Food Science and Technology

Edited by

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WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

IUFoST

This edition first published 2009

Blackwell Publishing was acquired by John Wiley & Sons in February 2007. Blackwell's publishing programme has been merged with Wiley's global Scientific, Technical, and Medical business to form Wiley-Blackwell.

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Editorial offices
9600 Garsington Road, Oxford, OX4 2DQ, United Kingdom
2121 State Avenue, Ames, Iowa 50014-8300, USA

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Library of Congress Cataloging-in-Publication Data

Food science and technology / edited by Geoffrey Campbell-Platt.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-632-06421-2 (hardback : alk. paper)
1. Food industry and trade. 2. Biotechnology. I. Campbell-Platt, Geoffrey.
II. International Union of Food Science and Technology.
[DNLM: 1. Food Technology. 2. Biotechnology. 3. Food Industry.
4. Nutritional Physiological Phenomena. TP 370 F6865 2009]
TP370.F629 2009
664–dc22
2009001743

A catalogue record for this book is available from the British Library.

Set in 9.5/12pt Palatino by Aptara® Inc., New Delhi, India
Printed and bound in Singapore by Fabulous Printers Pte Ltd

1 2009

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5 Food biotechnology

Cherl-Ho Lee

Key points

- The history of food biotechnology including alcohol fermentation, acid fermentation, bread fermentation and amino acid/peptide fermentation throughout the world.
- Recent developments in enzyme technology and the industrial production of amino acids, nucleic acids and organic acids.
- The basis of genetic engineering and tissue culture used in modern food biotechnology.

5.1 History of food biotechnology

Biotechnology has been broadly defined as the utilization of biologically derived molecules, structures, cells or organisms to carry out a specific process (Wasserman *et al.*, 1988). Many conventional food processing techniques utilize living organisms and bioactive molecules especially in the brewing and fermentation industries. Alcoholic food and drinks, cheese, yogurt, lactic acid fermented vegetables, soybean sauce and fish sauce have been made for thousand years by using naturally occurring microorganisms which grow in a specific environmental condition. Plant enzymes such as malt have been used in the brewing industry long before man acquired the knowledge of enzyme chemistry.

Traditional food fermentation technologies are based on the natural process whereby wet foodstuff undergoes microbial degradation, and when it is edible we call it fermented food and when it is not we call it spoiled or putrid food. Man has acquired fermentation skills over a long time and has developed unique technologies suitable for the specific environment and raw materials available in different regions of the world. The first product of fermentation man discovered was alcoholic fermented fruits, which contain sugar that is fermented by natural yeast to make alcohol. More sophisticated fermentation skills using cereals to make alcohol were developed later; beer in Egypt and rice wine in Northeast Asia, both in around 4000 BC (Owades, 1992; Lee, 2001). The oldest known written recipe was found on a 4000-year-old Mesopotamian clay tablet, for beer (Owades, 1992). The Babylonians made 16 kinds of beer, using barley, wheat and honey. The Chinese book *Shijing* (1100—600 BC) has a poem describing a 'thousand wines of Yao', a legendary nation of China in around 2300 BC. It appears that the fermentation technology in Northeast Asia must have been invented by the littoral foragers of the primitive Pottery Age (8000–3000 BC) before agriculture began (Lee, 2001).

Drying and fermentation were the most important food preservation technologies until the industrial revolution in the seventeenth century in most regions of the globe from temperate to tropical regions. The world of microorganisms was opened to human beings with the invention of the microscope by Antonie van Leeuwenhoek (1632–1723), and the scientific control of fermentation began with the studies of Louis Pasteur (1822–1895). Pasteur showed that good wine batches had certain types of ferment (microorganism), and bad batches had other types of ferment. By heating juices at 63°C for 30 min he could kill the bad ferments and after cooling the juice he could consistently produce satisfactory wine by inoculating ferments from good wine batches into the juice. This idea was also applied in the pasteurization process of milk, which contributed greatly to the improvement of food hygiene.

Enzymes, the biocatalysts, have been known since the early seventeenth century from observation of their role in digestion and fermentation processes. However, isolation of the crystalline form of enzyme was first achieved with urease in 1926. Thereafter amylase, carboxypeptidase, papain and pepsin were isolated from plants, animals and microorganisms. With the development of enzyme technology, the conventional industrial enzymes originating from plants and animals were substituted by microbial enzymes. Chymotrypsin from microorganisms containing the milk clotting enzyme chymosin has partially replaced rennet from the stomach lining of young calves. When genetic engineering techniques developed in the 1970s, the first application of GMO in food was the production of food enzymes.

Using GMO microorganisms, numerous food enzymes have been developed which have higher activity and are more tolerant of extreme working conditions such as high temperature. The production of biotech crops, especially corn and soybean, has increased rapidly since their first marketing in 1995. The cultivation area of GM crops reached over 120 million hectares in a total of 23 countries in 2007 (James, 2007). Table 5.1 summarizes the milestone events of food biotechnology through history.

5.2 Traditional fermentation technology

The traditional fermented foods of the world can be classified by the materials obtained from bioconversion, such as alcohol fermentation, acid fermentation, carbon dioxide (bread) fermentation and amino acid/peptide fermentation from protein (Steinkraus, 1993; Lee, 2001). Depending on the raw materials used numerous varieties of fermented food are made in each class of fermentation (Steinkraus, 1983). For example, in alcohol fermentation, wine from grape, cider from apple, toddys from palm sap, beer from barley or corn, *chongju* from rice and even *mayuchu* from horse milk are made. Furthermore, by distillation, brandy, rum, vodka, whisky and soju are produced. Figure 5.1 shows a traditional fermented food map of the world.

The traditional societies can be divided by the indigenous fermented foods they produce; for example, the cheese/yogurt culture of the Middle East, Northern Africa and Europe, fish sauce culture of Southeast Asia and soybean sauce culture of Northeast Asia. These products are made by the breakdown of proteins to produce the umami (meaty) taste of amino acids and peptides, and form the basic flavor of the meals and condiments characterizing the dietary culture of these different regions.

5.2.1 Alcohol fermentation

Alcoholic beverages have played an important role in human spiritual and cultural life both in Western and Eastern societies. Unlike in Europe and the Middle East, where most of the indigenous alcoholic beverages are produced from fruits, in the Asia-Pacific region alcoholic beverages are produced from cereals and serve as an important source of nutrients. European beer uses barley malt as the major raw material, while Asian alcoholic beverages utilize mold-grown *nuruk* made from rice or wheat as the fermentation starter.

Alcohol is produced from glucose by the action of the alcohol producing yeast, *Saccharomyces cerevisiae*. In 1810 Gay-Lussac established the fermentation formula:

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$

The general processes of alcohol fermentation of wine, beer and rice wine are presented as follows:

$$\text{Fruit juices} \xrightarrow{\text{yeasts}} \text{Wine}$$

$$\text{Barley} \xrightarrow{\text{sprouting}} \text{Malt} \xrightarrow{\text{yeasts}} \text{Beer}$$

$$\text{Rice} \xrightarrow{\text{molds}} \textit{Nuruk} \xrightarrow{\text{yeasts}} \textit{Chongju}\ \text{(rice wine)}$$

Table 5.1 Milestones in food biotechnology.

Date	Milestone in food biotechnology
6000 BC	Use of earthenware for cooking and storage in Northeast Asia Yeasts employed to make wine and beer in the Middle East
4000 BC	Leavened bread produced with the aid of yeasts in Egypt Fungal fermentation of cereals in earthen jars in Northeast Asia Salt fermentation of marine products and plants in earthen jars Curdling of milk in skin bags for cheese making in the Middle East
2000 BC	Fermentation starter 'nuruk' employed for rice wine 'Thousand rice-wines in Yao' of China Use of soybean as food in South Manchuria and the Korean Peninsula
200 BC	*Bacillus subtilis* employed to ferment soybean 'shi'
1680	Antoni van Leeuwenhoek invented the microscope and discovered microbes
1857	Louis Pasteur discovered anaerobic fermentation Pasteurization process began
1876	Pasteur proved microbial action on beer fermentation
1897	Buchner discovered that enzymes in yeast juice convert sugar into alcohol
1904	Pure cultured fermentation starter 'koji' developed in Japan
1912	Industrial chemicals (acetone, butanol, glycerol) obtained from bacteria
1928	Alexander Fleming discovered penicillin
1953	Industrial production of glutamate by soil bacteria Double helix structure of DNA revealed by Watson and Crick
1960	Industrial enzyme production from microorganisms
1965	Borlaug's Green revolution
1973	DNA recombination by Cohen and Boyer
1975	Hybridomas which make monoclonal antibodies first created
1976	US NIH guidelines on genetic engineering
1982	Genetic engineered insulin approved for use in diabetics in the US and UK First approval for release of GM microbes into the environment
1994	Introduction of GM Flavr Savr Tomato on market by Calgene, Inc. Herbicide-tolerant Round-Up Ready GM soybean by Monsanto Co.
1996	Herbicide-tolerant and insect-resistant YieldGard GM corn on market
2000	Development of Golden Rice™

Malt contains amylases and is able to break down starch into fermentable sugars. The fermentation starter, *nuruk* in Korea, is made by growing molds on cereals, either raw or cooked, to digest starch into sugars, which are then consumed by the yeast to produce alcohol. Rice-wine fermentation is therefore called a two-step fermentation process. Table 5.2 summarizes the names of cereal fermentation starters used in Asia-Pacific regions, their ingredients and the microorganisms involved.

5.2.1.1 Wine

Wine is the fermented product of grape, mainly of cultivars of *Vitis vinifera*. Fermented juices of many other fruits, for example apples, berries, peaches and even herbs, are also called wine. The distinctive character of various wines depends on the composition of the raw material, the nature of the fermentation process, and processing and aging treatments. Table wines with excess carbon dioxide include white,

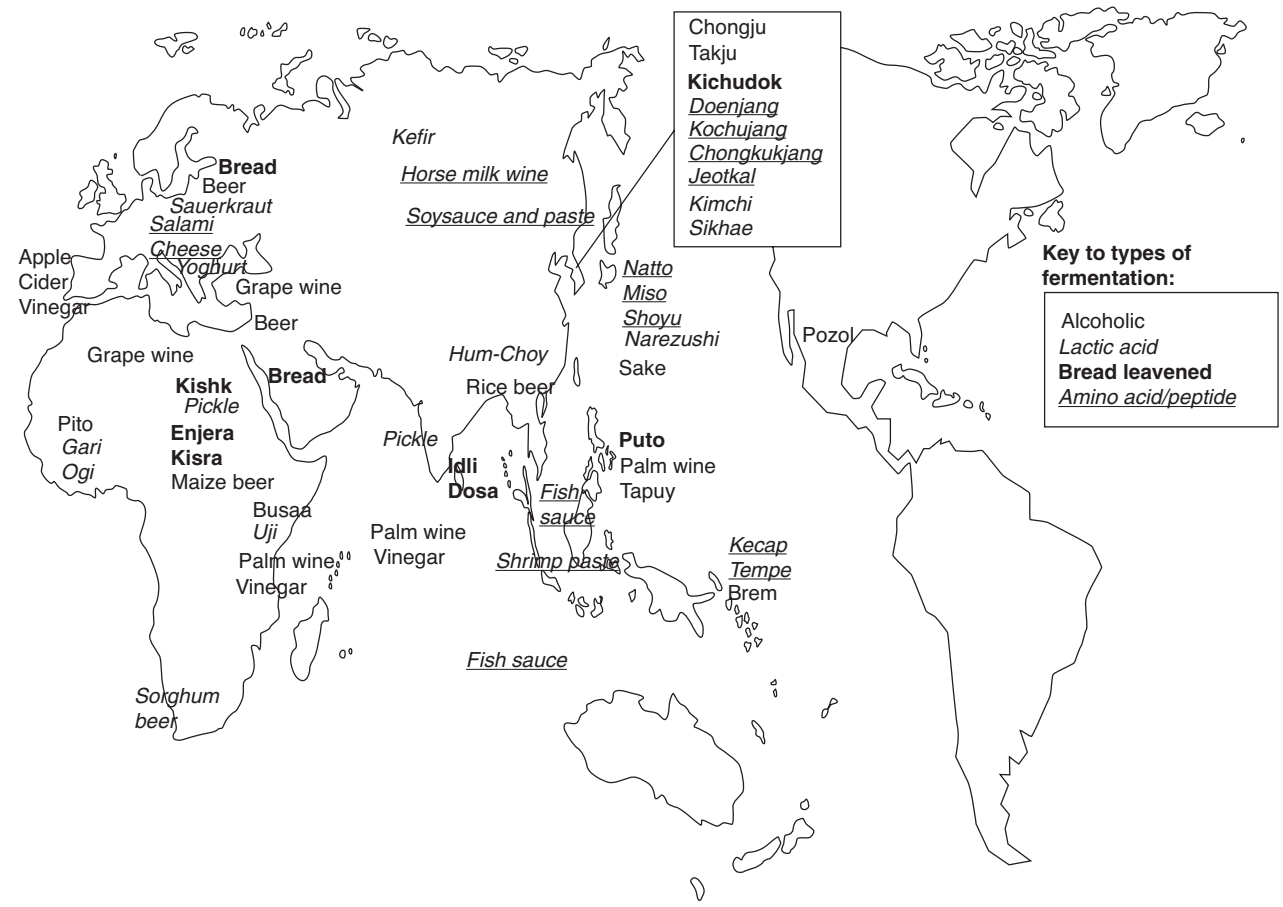


Figure 5.1 Traditional fermented food map of the world.

Table 5.2 Names of fermentation starters in different countries and the major ingredients used (Lee, 1998).

Country	Name	Ingredients commonly used	Shape	Microorganisms
China	Chu	Wheat, barley, millet, rice (whole grain, grits, flour or cake)	Granular	*Rhizopus* *Amylomyces*
Korea	Nuruk	Wheat, rice, barley (whole grain, grits or flour)	Large cake	*Aspergillus* *Rhizopus* Yeasts
Japan	Koji	Wheat, rice (whole grain, grits or flour)	Granular	*Aspergillus*
Indonesia	Ragi	Rice (flour)	Small cake	*Amylomyces* *Endomycopsis*
Malaysia	Ragi	Rice (flour)	Small cake	No data available
Philippines	Bubod	Rice, glutinous rice (flour)	Small cake	*Mucor* *Rhizopus* *Saccharomyces*
Thailand	Loogpang	Bran	Powder	*Amylomyces* *Aspergillus*
India	Marchaa	Rice	Flat cake	*Hansenula* *Mucor* *Rhizopus*

pink and red sparking wines, with or without Muscat flavor (champagne, spumante, Sekt, etc.). Non-sparkling white table wines are dry or sweet with regional, varietal and proprietary names (Riesling, Chardonnay, Chablis, Sauternes, etc.) Pink and red table wines are among the most important in terms of volume or consumer demand and have varietal, regional and proprietary names (Cabernet Sauvignon, Pinot noir, Burgundy, Bordeaux, etc.). There are also numerous dessert wines named after the grape variety from which they are produced, the processes and the added flavors and herbs (Amerine *et al*., 1980).

Wines are produced in many different ways. In general, grapes are washed, removed from the stem and macerated. The must is stored in a fermentation vat. The normal flora of ripe grapes and the winery contain sufficient yeast to initiate fermentation. However, the addition of pure actively growing yeast cultures or pressed yeast is common and desirable, and numerous strains of yeast for wine making are available in the market. To prevent growth of undesirable microorganisms 25–100 mg/L sulfur dioxide (in the form of potassium metabisulfite) is added to the must about 2 hours before the yeast. Sulfur dioxide acts as a selective antiseptic for bacteria and wild yeasts and permits the more rapid growth of the added yeast.

The optimum temperature for fermentation differs in red and white wines; red musts are fermented at up to 26.7°C and whites to 15°C. Red musts are fermented on the skins until the maximum color is extracted, often 5–10 days. The solid material, now called the marc or pomace, is transferred to the press. The fermentation of white musts, at the lower temperature, may last several weeks. A basket press or Willmes press in the larger wineries is often used for the filtration. For fining of the filtrate gelatine solution or egg white is added to combine with tannins and related compounds and precipitate. In order to remove excess potassium acid, tartrate wines are stored at a low temperature of approximately –2°C. The clarified wines are bottled and stored in a cool place for aging.

The maximum sugar accumulated by grapes during ripening depends on the variety of grape and the climate of the region where the grapes are grown. For most varieties ca. 15–25% sugar is reached, but late-harvest fruits may have 30–40% sugar. The actual percentage of alcohol in the finished wine depends not only on the sugar content of the must (crushed grapes) but also on the completeness of the fermentation, and in the case of dessert wines, the amount of alcohol added during or after the fermentation. At least 9 vol % of alcohol is needed to prevent rapid acetification of the finished product. When the sugar content of the grapes is too low to attain this percentage alcohol (1% sugar yields 0.55% alcohol according to the fermentation formula), sugar or grape concentrate may be added. The process, called chaptalization, is normal in cool-climate regions (eastern United States, Germany, France (Burgundy), etc.) but is prohibited in other areas (California, Spain, Italy, etc.).

5.2.1.2 Beer

Beer is an alcoholic beverage derived from barley malt, with or without other cereal grains (rice, corn, sorghum and wheat), and flavored with hops. Malt is made by three steps: the steeping of barley, germination and drying. By keeping steeped barley (45% moisture) in a humid and dark place for 4–6 days the barley starts to germinate and produce starches, splitting enzymes, α-amylase and β-amylase, and also protease and cellulase. When the modification is completed, the malt is dried. The drying stabilizes the malt and allows beer to be produced year round anywhere, even where barley is not grown. This differs from wine, which is only produced seasonally and only near grape-growing areas.

Beer production involves three distinct stages: brewing, fermentation and finishing. An extract of the crushed malt and the grains selected is prepared to make wort. This step takes about 4–10 hours at around 50°C and is often referred to as brewing. The mashing temperature and duration vary with the mashing systems. Mashing converts insoluble starch into fermentable sugars, maltose and glucose, and proteins into peptides and amino acids. The converted mash is separated into liquid (wort) and insoluble husk by filtration. The clear wort is boiled in a kettle. During the heating period hops are added. The insoluble humulones in the hops undergo a chemical rearrangement during this process to form isohumulones, which are soluble in water and impart to beer a palate-cleansing bitterness that provides beer with its unusual property of drinkability (Owades, 1992).

The next stage is fermentation, the conversion of wort by yeast into beer. Yeast is added to a cool wort containing oxygen, fermentable sugars and various nutrients including amino acids. The two main types of beer, lager and ale, are fermented with different strains of yeast. Lager is produced by bottom-fermenting *Saccharomyces uvarum* (*carlsbergensis*) at

fermentation temperatures between 7 and 15°C, and at the end of fermentation, these yeasts flocculate and collect at the bottom of the fermenter. For the production of ale, top-fermenting yeast, *Saccharomyces cerevisiae*, is used at fermentation temperatures between 18 and 22°C. *Saccharomyces cerevisiae* is less flocculent and collected for reuse from the surface of the fermenting wort (Russell and Stewart, 1995). The differentiation of lagers and ales on the basis of bottom and top cropping has become less distinct with the advent of vertical conical bottom fermenters and centrifuges.

The fermented beer may be finished in several ways. The simplest and most widely used method is merely to transfer the beer to another tank, chilling it en route, and keeping it for 7–14 days, which is called 'rest'. During this period much of the still suspended yeast settles, and some harsh, sulfury notes and undesirable flavor compounds, notably diacetyl, are removed. After finishing, the beer is filtered, always in the cold, through diatomaceous earth as the filter medium. For sterile filtration to remove all yeast and lactobacilli, another diatomaceous earth filter, cotton fibers, a porous plastic sheet or a ceramic filter are used as the retaining barrier. If packaged beer has not been sterile-filtered, it must be pasteurized, as beer is a fertile medium for many microbes. The pasteurization may be done just before filling (bulk pasteurization) or after filling in long tunnels with hot-water sprays (tunnel pasteurization). Bulk pasteurization takes about a minute, tunnel pasteurization about an hour.

5.2.1.3 Rice wine

Rice wine is a generic name for alcoholic beverages made from cereals, mainly rice, in East Asia. Traditional alcoholic beverages vary from crystal-clear products to turbid liquid or thick gruels and pastes. Clear products which are generally called *shaosingjiu* in China, *chongju* in Korea and *sake* in Japan contain around 15% alcohol and are designated as rice wine, while turbid beverages, *takju* in Korea and *tapuy* in the Philippines, contain less than 8% alcohol along with suspended insoluble solids and live yeasts, and are referred to as rice beer. Examples of alcoholic beverages prepared from cereals in the Asia-Pacific region are listed in Table 5.3 (Lee, 2001).

The process of cereal alcohol fermentation using *nuruk* involves two-step fermentation: a solid state fermentation growing mold on raw or cooked cereals which is called *nuruk*, and mashing the *nuruk* with additional cereals to produce alcohol using yeast. The dried and powdered *nuruk* is mixed with water and stored in a cool place for several days to make a 'mother' brew. During this period the microbial amylases and proteases are activated and convert starches into sugars. The acid-forming bacteria in *nuruk* produce organic acids to bring the pH below 4.5. About 2–3 volumes of cooked grains and water are added to the mother brew to prepare a first fermentation mash. By the addition of new cooked grains and water to the mash the volume of production increases and the alcohol concentration and quality of the final product enhances. Multiple brews prepared by adding newly cooked grains to the fermenting mash two, three, four and up to nine times have been described in the old literature (Yoon, 1993).

Newly cooked cereals are added at the end of each step of the fermentation process. The incubation period for each step of the brewing process varies from 2 days to 1 month depending on the fermentation temperature. Low temperatures (ca. 10°C) are better for improving the taste and keeping quality of rice wine. Traditionally rice wines are prepared in late autumn or early spring, when ambient temperatures are below 10° in the Far East. The volume of wine produced is approximately the same as that of the raw grain used (Rhee *et al*., 2003).

The traditional method of rice-wine brewing was industrialized by Japanese brewers in the early twentieth century, who adopted pure starter culture, rice *koji* and manufacturing technology from Europe and transferred it to Korea and China. Industrial production of rice wine uses pure cultured starter, *koji*, by the steaming of polished rice, inoculation of mold, *Aspergillus oryzae* or *kawachii*, and incubation at 25–30°C for 2–3 days. The mother brew is made by mixing *koji*, yeast seed mash and water followed by incubation for 3–4 days at 20°C. The main brew is made by adding ca. 10 times the volume of cooked rice and water to the mother brew and fermenting for 2–3 weeks. The fermented mash is filtered to obtain clear liquid and aged in a cool place for 1–2 weeks. It is filtered again and then bottled and pasteurized (Rhee *et al*., 2003).

Rice beers are produced at a higher temperature of fermentation (ca. 20°C). The fermentation starter powder is mixed with cooked cereals (rice, wheat, barley or corn) and water, and then incubated at approximately 20°C for 2–3 days, following which it is filtered through a fine mesh sieve or cloth. These beers are usually prepared by either single or double

Table 5.3 Examples of cereal alcoholic beverages in the Asia-Pacific region (Lee, 2001).

Product	Country	Major ingredients	Microorganisms	Appearance and usage
Rice wine				
Shaosingjiu	China	Rice	*Sac. cerevisiae*	Clear liquid
Chongju	Korea	Rice	*Sac. cerevisiae*	Clear liquid
Sake	Japan	Rice	*Sac. sake*	Clear liquid
Rice beer				
Takju	Korea	Rice, wheat	Lactic acid bacteria *Sac. cerevisiae*	Turbid liquid
Tapuy	Philippines	Rice, glutinous rice	*Saccharomyces* *Mucor* *Rhizopus* *Aspergillus* *Leuconostoc* *Lb. plantarum*	Sour, sweet liquid, paste
Brem bali	Indonesia	Glutinous rice	*Mucor indicus* *Candida*	Dark brown liquid, alcoholic
Alcoholic rice paste				
Khaomak	Thailand	Glutinous rice	*Rhizopus* *Mucor* *Saccharomyces*	Semi-solid, sweet, alcoholic
Tapai pulut	Malaysia	Glutinous rice	*Chlamydomucor* *Hansenula*	Semi-solid, sweet, alcoholic
Tape-ketan	Indonesia	Glutinous rice	*Asp. rouxii* *Saccharomycopsis burtonii*	Sweet/sour, alcoholic paste
Lao-chao	China	Rice	*Rhizopus* *Asp. rouxii*	Paste
Alcoholic rice seasoning				
Mirin	Japan	Rice, alcohol	*Asp. oryzae* *Asp. usamii*	Clear liquid seasoning

brew. Cereal beers are abundant in micronutrients, such as vitamin B groups formed during the fermentation, and provide rapid energy supplements with the ethyl alcohol and partially hydrolyzed polysaccharides (Lee, 1998).

5.2.2 Acid fermentation

Lactic acid fermentation is probably one of the first biological processes from which human beings discovered the benefits of fermentation (Lee, 1998). The sour ferments of flour dough, milk, cereals and vegetables have been used for the enhancement of keeping quality and palatability of food from prehistoric times. The fermentation of dairy products in Europe has been widely studied over the past century, and the processes have been highly standardized and industrialized to ensure efficient production of safe and nutritious food products. Caucasian yogurt and Middle-Eastern cheese have become every-day food for people in Europe, America and Oceania and they are considered as gourmet foods for wealthy people in Asia and Africa. However, little scientific research has been carried out on other types of fermented foods, which have contributed greatly to diets in East-Asia and Africa (Lee *et al*., 1994).

The most important microorganisms for acid fermented food are the lactic acid bacteria and these are differentiated into four genera: *Streptococcus*, *Pediococcus*, *Lactobacillus* and *Leuconostoc*. In addition, *Bifidobacterium*, belonging to the order *Actinomycetales*, is also important for dairy products. *Streptococcus*,

Pediococcus and some of *Lactobacillus* are homolactic, while *Leuconostoc* and *Bifidobacterium* are heterolactic. The metabolic pathways of glucose in lactic acid bacteria vary: glycolysis, the bifidus pathway and the 6-P-gluconate pathway.

Glycolysis:

$$C_6H_{12}O_6 \xrightarrow{\text{Homolactic bacteria}} 2\,CH_3\text{-}CHOH\text{-}COOH$$

Bifidus pathway:

$$\text{Glucose} \xrightarrow{\text{Bifidobacteria}} \text{Lactic acid} + \text{Acetic acid}$$

6-P-Gluconate pathway:

$$\text{Glucose} \xrightarrow{\text{Heterolactic bacteria}} \text{Lactic acid} + \text{Acetic acid (ethanol)} + \text{Carbon dioxide}$$

5.2.2.1 Lactic acid fermented milk products

Cultured milk products are produced by the lactic acid fermentation of milk using various bacterial cultures. Fermented milk products originated in the Near East and then spread to parts of southern and eastern Europe. Today cultured milk products in various forms have been introduced throughout the world even in regions where milk is not a traditional food like Korea and Japan. There are great differences in cultured products depending on the variations in the starter cultures used and manufacturing principles. However, most culture products use the following basic manufacturing steps:

1. culture of starter preparation;
2. treatment of product, such as pasteurization, separation and homogenization;
3. inoculation with bacterial cultures;
4. incubation;
5. agitation and cooling;
6. packaging.

Table 5.4 lists the world's principal cultured milk products including type, location and bacterial culture used (McGregor, 1992).

5.2.2.2 Lactic acid fermented cereals and starchy tubers

Lactic acid fermentation of bread dough improves the keeping quality and flavor of the baked products. It also enhances the palatability of bread made from low-grade flours and underutilized cereals. Acid-fermented breads and pancakes are an important staple food for people in Africa and some parts of Europe and Asia (Lee, 1994). Sour bread is a typical German food and Scandinavian rye bread is highly favored by the Nordic people. The Indian *idli* bread types (*idli, dosa, dhokla, khaman*) are important staple foods of the Indian and Sri Lankan people and they are consumed three or four times a week at breakfast and supper. *Idli* is a small, white, acid leavened, steamed cake made by bacterial fermentation of a thick batter prepared from rice and dehulled black gram. Similar products are made from rice in the Philippines (*puto*) and in Korea (*kichudok*). *Puto* is made using year-old rice and the batter is neutralized in the course of fermentation. In Sri Lanka *hopper* is prepared from acid fermented dough made with rice or wheat and coconut water. In hopper fermentation, a very large inoculum of baker's yeast or coconut toddy, which includes acid-producing bacteria, is added. Table 5.5 lists various types of acid fermented bread, pancakes, porridges and starch materials used in different regions (Lee, 1994).

Acid porridges prepared from cereals are eaten in various regions of the world, particularly in Africa, where porridges may represent the basic diet (Table 5.6). Nigerian *ogi*, Kenyan *uji* and Ghanaian *kenkey* are examples of porridges prepared by the acid fermentation of maize, sorghum, millet or cassava, followed by wet-milling, wet-sieving and boiling.

Acid fermentation is also used to produce food starches with extended shelf-life, resistance to infectious microorganisms and palatable flavor in different regions of the world. Nigerian *Gari*, Ethiopian *Kocho*, Chinese mungbean starch and Mexican *pozol* are important acid fermented starch ingredients used for the preparation of porridges, steamed cakes, pastes, noodles, soups and drinks (Table 5.7).

Most countries in Asia produce mungbean starch, and mungbean starch noodles are a staple of the Chinese diet. The manufacturing process for mungbean starch involves acidic bacterial fermentation. The mungbeans are hydrated by soaking in water inoculated with 12-hour steeped water from a previous fermentation to ensure acidification of the beans. The

Table 5.4 Examples of cultured milk products (McGregor, 1992).

Product	Location	Bacteria
Acidophilus	Europe, North America	*Lactobacillus acidophilus, Bifidobacterium bifidum*
Bulgarian buttermilk	Europe	*Lactobacillus bulgaricus*
Buttermilk	North America, Europe, Middle East, North Africa, Indian subcontinent, Oceania	*Lac. lactis* Subsp. *cremoris*, *Lac. lactis* subsp. *diacetylactis, Leuconostoc cremoris*
Filmjolk	Europe	*Lactococcus lactis* subsp. *cremoris, Lac. lactis, Lac. lactis* subsp. *diacetylactis, Leuc. cremoris, Alcaligenes viscosus, Geotrichum candidum*
Flummery	Europe, South Africa	Naturally present lactic bacteria
Ghee	Indian subcontinent, Middle East, South Africa, Southeast Asia	*Streptococcus, Lactobacillus* and *Leuconostoc* sp.
Junket	Europe	*Lactococcus* and *Lactobacillus* sp.
Kefir	Middle East, Europe, North Africa	*Streptococcus, Lactobacillus* and *Leuconostoc* sp., *Candida Kefyr, Kluyveromyces fragilis*
Kishk	North Africa, Middle East, Europe, Indian subcontinent, East Asia	*Streptococcus, Lactobacillus* and *Leuconostoc* sp.
Kolatchen	Middle East, Europe	*Lac. lactis, Lac. lactis* subsp. *diacetylactis, Lactococcus lactis* subsp. *cremoris, Saccharomyces cerevisiae*
Koumiss	Europe, Middle East, East Asia	*Lac. lactis, Lb. bulgaricus, Candida Kefyr, Torulopsis*
Kurut	North Africa, Middle East, Indian subcontinent, East Asia	*Lactobacillus* and *Lactococcus* sp., *Saccharomyces lactis, Penicillium*
Lassi	Indian subcontinent, East Asia, Middle East, North Africa, South Africa, Europe	*S. thermophilus, Lb. bulgaricus,* sometimes yeast
Prokllada	Europe	*Streptococcus* and *Lactobacillus* sp.
Sour cream	Europe, North America, Indian subcontinent, Middle East	*Lactococcus lactis* subsp. *cremoris, Lac. lactis* subsp. *diacetylactis*
Yakult	East Asia	*Lactobacillus casei*
Yogurt	Worldwide	*S. thermophilus, Lb. bulgaricus*

principal microorganisms found in the steep-water are *Leuconostoc mesenteroides*, *Lactobacillus casei*, *Lb. cellobiosus* and *Lb. fermentum.* The lactic fermentation, which reduces the pH to about 4.0, protects the beans from spoilage and putrefaction which would otherwise occur in ground bean slurries (Steinkraus, 1983).

Thai rice-noodle, *Khanom Jeen*, is also made from acid fermented raw rice. Soaked rice is drained and fermented for at least 3 days before grinding, and *Lactobacillus* sp. and *Streptococcus* sp. are involved in the acid fermentation.

5.2.2.3 Acid fermented vegetables

Acid fermented vegetables are important sources of vitamins and minerals. *Leuconostoc mesenteroides* has been found to be important in the initiation of the fermentation of many vegetables, i.e. cabbages, beets, turnips, cauliflower, green beans, sliced green tomatoes, cucumber, olives and sugar beet silages. In vegetables, *Leuc. mesenteroides* grows more rapidly and over a wider range of temperatures and salt concentrations than any other lactic acid bacteria. *Leuconostoc mesenteroides* produces carbon dioxide and acids, which quickly lower the pH, thereby inhibiting the development of undesirable microorganisms and the activity of their enzymes, which may soften the vegetables. The carbon dioxide produced replaces air and provides anaerobic conditions favorable for the stabilization of ascorbic acid and the natural color of the vegetables. The growth of this species modifies the environment, making it favorable for the

Table 5.5 Examples of acid-leavened bread and pancakes

Product	Country	Major ingredients	Microorganisms	Usage
Sourbread	Germany	Wheat	Lactic acid bacteria Yeast	Sandwich bread
Ryebread	Denmark	Rye	Lactic acid bacteria	Sandwich bread
Idli	India Sri Lanka	Rice Black gram	*Leuc. mesenteroides* *Enterococcus faecalis*	Steamed cake
Puto	Philippines	Rice	*Leuc. mesenteroides* *E. faecalis*	Steamed cake
Kichudok	Korea	Rice	Yeast	Steamed cake
Enjera	Ethiopia	Tef or other cereals	*Leuc. mesenteroides* *P. cerevisiae* *Lb. plantarum* *Sac. cerevisiae*	Pancake
Kisra	Sudan	Sorghum Millet	*Lactobacillus* sp. *Acetobacter* sp. *Sac. cerevisiae*	Pancake
Kishk	Egypt	Wheat + milk	*Lb. casei* *Lb. brevis* *Lb. plantarum* *Sac. cerevisiae*	
Hopper	Sri Lanka	Rice + coconut water	Yeast Lactic acid bacteria	Steam-baked pancake

growth of other lactic acid bacteria. The high acidity produced by the species and other subsequent lactic acid bacteria inhibits the growth of *Leuc. mesenteroides*. *Leuconostoc mesenteroides* converts glucose to approximately 45% levorotatory D-lactic acid, 25% carbon dioxide and 25% acetic acid and ethyl alcohol. Fructose is partially reduced to mannitol and is then readily fermented to yield equimolar quantities of lactic acid and acetic acid. The combination of acids and alcohol is conducive to the formation of esters that impart desirable flavors.

Table 5.8 shows examples of acid fermented vegetables produced in different regions of the world. The difference between sauerkraut and *kimchi* is the preferred end point of fermentation. The best tasting *kimchi* is attained before overgrowth of *Lb. brevis* and *Lb. plantarum* with an optimal product pH of 4.5. The overgrowth of *Lb. brevis* and *Lb. plantarum* diminishes

Table 5.6 Examples of acid fermented cereal gruels and non-alcoholic beverages.

Product	Country	Major ingredients	Microorganisms	Usage
Ogi	Nigeria	Maize, sorghum, or millet	*Lb. plantarum* *Corynebacterium* sp. *Acetobacter* Yeast	Sour porridge Baby food Main meal
Uji	Kenya Uganda Tanzania	Maize, sorghum, millet, or cassava flour	*Leuc. mesenteroides* *Lb. plantarum*	Sour porridge Main meal
Mahewu	South Africa	Maize + wheat flour	*Lac. lactis* *Lactobacillus* sp.	Sour drink 8–10% DM
Hulumur	Sudan	Red sorghum	*Lactobacillus* sp.	Clear drink
Busa	Turkey	Rice, millet	*Lactobacillus* sp.	

Table 5.7 Examples of acid fermented starch ingredients.

Product	Country	Major ingredients	Microorganisms	Usage
Gari	Nigeria	Cassava	*Leuconostoc* *Alcaligenes* *Corynebacterium* *Lactobacillus*	Staple Cake Porridge
Mungbean starch	China Thailand Korea Japan	Mungbean	*Leuc. mesenteroides* *Lb. casei* *Lb. cellobiosus* *Lb. fermenti*	Noodle
Khanom-jeen	Thailand	Rice	*Lactobacillus* sp. *Streptococcus* sp.	Noodle
Pozol	Mexico	Maize	Lactic acid bacteria *Candida*	Porridge
Me	Vietnam	Rice	Lactic acid bacteria	Sour food ingredient

product quality, but sauerkraut production depends on these organisms. The fermentation is manipulated by the salt concentration and temperature. The optimal range of salt concentration of sauerkraut is 0.7, approximately 3.0%, while that of *kimchi* is 3.0, approximately 5.0% (Lee, 1994).

5.2.2.4 Acid fermented fish and meat

The storage life of perishable fish and meats can be extended by acid fermentation with added carbohydrates and salt. In Scandinavian countries most traditional low-salt fermented fish products are transformed into pickled products in vinegar. These products generally require low-temperature storage. On the other hand, most Asian products are lactic fermented with added cereals, as shown in Table 5.9.

Rice, either cooked or roasted, is the most frequently used carbohydrate source, but other sources such as millet in *sikhae* are also used. In some cases fruits and vegetables, for example tamarind in *Bekasam* for the reduction of pH, and garlic and pepper in *sikhae*, are added. The antimicrobial effect of garlic to some putrefactive microorganisms such as *Bacillus* in lactic fermented fish products has been demonstrated (Souane *et al*., 1987).

Fermented sausages, salami in Europe, *nham* in Thailand and *nem-chua* in Vietnam, are also made by a process involving lactic acid bacteria. Starter cultures for salami fermentation are isolated from fermented

Table 5.8 Examples of acid fermented vegetables produced in different regions of the world.

Product	Country	Major ingredients	Microorganisms	Usage
Sauerkraut	Germany	Cabbage, salt	*Leuc. mesenteroides* *Lb. brevis*	Salad Side dish
Kimchi	Korea	Korean cabbage, radish, various vegetables, salt	*Leuc. mesenteroides* *Lb. brevis* *Lb. plantarum*	Salad Side dish
Dhamuoi	Vietnam	Cabbage, various vegetables	*Leuc. mesenteroides* *Lb. plantarum*	Salad Side dish
Dakguadong	Thailand	Mustard leaf	*Lb. plantarum*	Salad Salt side dish
Burong mustala	Philippines	Mustard	*Lb. brevis* *P. cerevisiae*	Salad Side dish

Table 5.9 Examples of acid fermented seafood and meat products (Lee, 1994).

Product	Country	Major ingredients	Microorganisms	Usage
Sikhae	Korea	Sea water fish, cooked millet, salt	*Leuc. mesenteroides* *Lb. plantarum*	Side dish
Narezushi	Japan	Sea water fish, cooked millet, salt	*Leuc. mesenteroides* *Lb. plantarum*	Side dish
Burong-isda	Philippines	Freshwater fish, rice, salt	*Lb. brevis* *Streptococcus* sp.	Side dish
Pla-ra	Thailand	Freshwater fish, salt, roasted rice	*Pediococcus* sp.	Side dish
Balao-balao	Philippines	Shrimp, rice, salt	*Leuc. mesenteroides* *P. cerevisiae*	Condiment
Kungchao	Thailand	Shrimp, salt, sweetened rice	*P. cerevisiae*	Side dish
Nham	Thailand	Pork, garlic, salt, rice	*P. cerevisiae* *Lb. plantarum* *Lb. brevis*	Pork meat in banana leaves
Sai-krok-prieo	Thailand	Pork, rice, garlic, salt	*Lb. plantarum* *Lb. salivarius* *P. pentosaccus*	Sausage
Nem-chua	Vietnam	Pork, salt, cooked rice	*Pediococcus* sp. *Lactobacillus* sp.	Sausage

fish products in Korea as well as other Asian countries (Lee, 2001).

5.2.2.5 Vinegar

Vinegar fermentation is as ancient as alcoholic fermentation, since acetic acid is produced in any natural alcoholic fermentation upon exposure to the air.

$$\text{Ethanol in fruits, wine, toddy, rice-wine} \xrightarrow{\textit{Acetobacter aceti}} \text{Acetic acid}$$

Vinegars are produced from fruits in Europe, from tropical fruits, such as coconut, sugar cane and pineapple in the Asia Pacific region and from cereals in Northeast Asia. Cereal vinegars may be divided into three classes: rice vinegar, rice-wine filter-cake vinegar and malt vinegar. The indigenous processes are natural or spontaneous fermentation brought about by the growth of *Acetobacter aceti* on alcoholic substrates under aerobic conditions. Traditionally, degraded or poor quality wines were used for the production of low-grade vinegars at the household level. Today, vinegars of high quality standards are produced by industry.

Commercial vinegar is prepared from rice-wine filter-cake in Far-Eastern countries. Filter cakes from rice-wine factories are collected and packed tightly into a storage tank for 1–2 years. The filter-cake contains large amounts of unused carbohydrate and proteins, which are further hydrolyzed by inherent microorganisms and enzymes during storage, converting them into alcohol and other nutrients and flavor substances. The cake is slurried in 2–3 volumes of water prior to filtration. The filtrate is heated to 70°C, and cooled by mixing with fresh vinegar mash to a temperature of 36–38°C, and then fermented with *Acetobacter* for 1–3 months. It is further aged at the ambient temperature for 3–6 months and filtered to obtain clear vinegar (Lee, 2001).

5.2.3 Bread fermentation

Baking, brewing and enology all depend on the ability of yeasts to carry out anaerobic fermentation of sugars, yielding CO_2 and ethanol. In brewing and wine-making, alcohol is the prime product of interest, while in baking the leavening effect of CO_2 is more important. Breads are divided into two groups, leavened loaf bread and unleavened flat bread. Traditionally leavening is due to the products of fermentation, carbon dioxide and ethanol produced by yeast. Although the CO_2 generating chemical leavening agents such as food acid and soda (sodium bicarbonate) can replace yeast, biological leavening imparts physicochemical modification of dough constituents and flavor development.

Originally sour doughs were used for the production of all type of breads because commercial baker's yeast was not available. Baker's yeast was introduced in the market at the beginning of the twentieth century. Industrially produced yeasts are strains of the top-fermenting species *Saccharomyces cerevisiae* grown on molasses in an aerobic fed-batch fermentation. The optimum temperature for the growth and fermentation of baker's yeast is between 28 and 32°C, and the optimum pH is 4–5. Leavening of dough requires the addition of 1–6% yeast based on the weight of flour. The exact percentage depends on the recipe, the process and the quality of the flour and yeast as well as the operation conditions (Spicher and Brummer, 1995).

The production of baked goods consists of preparation of raw materials, dough formation (kneading, maturing), dough processing (fermentation and leavening, dividing, molding and shaping), baking in the oven and final preparation (slicing, packaging, etc.). Bread dough is fermented for a sufficiently long time to permit the yeast to act on the assimilable carbohydrates and convert them into alcohol and carbon dioxide as the principal end product. By the end of the proofing period the aqueous phase of the bread is saturated with CO_2 and the volume has roughly doubled owing to the pressure of CO_2 that has diffused to air cells. At the beginning of baking the loaf further expands (oven spring) by the expansion of air and steam during heating, and then at some temperature the matrix sets, expansion stops, and starch gelatinization, crust coloring and flavor development take place. The magnitude of oven spring depends on two factors: (1) the generation and expansion of gases, and (2) the amount of time available for loaf expansion before the structure sets. The first factor is primarily a function of yeast fermentation; the second factor is affected by dough components such as shortening, surfactants, gluten protein and flour lipids (Stauffer, 1992).

5.2.4 Amino acid/peptide fermentation

Fermented protein foods are used mainly for flavor-enhancing condiments and gourmet food ingredients due to the meaty and appetite-stimulating flavor of protein hydrolysate, which is formed during the fermentation. The type of indigenous fermented protein food is decided primarily by the availability of the raw material in the specific climatic and geographical conditions. Cheese is made in the Middle East and Europe where animals are the main food source. Fermented soybean products, for example soybean sauce and paste, are used in Northeastern Asian countries, and fermented fish products in the Asia-Pacific region.

5.2.4.1 Cheese

The worldwide number of cheese varieties has been estimated at 500, and there are several methods of classification. Cheeses can be divided by their texture: very hard (Parmesan, Romano), hard (Cheddar, Swiss), semisoft (Brick, Muenster, blue, Harvarti), soft (Brie, Camembert, feta) and acid (cottage, cream, Ricotta). A broad look at cheeses might divide them into two large categories, ripened and fresh. More technical classifications are also used, for example, those based on coagulating agent: rennet cheese (Cheddar, Brick, Muenster), acid cheese (cottage, Quarg, cream), heat-acid (Ricotta, Sapsago) and concentration-crystallization (Mysost) (Nuath *et al*., 1992).

Cheese is manufactured by coagulating or curdling milk, stirring and heating the curd, draining off the whey, and collecting or pressing the curd. Characteristic flavor and texture are formed during the ripening of cheese depending on the type of starter culture and microorganisms involved as well as the coagulating agent and salting methods. Depending on the variety, the milk is pasteurized (generally at about 72°C for 16 sec), and a bacterial starter culture is added to the milk, which is at 30–36°C. The inoculated milk is generally ripened at the temperature for 30–60 min to allow the lactic acid bacteria to multiply sufficiently for their enzyme system to convert lactose to lactic acid. After ripening a milk-coagulating agent is added. For blue cheese, the mold (*Penicillium* sp.) is added to the starting milk or to the drained curds.

The starter cultures are organisms that ferment lactose to lactic acid and other products. These include *Streptococci, Leuconostocs, Lactobacilli* and *Streptococcus thermophilus*. Starter cultures also include *Propionibacteria*, brevibacteria and mold species of *Penicillium*. The latter organisms are used in conjunction with lactic acid bacteria for a particular characteristic of cheese, for example, the holes in Swiss cheese are due to *Propionibacteria*, and the yellowish color and typical flavor of brick cheese is due to *Brevibacterium linens*.

Coagulation of milk is essential to cheese making. Most proteolytic enzymes can cause milk to

coagulate. Rennet (chymosin, EC 3.4.23.4) is widely used for milk coagulation for cheese making. However, due to the shortage of calf's chymosin, commercial rennet may include blends of chymosin and pepsin extracted from the stomach of other animals such as pig. Microbial rennets with similar functionality are also prepared from *Mucor miehei*, *Mucor pusillus* and *Endothia parasiticus*. Proteases from plants are known to coagulate milk but are not used in commercial cheese making.

5.2.4.2 Fish sauce and paste

Fish fermentation is an old technology used for the preservation of freshwater and marine animals, which are highly perishable and localized in production and seasonally fluctuating in catch (Ruddle, 1993). The technology appears to have evolved with the availability of salt and a non-pastoral way of life. There is a strong correlation throughout the world between the use of fermented fish products and the use of cereals, especially rice, and vegetables (Ishige, 1993). Although the use of fermented fish products is nowadays mainly confined to East and Southeast Asia, traces of this technology can be found throughout old human civilizations.

Aging salted (cured) fish in a container or earthen jar for longer period produces fish sauce. The enzymes in the gut and from the halophilic microorganisms grown in the system decompose fish meats, and the exuded liquid (protein hydrolysate) is fish sauce. The hydrolysate mainly consists of amino acids and peptides, which form the characteristic meaty flavor of fish sauce. In the case of Korean *Jeotkal* fermentation containing 20% salt, the total number of viable cells increases for the first 40 days, mainly attributed to the growth of *Pediococcus* and *Halobacterium*. The concentrations of soluble-N and amino-N increase steadily during the first 60 days and it coincides with the development of optimum taste. The volatile basic-N content increases in two steps, and the second step increase causes the taste deterioration, which is related to the maximum growth of yeast (Lee *et al*., 1993).

Depending on the amount of salt added, the products are classified as high-salt (> 20% salt of total weight), low-salt (6–18% salt) and no-salt products, as shown in Fig. 5.2. When the salt concentration is higher than 20% of total weight, pathogenic and putrifactive microorganisms cannot grow and the product does not need other preservative means. The first criterion for the subdivision of this group is the degree of hydrolysis, which is influenced by fermentation time and temperature, added enzyme sources and water content. The fully hydrolyzed liquid is fish sauce. The name cured fish is confined to represent the partially hydrolyzed fish products which retain the original shape of fish immersed in the exuded liquid, and this form as such is frequently used as a side dish for rice meals. Fish paste is characterized by the salted fish being partially dried in order to restrict the degree of hydrolysis and comminuted to produce the homogeneous, solid condiment. Each class can be further subdivided by the kind of raw materials, such as fish species and portion of fish, and thus there are numerous kinds of products (Lee, 1989).

Many Asian countries produce salt cured and dried fish products, for example, *plakem* in Thailand, *jambalroti* in Indonesia, Maldive fish in Sri Lanka and *gulbi* in Korea, but the role of fermentation in these products is not fully understood. Fish fermentation without added salt is not a common practice. In some local specialties, half-spoiled fish or alkaline fermentation in leafy plant ash is used. The propagation of mold in dried bonito (katsuobushi) processing in Japan is another example of non-salt fish fermentation .

Most countries in East and Southeast Asia have fish sauce, but the flavor, physical properties and raw materials used vary. Depending on the degree of hydrolysis or fermentation time and the separation method, two types of sauce, namely, clear and turbid, are produced. *Ngan-pya-ye, nuoc-man, nampla, shottsuru* and *yu-lu* are clear fish sauces, while *budu, patis, ketjap-ikan* and *jeot-kuk* are turbid. Some turbid sauces are

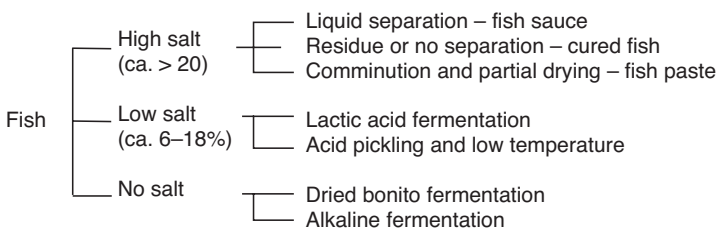


Figure 5.2 Classification of fermented fish products.

obtained from the exuded liquid of cured fish, for example, *patis* from *bagoong* production in the Philippines and *jeotkuk* from *jeotkal* production in Korea. In Northeast Asia cured fish products are more important than fish pastes. Fish pastes, especially those made from shrimp and planktonic animals such as *Seinsa ngapy*, *belacan*, *trassi*, *prahoc* and *kapi*, are important in Southeast Asian diets.

5.2.4.3 Fermented soybean products

At the early stage of soybean utilization the Northeastern *Dong-yi* probably first invented *shi*, the old Chinese term for Korean *meju*, by keeping cooked soybeans in a pottery jar. Cooked soybeans grown with mold and bacteria, which is called *meju*, are immersed in brine to leach out the protein hydrolysate, and the liquid part is soybean sauce (*kanjang*) and the residue is soybean paste (*doenjang*).

The traditional fermented soybean products are divided into three groups based on the type of fermentation starter used: *Shi* made from loose type soybean *meju*, *Maljang* from cake type soybean *meju* and *Jang* from soybean mixed with other cereals. The propagation of these products in the Northeastern region, namely China, Korea and Japan, and their variations are shown in Fig. 5.3. According to S.W. Lee (1990), fermented soybean products were first introduced to China in the first century BC and to Japan in the sixth century AD. Varieties of products have been developed and have disappeared throughout history.

Korean kanjang *and* doenjang

Meju is prepared from cooked soybean. Soybeans are soaked in water overnight, cooked for 2–3 hours and mashed by pounding. The mash is then shaped like a brick or a ball, dried in the sun and kept in a stack covered during the night for several days. During this period, the surface is grown with mold, especially *Aspergillus oryzae*, and the inside with bacteria, typically *Bacillus subtilis*. The enzymes from mold and bacteria hydrolyze the soybean proteins into amino acids, and the carbohydrates into sugars and organic acids. The amino acids and sugars interact with each other during browning reaction, resulting in the characteristic dark brown color and meaty flavor. Properly fermented *meju* is immersed in brine in an earthen jar and ripened for several months. The brown color and

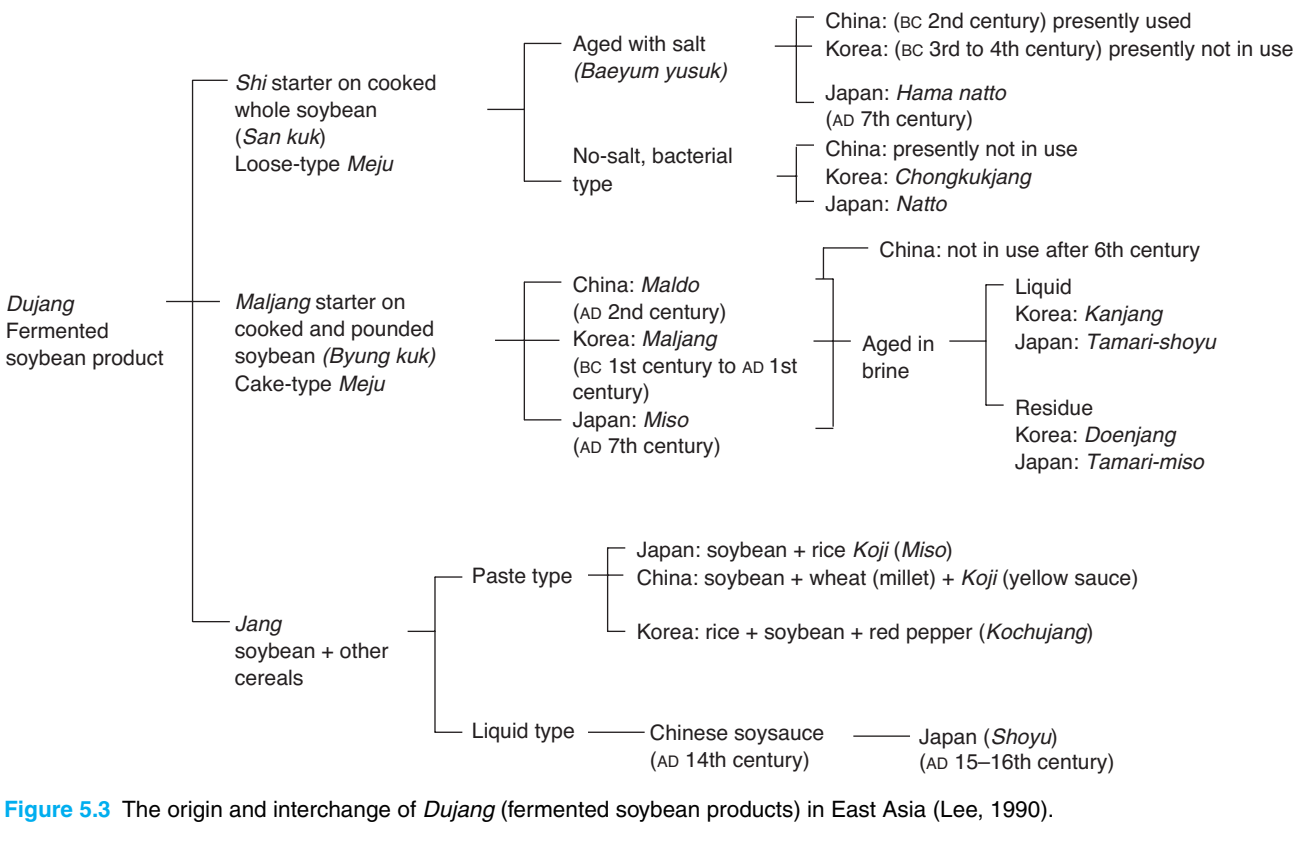


Figure 5.3 The origin and interchange of *Dujang* (fermented soybean products) in East Asia (Lee, 1990).

meaty flavor leach out into the brine. During this period, salt-tolerant yeasts grow in the mash, especially *Saccharomyces rouxii*, which produces the aroma of soy sauce. The liquid part is soy sauce and the precipitates are soybean paste. Soy sauce so produced is boiled once and stored in an earthen jar for years. The flavor of soy sauce gets richer as the storage time increases, just as the flavor of wine becomes smoother as it get old. It has been said in Korea that the taste of food in a household is decided by the taste of the household's fermented soybean products.

Japanese shoyu *and* miso

Japanese people modified the *meju* preparation method in the early twentieth century by controlled fermentation technology using a pure culture of mold isolated from the traditional starter (Shettleff and Aoyaki, 1976). The mold, normally *Aspergillus oryzae*, is grown on cooked rice or cooked wheat grits to make *koji*. It is mixed with cooked soybean for further fermentation, and then ripened in the brine. Soybean paste (*miso*) and soy sauce (*shoyu*) are made separately; for *shoyu*, *koji* is made with cooked defatted soybean flake and wheat grits and then mixed in brine for aging. After 4–6 months of aging, it is filtered to obtain shoyu, the liquid part, and the solid part is discarded. Miso is prepared by using koji made from cooked rice or other cereals mixed with cooked soybean and salt, and then mashed into a paste and ripened. These processes are easy for industrialization of the products. The flavor of Japanese *shoyu* and *miso* is mild and sweet compared to Korean counterparts. Korean people prefer the strong flavor of traditional soy sauce and soybean paste, the same as European people distinguish Roquefort from processed Cheddar cheese.

Korean chongkukjang *and Japanese* natto

Soybean is cooked and covered with straw mat or cloth, and placed on the warm stone floor, *ondol*, for 3–4 days until the mucous string is formed. It is mixed with chopped ginger, chopped garlic and salt, and pounded slightly until the bean kernels are separated into halves, and then stored in an earthen jar. The strong smell of fermented soybean is partially masked by the ginger and garlic smell, and creates the characteristic *chongkukjang* flavor. The spicy seasoning is thus prepared in 3–4 days, while ordinary soybean paste, *doenjang*, which uses *meju* as fermentation starter, takes over 6 months for complete ripening. In this respect, chongkukjang is a rapid fermentation method. The mucous substance in *chongkukjang* is peptido-polysaccharide produced by *Bacillus subtilis.*

Japanese *natto* is a modified form of *chongkukjang*. *Natto* is fermented soybean grown with *Bacillus subtilis* on cooked soybean. The fermented soybean with mucous string is consumed directly without further processing, so it is a non-salt fermented product. However, natto is not generally accepted by Korean people. It is always mixed with spices and used for the cooking of vegetable stew as a meaty flavored condiment. The amount of *chongkukjang* added to the stew is large enough to supplement protein in the diet significantly. *Chongkukjang* was also called *Jeonkukjang* in the old days. 'Chongkuk' means the Chinese kingdom 'Qing', while 'Jeonkuk' means 'a country at war' or a combat zone. What all these names imply is that this product was made in extraordinary situations, for example during war time or famine conditions, for the urgent supply of a nutritious savory food ingredient.

Korean kochujang

The basic tastes of European people are sweet, sour, bitter and salty and Japanese people add umami, the meaty taste. Korean people add another: hot or pungent taste. The most remarkable difference of Korean food compared to food of neighboring Japan and China is the strong pungent taste of red pepper in most Korean dishes.

Kochujang is a unique hot bean paste seasoning popular in Korea. It is made from fermented soybean starter, *meju* and malt made from barley. Malt powder is mixed with cereal porridge made from rice, glutinous rice or barley. The enzymes in malt hydrolyze the starch into sugars and reduce the consistency of the mixture. *Meju* powder, red pepper powder and salt are added to the partly saccharified porridge with thorough mixing to make a paste, and put in an earthen jar. The top is covered by salt in order to prevent mold growth. The jar is placed in a sunny place for further fermentation. The proteins in soybean and cereals degrade into amino acids to produce the meaty flavor. During fermentation a wonderful harmony of the meaty flavor from hydrolyzed proteins and the sweet taste of hydrolyzed starches with the pungent taste of red pepper and salty taste is achieved, and a new characteristic flavor stimulating the appetite of Koreans is formed.

Tempe

Tempe is found in all parts of Indonesia but is particularly important in Java and Bali. It is also produced in some Malaysian villages and in Singapore. *Tempe* is a white, mold-covered cake produced by fungal fermentation of dehulled, soaked in water and partially cooked soybean cotyledons (Steinkraus, 1983). It is packed in wilted banana leaves and sold in the market. Essential steps in the preparation of tempe include cleaning the beans, soaking in water, dehulling and partial cooking of the dehulled beans. Dehulling is important for the growth of mold on the surface of cotyledons. Soybean is not necessarily cooked fully, because subsequent mold growth is able to softening the texture. Under the natural conditions in the tropics, *tempe* production involves two distinct fermentations: bacterial acidification of the beans during soaking and fungal overgrowth of the cooked bean cotyledon by the mold mycelium. A previous batch of sporulated *tempe* or sun-dried pulverized *tempe* powder (1–3 g) is sprinkled over the cooked and drained soybean cotyledon (1 kg) and thoroughly mixed to distribute the mold spores over the surface of all the beans. *Rhizopus oligosporus* is known as *tempe* mold, and the pure culture of strain NRRL2710 or CBS 338.62 can be used for the inoculum.

A handful of inoculated beans are placed on wilted banana leaves or other large leaves and packed. The leaf keeps the soybean cotyledon moist during the fermentation and allows for gaseous exchange. Incubation can be at temperatures from 25 to 37°C. The higher the incubation temperature, the more rapidly the *tempe* molds will grow. For example, 80 hours of incubation is required at 25°C, 26 hours at 28°C and 22 hours at 37°C. The *tempe* should be harvested as soon as the bean cotyledons have been completely overgrown and knitted into a compact cake. The cotyledons should be soft and pasty (not rubbery), and the pH should have risen to about 6.5.

Tempe should be consumed immediately after harvest. It can be stored for 1 or 2 days without refrigeration. If the *tempe* is not going to be consumed immediately, it should be deep-fried, in which form it remains stable for a considerable time, or it should be blanched by steaming and refrigerated. It can be stored after dehydration, either by sun-drying or by hot-air drying and keeping in plastic bags. Subsequent keeping quality is excellent because *tempe* contains a strong antioxidant produced by the mold and is resistant to the development of rancidity. *Tempe* is consumed fresh or in deep-fried form.

Chinese sufu

Chinese *sufu* (*tosufu, toufuru, fuyu* or *tauhuyi*) is a highly flavored, creamy bean paste made by overgrowing soybean curd with a mold belonging to the genus *Actinomucor, Rhizopus* or *Mucor* and fermenting the curd in a salt brine/rice wine mixture (Lee and Lee, 2002). In the West, *sufu* has been referred to as Chinese cheese. *Sufu* is usually sold in red or white blocks 2–4 cm^2 by 1–2 cm thick, and the white *sufu* is untreated, while the red variety is colored with Chinese red rice, *hung chu*. The procedure for making *sufu* consists of five steps: preparation of soybean curd (tofu), preparation of molded *tofu* (*pehtze*), salting, fermenting in salt brine/rice wine, and processing and packaging.

Soybeans are cleaned, soaked in water and ground to make soybean milk slurry. The slurry is heated to boiling and filtered through cloth, and the residue is discarded. To the filtrated soymilk are added coagulants (calcium chloride/calcium sulfate mixture or sea salt brine) to make soybean curd. The amount of coagulant used to produce *tofu* for sufu manufacture is 20% higher than that used for regular *tofu*. Moreover, after the coagulates are mixed with soybean milk, the mixture needs to be agitated vigorously in order to break the coagulated protein into small pieces, after which it is set aside for 10 min to complete the process of coagulation. This process reduces the water content of the curd and makes the texture harder. If the water content is more than 60%, the inoculation of fungi is deferred until the water remaining on the curd surface is reduced by drying.

Pehtze is the soybean curd overgrown with the grayish hair-like mycelium of molds belonging to genera *Actinomucor*, *Rhizopus* or *Mucor*. These fungi are normal contaminants in rice straw. Traditionally inoculation was performed by placing the *tofu* on the rice straw, but this method does not always yield a high quality because of undesirable contaminating microorganisms. In spring or autumn when the ambient temperature is 10–20°C, white fungal mycelium is visible on the surface of the cubes after 3–7 days, at which point the cubes are taken out and immediately salted in large earthenware jars. Each layer of *pehtze* is sprinkled with a layer of salt, and after 3–4 days when the salt is absorbed, the *pehtze* is removed, washed with water and put into another jar for processing.

For processing, a dressing mixture, which varies for each type of *sufu*, is placed in the jar. To make red *sufu*, *anka koji* and soy mash are added; to make fermented rice (*tsao*) *sufu*, fermented rice mash is

added; to make Kwantung *sufu*, red pepper and anise are added in addition to salt and red *koji*. Alternate layers of *pehtze* and dressing mixture are packed into the jar until it is filled to 80% of its volume, then a brine with a concentration of approximately 20% NaCl is added. Finally, the mouth of the jar is wrapped with the sheath leaves of bamboo shoots and sealed with clay. After 3–6 months fermenting and aging, the *sufu* is ready for consumption (Steinkraus, 1983).

5.2.5 Other fermented products

5.2.5.1 *Chinese red rice* (Anka)

Anka, also known as *ang-kak*, *beni-koji* and red rice, is used in China, Taiwan, the Philippines, Thailand and Indonesia to color foods which include fish, rice wine, red soybean cheese, pickled vegetables and salted meats (Lee and Lee, 2002). It is a product of fermentation of rice with various strains of *Monascus purpureus* Went. A number of countries are gradually adopting this natural pigment to replace coal-tar dyes, as the latter have been implicated as carcinogens. The advantages of using *Anka* are that its raw materials are readily available, the yield is good, the color of pigment produced is consistent and stable, the pigment is water soluble, and there is no evidence of any toxicity or carcinogenicity.

Anka is produced at an industrial scale in Taiwan. Non-glutinous rice (1450 kg) is washed and steamed for 60 min. Water (1.8 hL) is sprayed on the rice, which is again steamed for 30 min. The steamed rice is mixed with 32 L of *chu chong tsaw*, a special variety of red rice inoculum in Taiwan, after cooling to 36°C, and heaped in a bamboo chamber. When the temperature of the rice rises to 42°C, it is spread on plates and shelved. *Anka* is produced by moistening the rice three times during incubation, followed by final drying; 700 kg of *Anka* is produced from 1450 kg of rice.

An uncommon phenomenon of the mold *Monascus purpureus* is the exudation of granular fluid through the tips of the hyphae. When the culture is still young, the freshly extruded fluid is colorless, but gradually changes to reddish-yellow and purple-red. The production of the red coloring matter is observed not only on the exuded granular fluid but also on the hyphal content within. The red coloring matter diffuses throughout the substrate. The dark red color consists of two pigments, the red monascorubrin ($C_{22}H_{24}O_5$) and the yellow monascoflavin ($C_{17}H_{22}O_4$). The strains of *Monascus purpureus* that are adopted in the production of red rice are only those that are capable of impregnating rice with a dark red color in the presence of water concentrations sufficiently low that no distortion is produced in the hydrated grains.

5.3 Enzyme technology

The application of enzymes in food processing is an important branch of biotechnology. Enzymes are the proteins that catalyze virtually all chemical reactions occurring in biological systems, and thousands of enzymes have been identified and characterized. Some of importance to the food industry include the production of high fructose corn syrups by using glucose isomerase, saccharification of starch by amylases in baking and brewing, juice clarification by using cellulases and pectinases, production of low lactose milk by using lactase, cheese making by rennin, and meat tenderization by proteases such as papain, bromelain and ficin. The cell wall degrading enzymes (hemicellulase and cellulase) are used to extract vegetable oil (olive and canola/rape seed) in an aqueous process by liquefying the structural cell wall components of the oil-containing crop. Table 5.10 summarizes some important food enzymes, their source, reaction specificity and applications in food processing.

In this chapter, enzymatic starch modification and protein hydrolysis will be discussed in detail.

5.3.1 Enzymatic starch modification

The major steps in the conversion of starch are liquefaction, saccharification and isomerization. During liquefaction the α-1,4 linkages of amylose and amylopectin are hydrolyzed at random by endo-α-amylase. This reduces the viscosity of the gelatinized starch and increases the dextrose equivalent (DE), a measure of the degree of hydrolysis of the starch. For saccharification to dextrose, a DE of 8–12 is commonly used, and the maximum DE obtainable is about 40 (Olsen, 1995).

β-Amylases are exo-enzymes which attack amylase chains, resulting in production of maltose from the non-reducing end. In the case of amylopectin the cleavage stops 2–3 glucose units from the α-1,6 branch points. Isoamylase and pullulanase hydrolyze α-1,6 glucosidic bonds of starch. When amylopectin is treated with pullulanase, linear amylose fragments are obtained.

Table 5.10 Some important food enzymes and their usage.

Name	Source	Action mode	Applications
α-Amylase	Malt, *Aspergillus, Bacillus* spp.	α-1,4 glycosidic linkage of amylase, amylopectin	Starch modification Brewing aid Reduce dough viscosity Prevent staling
β-Amylase	Malt, molds, bacteria	Split off β-maltose from non-reducing end of starch	Production of maltose syrup Brewing and baking aid
Glucoamylase	*Aspergillus*, *Rhizopus* spp.	Stepwise hydrolysis of α-1,4 linkages in starch	Production of glucose Analysis of starch content in food
Glucose isomerase	*Streptomyces*, *Actinoplanes*, *Bacillus* spp.	Conversion of glucose to fructose	Production of high-fructose corn syrup Immobilized form is used
Pullulanase	*Klebsiella pneumoniae*	α-1,6 bond of amylopectin	Production of maltose and malto-trios Removal of limit dextrins to produce high alcohol beer
Invertase (β-fructo-franosidase)	*Saccharomyces*, *Candida* spp.	Hydrolyze sucrose to glucose and fructose	Invert sugar syrup Chocolate coated sucrose candy Recovery of scrap candy Artificial honey Humectants
β-Glucanase	*Bacillus subtilis*, *Asp. niger*	β-1,3 or β-1,4 bonds of β-D-glucans	Solubilize barley gums in brewing Reduce viscosity of wort
Cellulase	*Trichoderma reesei*	Endo-cellulases to split β-1,4 linkage, exo-cellulases, cellobiases	Turn cellulosic waste into glucose to make ethanol Hydrolyze cellulose into β-dextrins and glucose
Pectinases (PG, PL, PE)	*Aspergillus* spp.	Split glycosidic bonds of pectin, endo/exo	Extraction and clarification of fruit juice
Lactase (β-galactosidase)	*Kluyveromyces marxianus*, *Asp. niger*	Hydrolyze lactose into glucose and galactose	Low-lactose dairy products Prevents crystallization of lactose in milk concentrate
Rennet (chymosin, pepsin)	Calf stomach, *Endothia parasitica, Mucor meihei*	Catalyze k-casein, destabilize casein micelle	Milk clotting in cheese making
Proteases	Plants (papain, ficin) Animal (trypsin) *Aspergillus niger, Bacillus* spp.	Hydrolyze peptide bond esterase activity	Meat tenderizer Chill-proof beer Recovery of scrap meat and fish Gluten modifier Decolorization of red blood cells
Lipases	*Mucor, Rhizopus*, *Aspergillus* spp.	Hydrolyze ester linkages of triglycerides	Accelerate cheese ripening Cheese flavor production

Maltodextrin (DE 15–25) produced from liquefied starch is commercially valuable for its rheological properties. Maltodextrins are used in the food industry as fillers, stabilizers, thickeners, pastes and glues. When saccharified by further hydrolysis using amyloglucosidase or fungal α-amylase, a variety of sweeteners can be produced having DE in the range 40–45 (maltose, 50–55 (high maltose) and 55–70 (high conversion syrup)).

5.3.2 Enzymic modification of proteins

The enzymic modification of proteins is an attractive means of obtaining better functional and nutritional properties of food proteins. The conversion of milk to cheese is an effect of the action of the protease of the microorganisms inhabiting the system. Enzymatic hydrolysis of milk proteins is used to produce non- and low allergic cow's milk products for baby food and highly digestible protein foods for hospital patients.

Protein structure is modified to improve solubility, emulsification and foaming properties, gelation and textural properties. Enzymatic processes provide several advantages compared to chemical treatments, including fast reaction rates in mild conditions with high specificity.

Proteases are classified according to their source of origin (animal, plant or microbial), their catalytic action (endo-peptidase or exo-peptidase) and the nature of the catalytic site. Based on a comparison of active sites, catalytic residues and three-dimensional structures, four major protease families have been recognized to date: the serine, the thiol, the aspartic and the metallo-proteases. The serin protease family contains two subgroups: the chymotrypsin-like and the subtilisin-like proteases. Many industrially important proteases are mixtures of the different types of proteases. This is especially the case for pancreatin, papain (crude), and some proteases from *Bacillus amyloliquefaciens*, *Aspergillus oryzae*, *Streptomyces* and *Penicillium duponti* (Olsen, 1995)

The degree of hydrolysis (DH) of enzyme-treated proteins determines the properties of relevance to food application. DH is measured by the pH-stat technique (Adler-Nissen, 1986). It is based on the principle that pH is kept constant during hydrolysis by means of automatic titration with a base, when the hydrolyzes are carried out under neutral to alkaline conditions. DH is calculated on the basis of the titration equations as follows:

$$DH = (h/h_{tot}) \times 100\%$$
$$DH = B \times Nb \times 1/a \times 1/MP \times 1/h_{tot} \times 100\%$$

where

B	is base consumption,
Nb	is the normality of the base,
a	is the average of the dissociation of the α-NH_2 groups,
MP	is the mass of protein,
h	is the equivalent peptide bonds cleaved per kilogram of protein (or milliequivalents per gram of protein),
h_{tot}	is the total number of peptide bonds in the protein substrate.

Proteases catalyze the hydrolytic degradation of the peptide chain. When a protease acts on a protein substrate, the catalytic reaction consists of three consecutive reactions:

1. formation of the Michaelis complex between the original peptide chain (the substrate) and the enzyme;
2. cleavage of the peptide bond to titrate one of the two resulting peptides;
3. a nucleophilic attack on the remains of the complex to split off the other peptide and to reconstitute the free enzyme.

The rate-determining step is the acylation step characterized by the reaction velocity constant k_{+2}.

Enzymatic hydrolysis of milk proteins and soybean proteins often produces bitter peptides (Kim *et al.*, 2003). The unpleasant bitterness can be managed by proper selection of the reaction parameters and the enzymes used. Bitterness is a complex problem, which can be influenced by many variables, for example, the hydrophobicity of the substrate, since the amino acid side chains containing hydrophobic groups become exposed due to the hydrolysis. DH is closely related to the bitterness of the protein hydrolysates.

5.3.3 Enzyme reaction kinetics

The activity of an enzyme is determined by many factors, including enzyme, substrate, and cofactor

concentrations, ionic strength, pH and temperature. For conversion of substrate (S) to product (P) by an enzyme (E), the reaction scheme can be simply represented as:

$$E + S \overset{ks}{\longleftrightarrow} ES \overset{kcat}{\longrightarrow} E + P$$

The reaction velocity (V) is then given by the Michaelis–Menten equation:

$$V = \frac{k_{cat}[E][S]}{K_m + [S]}$$

where K_m is the substrate concentration at which V equals one-half the maximum velocity (V_{max}), as shown in Fig. 5.4.

Integration of this equation with respect to time gives:

$$V_{\max} = K_m \ln\left(\frac{[S]}{[St]}\right) + ([S] - [S_t])$$

where S and S_t are the substrate concentrations at zero time and time t, respectively. This equation is particularly useful in industrial situations where a reaction is allowed to proceed to near completion or equilibrium. K_m and $V_{\max}$ can be determined by linearizing the above equation as follows, resulting in a Lineweaver–Burk plot (Karel and Lund, 2003):

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}[S]}$$

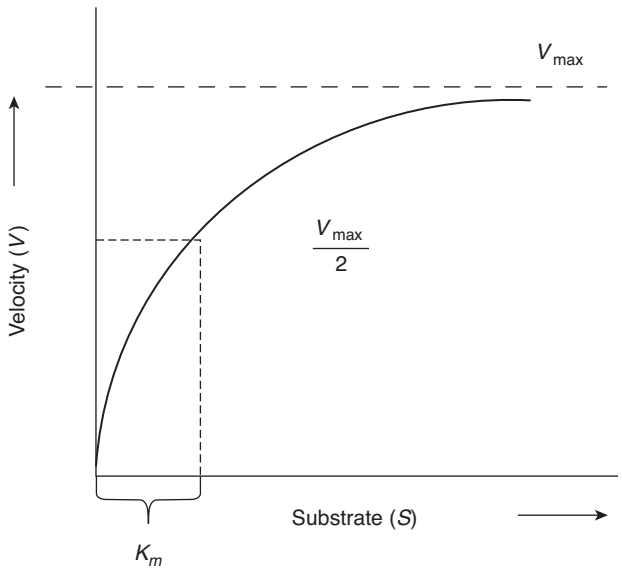


Figure 5.4 Enzyme-catalyzed reaction.

5.4 Modern biotechnology

The development of traditional fermentation technology and enzyme technology paved the way for modern biotechnology to achieve industrial mass production of amino acids, nucleic acids, organic acids and antibiotics in the twentieth century. The production of fine chemicals by fermentation methods has many advantages over chemical synthetic processes. It requires milder, safer and environmentally friendly reaction conditions, and provides higher productivity and wider varieties of physiologically active substances. Modern biotechnology was initially applied for the mass production of glutamate, the raw material of flavor enhancer, monosodium glutamate (MSG), in the 1950s in Japan, followed by the commercial production of other amino acids, nucleic acid-related substances and antibiotics. The modern fermentation process is carried out aseptically in the closed fermenter to form useful substances using microbial strains, and converts these to the higher-value-added chemicals either by enzymatic or chemical modification by necessity (Fig. 5.5). This so-called hybrid technology, combined biotechnology between fermentation and chemical/enzymatic modification, is recognized in both the food and fine chemical industries (Lim, 1999).

5.4.1 Amino acid production

Amino acids have many useful functions not only as nutrients but also as preventive pharmaceuticals. The world market for natural L-amino acids is led by glutamate (MSG), lysine, phenylalanine, methionine and glycine. MSG and feed additive amino acids (lys, met, thr, trp) account for 98% of the market. Phenylalanine is an important raw material for the production of aspartam, the synthetic sweetener.

Glutamate is produced from glucose (industrially raw sugar) through the Embden Meyerhoff Pathway (EMP) and the TCA cycle in the cells of *Corynebacterium glutamicum* or *Brevibacterium lactofermentum* (or *flavum* or *thiogenitalis*), as shown in Fig. 5.6.

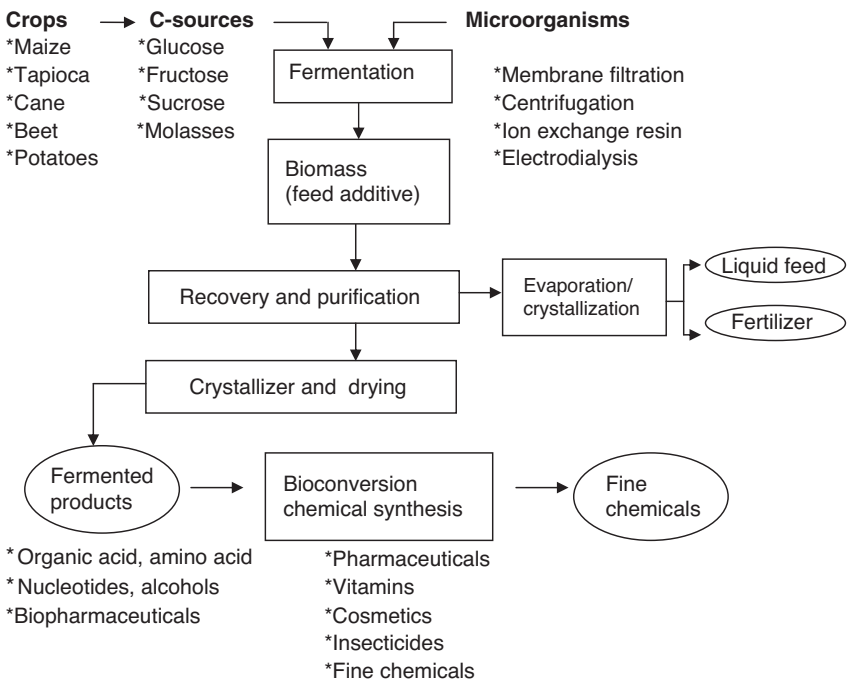


Figure 5.5 Schematic flow diagram of the fermentation bioindustry.

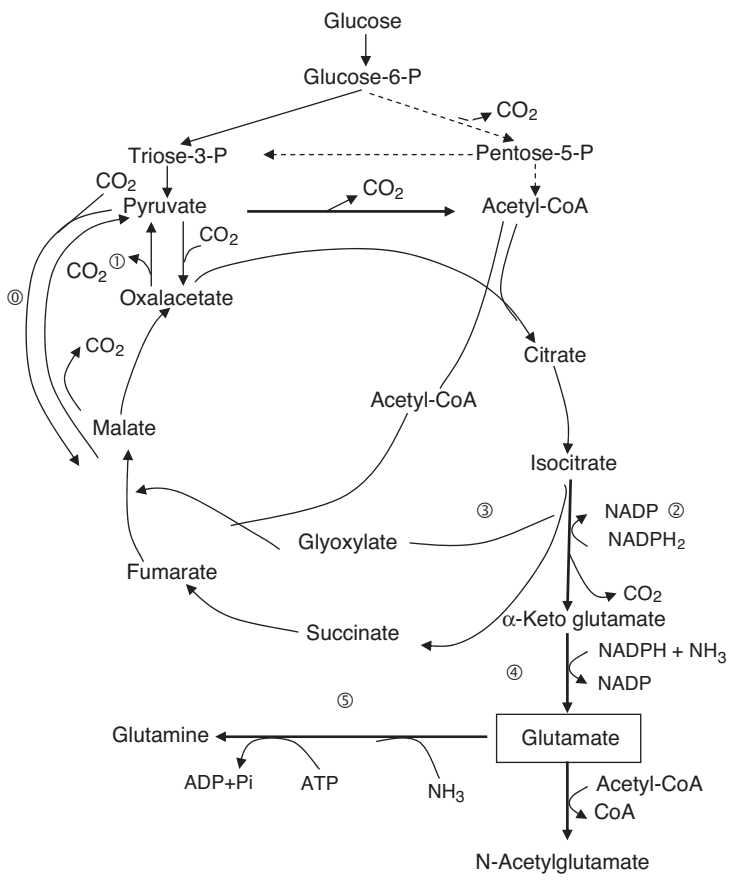


Figure 5.6 Biosynthetic pathways of glutamate production.

In order to convert glucose into amino acid nitrogen source (NH_4^+), energy (NADP) and oxygen are needed:

$$C_{12}H_{12}O_6 + NH_3 + O_2 \rightarrow C_5H_9O_4N + CO_2 + 3H_2O$$

One mole glucose produces 1 mol glutamate, but in the large fermenter the yield of glutamate is 60–67%.

MSG production involves fermentation, recovery, purification, crystallization, drying and packaging (Fig. 5.7).

Lysine is produced by *Corynebacterium glutamicum* or *Brevibacterium flavum* and their mutants. Aromatic amino acids, mainly phenylalanine, are produced by *Escherichia coli*, *Bacillus subtilis* and *B. flavum*.

5.4.2 Nucleic acid production

Nucleic acid is polynucleotide composed of a pentose (ribose or deoxyribose), phosphorus and a base unit. Base units are purines (adenine, guanine and hypoxanthine) and pyrimidines (thiamine, cytosine and uracil). Among the nucleic acid related substances, 5′-IMP (inosine monophosphate) and 5′-GMP (guanine monophosphate) are important for their flavor-enhancing properties, especially for their synergenic effect with MSG. Nucleic acids for flavor enhancement can be produced in many different ways. RNA is extracted from yeast cell mass and decomposed either chemically or by enzymatic method and then by deamination or phosphorylation to produce IMP, GMP and AMP. Another method is to produce inosine and guanine from carbohydrate substrate by *Bacillus subtilis* and then phosphorylation to make IMP and GMP. Direct fermentation process involves *Brevibacterium aminogenesis* fermentation on carbohydrate sources. Figure 5.8 shows the different methods of industrial nucleic acid production.

5.4.3 Organic acids production

Organic acids are also produced by industrial fermentation process or chemical synthetic methods. Over 70 different organic acids are produced by

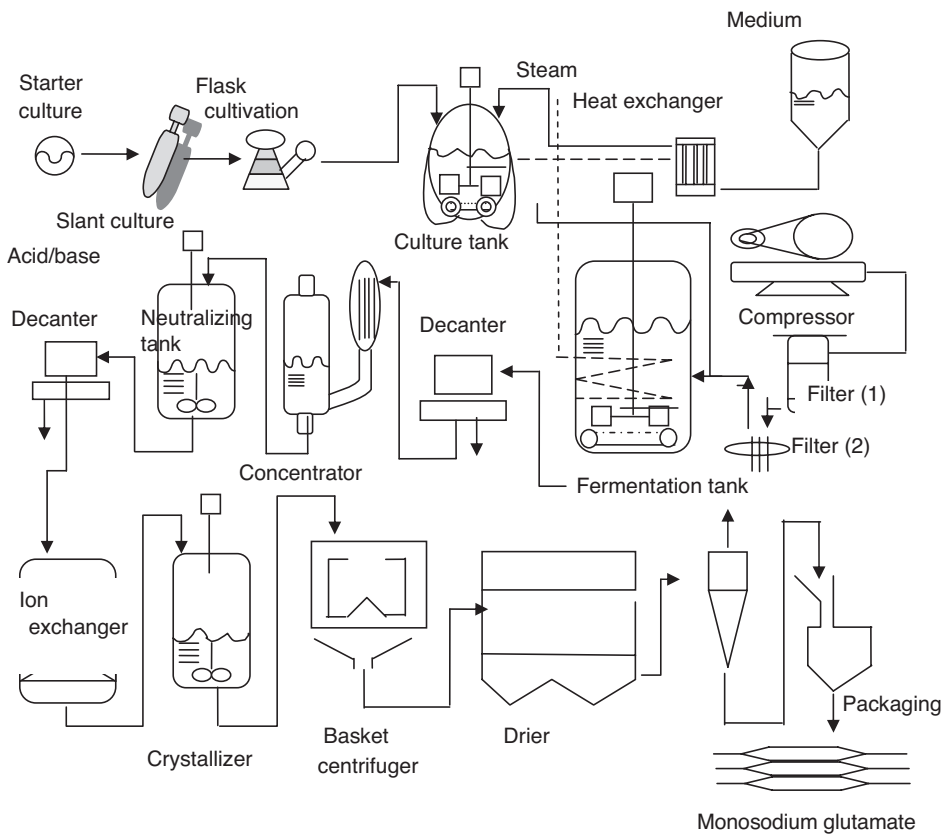
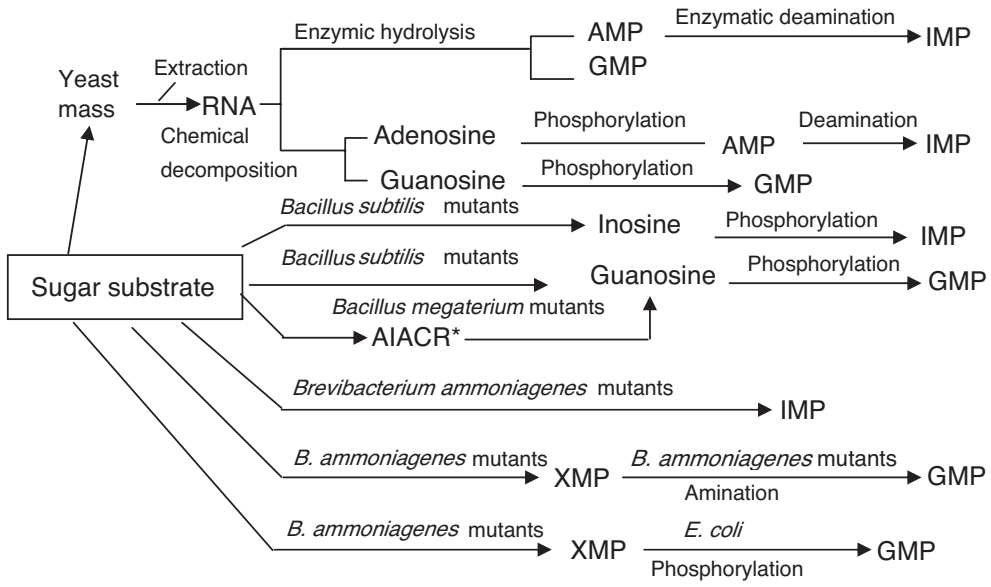


Figure 5.7 Flow diagram of the industrial production of glutamate.



*5-amino-4-imidazole-carboxamide riboside

Figure 5.8 Production methods of flavor enhancer nucleic acids.

fermentation process. Table 5.11 summarizes the microbial strains used for the production of organic acids and their production yield from carbohydrate source.

Enzyme technology is a field aiming at production and improvement of enzymes of interest to the food industry. Modern biotechnology provides the means to select useful enzymes efficiently from conventional fermentation processes.

By using PCR techniques the newly developed enzymes are easily identified. The physiological functions of novel food ingredients are screened and confirmed rapidly by cell culture techniques.

Table 5.11 Microbial strains and yields of industrial organic acid production.

Acids	Microorganisms	Yield (%) (C source)
Acetic acid	*Acetobacter aceti*	95 (Ethanol)
Propionic acid	*Propionibacterium shermanii*	69 (Glucose)
Pyruvic acid	*Pseudomonas aeruginosa*	50 (Glucose)
Lactic acid	*Lactobacillus delbrueckii*	90 (Glucose)
Succinic acid	*Cytophaga succinicans*	57 (Malic acid)
Tartaric acid	*Gluconobacter suboxydans*	27 (Glucose)
Fumaric acid	*Rhizopus delemar*	58 (Glucose)
Malic acid	*Lactobacillus brevis*	100 (Glucose)
Itaconic acid	*Aspergillus terreus*	60 (Glucose)
α-Ketoglutaric acid	*Candida hydrocarbofumarica*	84 (N-paraffin)
Citric acid	*Aspergillus niger*	85 (Glucose)
	Candida lipolytica	140 (N-paraffin)
L(+)-Isocitric acid	*Candida brumptii*	28 (Glucose)
L(–)-Alloisocitric acid	*Penicillium purpurogenum*	40 (Glucose)
Gluconic acid	*Aspergillus niger*	95 (Glucose)
2-Ketogluconic acid	*Pseudomonas fluorescens*	90 (Glucose)
D-Araboascorbic acid	*Penicillium notatum*	45 (Glucose)
Kojic acid	*Aspergillus oryzae*	50 (Glucose)

5.5 Genetic engineering

Modern biotechnology is represented by the production of genetically modified organisms and their use in bioindustry. Since GM foods were first introduced in the 1980s, a quiet revolution in the food supply system has been going on. In 2001 46% of the world's soybean cultivated land and 7% of the world's corn fields were sown with transgenic crops (International Service for the Acquisition of Agri-biotech Applications, 2002). Among the 150 kinds of microbial enzymes in use in food production, over 40 food enzymes are now produced from GM microorganisms.

5.5.1 DNA transcription

Each protein is coded for by a piece of deoxyribonucleotide (DNA), commonly known as a gene. In most instances, DNA is located in the chromosome, although in some bacteria important DNA may be found on extrachromosomal elements called plasmids. In plants, mitochondrial and chloroplast DNA as well as nuclear DNA are important. DNA is composed of linear chains of nucleotide bases; adenine pairs with thymine (A-T) and guanine with cytosine (G-C). In DNA double helix, two strands of nucleotides twist round each other and the strands are linked by bonds between bases in each strand composed of alternating sugar and phosphate sections.

DNA copying is made by breaking the bonds between the bases on each strand and picking up to the free bases new nucleotides from a pool of such molecules provided by the cell. This process occurs with both parent strands and thus two identical DNA molecules are created from one original. The specific paring of the bases guarantees faithful reproduction. The strands of DNA separate and the messenger RNA molecule is built up according to the instructions contained in the DNA strand. As in DNA, the C and G bases pair together, but in mRNA a different base, uracil (U), replaces T as the partner of adenine (A). When the mRNA molecule is complete, it peels off the DNA template and moves to the protein assembly unit 'ribosome'. Ribosomes are built up from several sorts of protein plus a form of RNA called ribosomal RNA. Once the ribosomes have grasped the mRNA, the third type of RNA, transfer RNA (tRNA), comes into action.

There are many sorts of tRNA and each is able to recognize certain codons on the mRNA. Furthermore, each type of tRNA carries with it just one specific type of amino acid. The translation of genetic code depends on the fact that one end of a tRNA molecule recognizes specific codons, while the other end of the same tRNA molecule carries a particular amino acid. The tRNA deposits the amino acid it has brought on the last amino acid in the growing protein chain. The mRNA then moves another notch along the ribosome, exposing the next codon, and so the process continues. This process is known as transcription and translation.

Figure 5.9 shows mRNA translation for protein synthesis (Prentis, 1984).

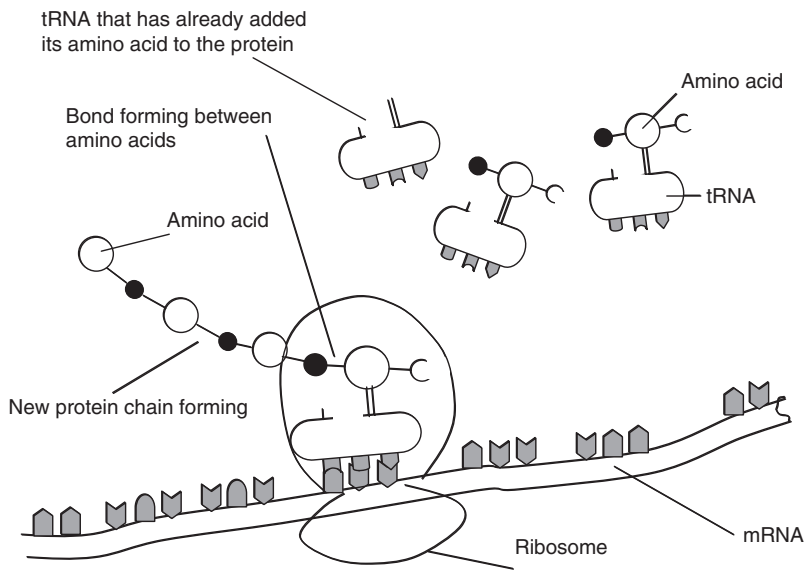


Figure 5.9 Schematic diagram of DNA transcription for protein synthesis.

5.5.2 Recombinant DNA technique

Recombinant DNA is made by excising a specific gene from one organism and inserting it into another organism. The development of gene transfer techniques requires a surgically precise means to cut and rejoin pieces of DNA. The discovery of restriction enzymes and ligases, both derived from bacteria in the early 1970s, enabled this. Restriction enzymes are able to cut DNA at specific sites, and ligases are enzymes which rejoin DNA fragments.

Figure 5.10 shows gene cloning in a simple bacterial system (Harlander, 1987). The process of introducing a gene into a host cell is known as transformation. The proper expressions or functions of transferred genes are tested for conformation. Procedures for transformation and expression in some simple bacteria and unicellular fungi such as *Escherichia coli* and yeast are now well established. Plants and filamentous fungi are more difficult to transform than bacteria or yeast. This is due in part to an increase in the number of chromosomes and the amount of DNA, and to more highly regulated mechanisms of transcription and translation.

Genetic engineering has been applied widely, among others, to crop improvement for high yield, disease resistance, herbicide tolerance and storage quality enhancement, as shown in Table 5.12.

Table 5.12 Application of genetic engineering in the food supply industry.

Agronomic application	Food technology application
Insect protection	Microbial strain improvement for enzyme production
Disease resistance	Ripening modification of fruits and vegetables
Herbicide tolerance	Increased levels of and modification of starch
Virus resistance	Increased levels of and modification of oils
Fungal disease resistance	Improved protein content and quality
Resistance to storage pests	Higher vitamin and mineral contents
Resistance to cold and draught	Reduced cyanogenic glucosides
Nitrogen fixation capability	Improved quality/ processing traits

The most immediate applications of genetic engineering in food technology have been in the dairy, baking and brewing industries. The gene for calf rennin has been isolated and cloned into yeast and fungi

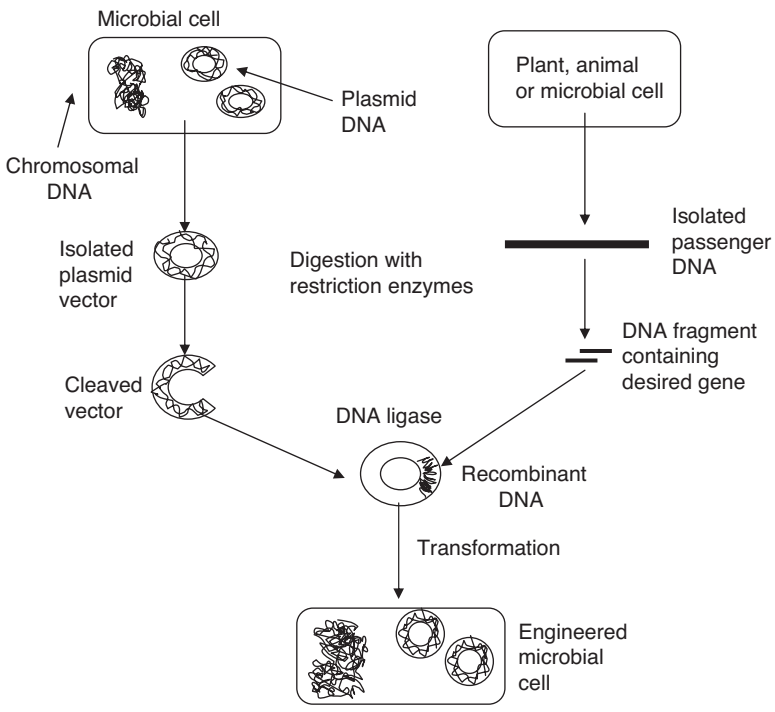


Figure 5.10 Gene cloning in a simple bacterial system.

Table 5.13 Examples of commercial food enzymes from GM microorganisms.

Enzyme	Production organism	Food application
Alpha-acetolactate decarboxylase	*Bacillus amyloliquefaciens* or *subtilis*	Beverages
Alpha-amylase	*Bacillus amyloliquefaciens* or *subtilis*	Baking, beverages
Aminopeptidase	*Trichoderma reesei* or *longibrachiatum*	Cheese, dairy
Arabinofuranosidase	*Aspergillus niger*	Beverages
Beta-glucanase	*Bacillus amyloliquefaciens* or *subtilis*	Beverages
Catalase	*Aspergillus niger*	Egg-based products
Chymosin	*Aspergillus niger*	Cheese
Cyclodextrin-glucosyl transferase	*Bacillus licheniformis*	Starch
Glucoamylase	*Aspergillus niger*	Beverages, baking
Glucose isomerase	*Streptomyces lividans*	Starch
Glucose oxidase	*Aspergillus niger*	Baking
Hemicellulase	*Bacillus amyloliquefaciens* or *subtilis*	Baking, starch
Lipase, triacylglycerol	*Aspergillus oryzae*	Fats
Maltogenic amylase	*Bacillus amyloliquefaciens* or *subtilis*	Baking, starch
Pectin lyase	*Aspergillus niger*	Beverages
Pectinesterase	*Trichoderma reesei* or *longibrachiatum*	Beverages
Phospholipase A	*Trichoderma reesei* or *longibrachiatum*	Baking, fats
Phospholipase B	*Trichoderma reesei* or *longibrachiatum*	Baking, starch
Polygalacturonase	*Trichoderma reesei* or *longibrachiatum*	Beverages
Protease	*Aspergillus oryzae*	Cheese
Pullulanase	*Bacillus licheniformis*	Starch
Xylanase	*Aspergillus niger*	Baking, beverages

to produce calf rennin from microorganisms. The lactose utilization genes of *E. coli* have been cloned into *Sac. cerevisiae*, *Xanthomonas campestris* and other microorganisms so that the lactose in whey permeate can be converted into ethanol, single-cell protein or xanthan gum. Interspecies gene transfer and protein engineering have been applied to microbial enzyme modification, such as in the production of thermotolerant amylase.

Table 5.13 lists the enzymes produced by GM microorganisms on the market (Robinson, 2001). However, no GM microorganisms have been utilized directly in food production yet.

5.6 Tissue culture

Plant tissue culture, the propagation of plant tissue in aseptic nutrient media, has widespread application in food and ingredient production (Wasserman *et al.*, 1988). Figure 5.11 shows the tissue culture process (Harlander, 1987). Plant tissue culture plays an important role in plant research, particularly genetic engineering because of time saving. The introduction of new genes into plant tissue requires the generation of single plant cells, or protoplasts. After genetic material is introduced, the protoplasts are grown in tissue culture, and, ultimately, whole plants are regenerated from single cells. Fusion of two genetically dissimilar protoplasts followed by regeneration often results in plants with desirable properties.

Highly valued natural products such as flavors, colors, preservatives and nutritional supplements are biosynthesized efficiently using this technology. For example, the world's supply of vanilla beans does not satisfy the demand for vanilla flavor. The in-vitro biosynthesis of natural vanilla flavor would alleviate this problem (Moshy, 1986). Product yields in plant cell culture are often many-fold higher than those found in the native plant. Technologies used to accomplish this include mutation, adjustment of nutrient and hormone levels, addition of appropriate metabolic precursors and plant cell immobilization (Knorr and Sinskey, 1985).

5.7 Future prospects

The biotechnological revolution we have witnessed in the last century has opened up the possibility of processing food through specific reaction and producing food having specific function. The initial application of genetic engineering for agronomic benefits, such as resistance to herbicides and pesticides,

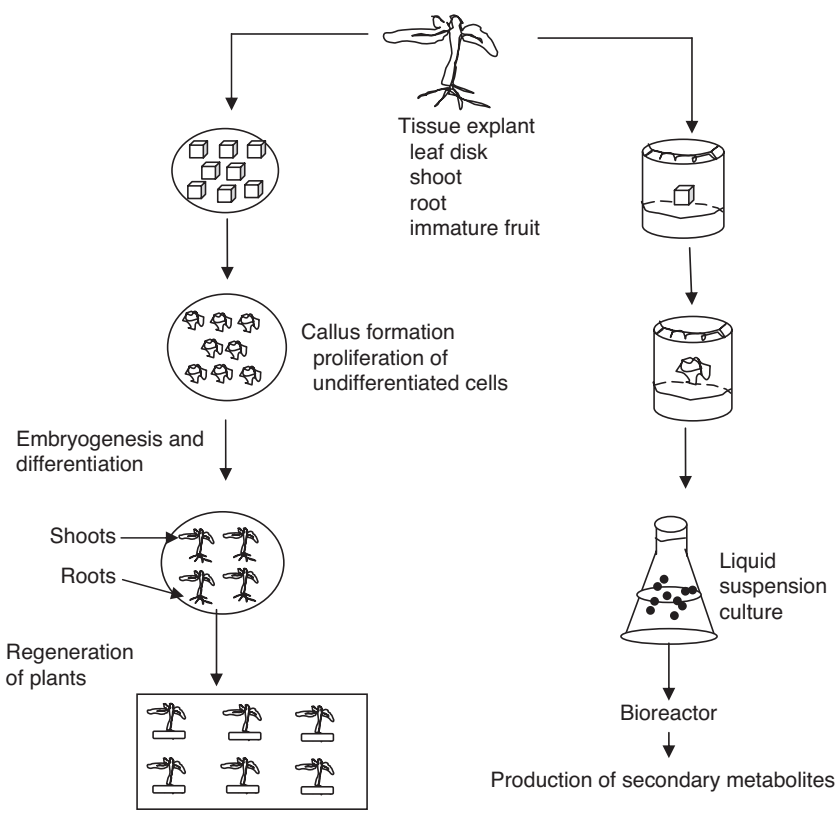


Figure 5.11 Schematic diagram of tissue culture.

shorter time between sowing and harvesting and increased yields, is now gradually changing focus to quality traits for producing better nutritional value and keeping quality. Although the direct use of GM microorganisms in food has not yet occurred in the market place, new and powerful enzymes derived from GM microorganisms are widely used in food processing. By knowing the genetic map of major food crops and the amino acid sequence of proteins, the tailor-making of food ingredients having specific function is now possible through biotransformation and biocatalysis (Lee, 2003).

Modern biotechnology provides the means to select useful enzymes efficiently from conventional fermentation processes. By using PCR technique the newly developed enzymes are easily identified. The physiological functions of novel food ingredients are screened and confirmed rapidly by cell culture technique.

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