are by far the most widespread in fruit juice technology, are simply poured into the juice tank while filling up the tank with the juice. The correct dosage is usually determined with the help of pretest in the laboratory. A mechanical stirrer in the tank leads to good distribution of the enzyme. After the enzyme reaction the juice is ready for clarification, filtration and concentration

The most important test method for pectin degradation is so called alcohol test. The test is simple and reliable. The degradation of starch is tested by iodine test.

Although enzymes are useful as catalysts in food processing, they may not be always suitable for practical application. Conventionally, an enzymatic reaction is carried out in a batch process. More over presence of residual enzyme in processed food may, in certain cases cause allergy. To overcome this problem, the emphasis on the modification of natural enzymes by immobilizing them, will help the fruit juice industry on a long term basis.