Food Applications
Starch processing

Sugars having various useful properties have been manufactured by the use of enzymes.

Characteristics and Functions of Sugar produced from Starch by the Use of Enzymes

1. Glucose

Glucose is manufactured by liquefaction of starch with \( \alpha \)-amylase (KLEISTASE ESNC, T10S) followed by treatment with pullulanase (KLEISTASE PLF3) and glucoamylase. Its sweetness is about 60% that of sugar, and it has a reducing property. It is used not only as a sweetener for foods e.g. drinks and cakes but also as an energy source for transfusions, and as a raw material for the chemical industry e.g. production of sorbitol, amino acids.

2. Maltose

Maltose is manufactured by liquefaction of starch followed by treatment with pullulanase (KLEISTASE PLF3) and \( \beta \)-amylase (\( \beta \)-Amylase F "Amano"). Its sweetness is about 40% that of sugar and its molecular weight is the same as that of sugar, hence maltose is used as a light sweetener in place of sugar for Japanese cakes. It is also used for transfusion because maltose can be an energy source not requiring insulin. Another use for maltose is as an ingredient of Maltitol.

3. Maltotriose

Maltotriose is produced by liquefaction of starch followed by treatment with maltotriose-producing amylase (AMT 1.2L) and pullulanase (KLEISTASE PLF3). Its sweetness is about 30% that of sugar. Maltotriose is characterized by its non-crystalline form and strong water-preservative property, and thus has a starch anti-staling effect. With these properties, it is often used for Japanese cakes that must maintain a certain percentage of moisture.

4. Branched oligosaccharide (Isomaltol oligosaccharide)

Branched oligosaccharide is an oligosaccharide containing a series of \( \alpha \)-1,6 bonds in its structure. It is produced by liquefaction of starch followed by treatment with \( \beta \)-amylase (\( \beta \)-Amylase F "Amano"), pullulanase (KLEISTASE PLF3), and transglucosidase (Transglucosidase L "Amano"). Its sweetness is about 40% that of sugar, and it is easily utilized by intestinal bacteria but not by bacteria in the mouth, so it does not promote dental caries.

5. Cyclodextrin

Cyclodextrin is a nonreducing cyclic dextrin produced from starch by treatment with cyclodextrin glucanotransferase (Cyclodextrin glucanotransferase "Amano"). There are several types such as \( \alpha \)-cyclodextrin (glucose number: 6, \( \alpha \)-CD), \( \beta \)-cyclodextrin (glucose number: 7, \( \beta \)-CD), and \( \gamma \)-cyclodextrin (glucose number: 8, \( \gamma \)-CD). The hollow portion of the molecule is hydrophobic, thus it has the property of enclosing various compounds. With this property cyclodextrin is used for stabilization of various materials, preservation of aroma, masking bitter tastes, and increasing solubility of slightly soluble materials.
6. Isomerized sugar

Isomerized sugar is a mixture of glucose and fructose. There are two types of liquid sugar; glucose-fructose liquid sugar whose fructose content is more than 42% (available by treatment of glucose with glucose isomerase) and fructose-glucose liquid sugar whose fructose content is more than 55% (available by addition of high-fructose fraction obtained by separation of the above-mentioned type of liquid sugar with ion-exchange resin). They are inexpensive and their sweetness is almost equivalent to that of sugar; thus they are used as a sweetener to replace sugar in beverages and ice cream, for instance.

7. Fructose

Fructose is manufactured by its separation from isomerized sugar by the use of ion-exchange resin. Fructose used to be manufactured by acidic decomposition of an artichoke, but with the change of manufacturing method, it has become inexpensive and usable in the food industry too. Its sweetness is higher than that of sugar at cold temperature, but decreases at higher temperatures. For its strong browning at heating, fructose is mainly used for ice cream. Fructose is used to sweeten some diet foods and is also used in sports drinks.

Enzymatic Degradation of Starch
Protein processing

In the field of protein processing, protease preparations are mainly used for improvements of taste, yield of extract, nutritional content, and physical properties. Enzymes contained in the protease preparations are classified broadly into two types. One is proteinase, which hydrolyzes large protein molecules to smaller peptide chains. Another is peptidase, which releases amino acids from the terminals of proteins and peptides.

The sources of proteases are Fungal (Aspergillus - filamentous fungus Rhizopus - phycomycetes), Bacterial (Bacillus) and extracts of rhizome (papaya, pineapple). The reason why so many different protease preparations are on the market is that the types of degradation differ according to the source of protease. For instance, protein is generally degraded to smaller molecules by proteases from filamentous fungi rather than proteases from bacterial sources.

* A guide for Selection of Protease Preparations * is shown below for selection of protease preparations supplied by Amano Enzyme Inc.

_selection guide for using of amano’s protease and peptidase preparations_

<table>
<thead>
<tr>
<th>Flavor</th>
<th>THERMOASE PC10F</th>
<th>Protease P “Amano” 6 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement of physical properties (viscosity, solubility, etc.)</td>
<td>PROTEIN SD-AY10</td>
<td>PROTEIN SD-NY10</td>
</tr>
<tr>
<td>Extract for seasoning (Meat taste extract etc.)</td>
<td>Protease A “Amano” 2 SD</td>
<td>Protease M “Amano” 2 SD</td>
</tr>
<tr>
<td>Hydrolyzates rich in amino acid (HVP)</td>
<td>Protease M “Amano” SD</td>
<td>Peptidase R</td>
</tr>
<tr>
<td>Nutrition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptide</td>
<td>Protease A “Amano” 2 SD</td>
<td>Protease M “Amano” 2 SD</td>
</tr>
<tr>
<td>Peptidase R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td></td>
<td></td>
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<tr>
<td>Enzyme Modified Cheese</td>
<td>Protease M “Amano” SD</td>
<td>Peptidase R</td>
</tr>
<tr>
<td>Acid casein</td>
<td>Protease A “Amano” 2 SD</td>
<td>Peptidase R</td>
</tr>
<tr>
<td>Infant milk (Hypo-allergenicity)</td>
<td>Whey protein</td>
<td>Protease A “Amano” 2 SD</td>
</tr>
<tr>
<td>All food protein hydrolysates</td>
<td>Removal of bitterness</td>
<td>Peptidase R</td>
</tr>
</tbody>
</table>

**Production of protein hydrolysate using proteases**

Protein (Meat, Fish, Milk, Soybean, Wheat, Yeast extract, etc)

(Fungal Protease) Protease M “Amano” SD

(Bacterial Protease) THERMOASE PC10F

Amino Acids, Peptides

Glutamine

Glutamic Acid

Effect of protease in casein hydrolyzation

Casein

Hydrolyzed Casein by Protease
Oil & Fat processing

Lipases are used for processing of oils and fats in the food industry. Lipases principally hydrolyze triglycerides into fatty acids and glycerol.

**Utilization of Lipase Reaction**

1. **Manufacture of dairy flavor**
   
   Enhancement of dairy flavor is closely related to fatty acids produced by hydrolysis of milk fats. Compositions of fatty acids after hydrolysis (i.e. short, medium or long chain) are different depending on the lipase due to differences in substrate specificity and positional specificity of lipases. These differences are used to produce a variety of aroma to enhance butter and cheese flavors in margarine, cakes, and snacks.

2. **Purity improvement of triglycerides**
   
   Lipase G "Amano" 50 decomposes partial glycerides into glycerol and fatty acids selectively, resulting in an increase in triglyceride content in the oils and fats.
   
   Immobilized Lipase G "Amano" 50 can be used to synthesize triglyceride from partial glycerides in an extremely micro-aqueous reaction system, which contributes not only purity improvement of triglycerides, but productivity.

3. **Concentration of PUFA (EPA, DHA)**
   
   EPA and DHA, well known as healthy Omega-3 fatty acids, are contained in fish oil.
   
   Lipase AY "Amano" 30SD can preferentially separate fatty acids besides EPA and DHA from oil to be able to produce high-content EPA and DHA oil.

**Selection Guide for Using of Amano's Lipase Preparations**

<table>
<thead>
<tr>
<th>Application</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Manufacture of Dairy Flavor</td>
<td>Lipase AY &quot;Amano&quot; 30SD, Lipase MER &quot;Amano&quot;</td>
</tr>
<tr>
<td>Milk, Butter, Cheese flavor</td>
<td>Lipase R &quot;Amano&quot;, Lipase DF &quot;Amano&quot; 15, Lipase A &quot;Amano&quot; 12</td>
</tr>
<tr>
<td>2. Purity Improvement of Triglycerides</td>
<td>Lipase G &quot;Amano&quot; 50</td>
</tr>
<tr>
<td>3. Concentration of PUFA (EPA, DHA)</td>
<td>Lipase AY &quot;Amano&quot; 30SD</td>
</tr>
<tr>
<td>4. Others</td>
<td>Lipase A &quot;Amano&quot; 12, Lipase DF &quot;Amano&quot; 15</td>
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</tbody>
</table>
Yeast extract

Yeast extract is a soluble concentrate extracted from yeast such as Saccharomyces species (Brewer’s yeast, Baker’s yeast) and Torula yeast, and is used widely as a flavor enhancer containing inosinic acid and guanylic acid. It is manufactured by autolysis with endogenous enzymes, or by enzymatic treatment using exogenous enzymes.

Flavor components are classified into a nucleic acid type, protein type, and trace component type. Main flavor components of a nucleic acid type are inosinic acid (IMP) and guanylic acid (GMP). Yeast extract is manufactured using exogenous enzymes in the following steps; (1) destruction of mainly cell walls with enzymes (YL-T “Amano” L) for extraction of RNA, (2) production of adenylic acid and guanylic acid by treatment with nuclease (Enzyme RP-1G) that degrades DNA and RNA, and (3) conversion of adenylic acid to tasty inosinic acid by treatment with deaminase (Deamizyme 50000G).

The flavor components of a protein type are amino acids and peptides. They are produced by treatment with protease (Protease M “Amano” SD) or peptidase (ProteAX, Peptidase R). Improvement of flavor is attained by converting glutamine released from yeast proteins to glutamic acid by treatment with glutaminase (GLUTAMINASE SD-C100S).

Typical Manufacture of Yeast Extract by Amano’s Enzyme
Others

- **Improvement of Yield of Refined Sugar**
  During the process of sugar refining, filtration becomes difficult and sugar crystallinity lowers if dextran is present in the materials. Dextranase (Dextranase L "Amano"), which degrades dextran, is used for improvement of the filterability and crystallinity of sugar.

- **Deoxygenation and Removal of Sugar**
  Glucose oxidase/catalase preparation (Hyderase) is used for prevention of oxidation caused by dissolved oxygen in beverage or atmospheric oxygen. It is also used to prevent browning caused by Maillard reaction between amino compounds such as protein and reducing sugars.

- **Lactose Degradation**
  People with lactose intolerance complain about abdominal distention, stomachache, or diarrhea when they drink milk. The causative substance is lactose, and low lactose milk is manufactured for these people. Lactase (Lactase F "Amano") is used for decomposition of lactose.

- **Oxidation of Polyphenol**
  Laccase (LACCASE M120) is an enzyme that catalyses oxidative polymerization of polyphenols with atmospheric oxygen. Active application developments utilizing this property is under progress in various fields, including breath freshening products (chewing gum etc.), hair dyeing, color enhancement in tea, promotion of lacquer drying, modification of polyester, and processing of waste water.

- **Baking**
  For baking application, lipase (Lipase DF "Amano" 15) use contributes to reduced need for emulsifying aids, since monoglycerides and diglycerides produced by lipase have emulsification properties.

- **Enhancement of Aroma**
  Beta-Glucosidase (Aromase) can improve and enhance aroma, such as tea, fruit, flower. Aroma components exists as glycosides, thus many of them are non-volatile. Wide specificity of Aromase can hydrolyze glycosides to enhance volatile aroma.
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