

Chapter 2

Literature review

1. Probiotics

Originally defined as a substance stimulating growth of other microorganisms, nowadays probiotics are redefined and restricted to a viable microbial agent, which beneficially affects the host possibly by improving indigenous microflora balance when used in animals or humans. Based on the latter meaning, several terms such as “friendly”, “beneficial” or “healthy” bacteria are commonly known for probiotics. Moreover, probiotics have been recently defined more precisely as “mono or mixed cultures of live microorganisms which beneficially affects the host possibly by improving indigenous microflora properties when used in animals or humans”. In relation to foods, probiotics are considered as viable food or dietary supplemented preparations to improve health of humans and animals. Accordingly, an impressive number of microbial genera and species are considered as probiotics (Table 2.1) (Holzapfel *et al.*, 2001).

Probiotics offer various potential therapeutic uses including (Anonymous, 2005d):

1. Replacing the "friendly" intestinal bacteria destroyed by antibiotics.
2. Aiding digestion and suppressing disease-causing bacteria.
3. Preventing and treating diarrhea, including infectious diarrhea, particularly from rotavirus (a virus that commonly causes diarrhea in children).

Table 2.1 Microorganisms considered as probiotics (Holzapfel *et al.*, 2001)

<i>Lactobacillus</i>	<i>Bifidobacterium</i>	Other lactic acid bacteria	Nonlactic acid bacteria
<i>L. acidophilus</i>	<i>B. adolescentis</i>	<i>Enterococcus faecalis</i> ¹	<i>Bacillus cereus</i> var. <i>toyoi</i> ^{1,2}
<i>L. amylovorus</i>	<i>B. infantis</i>	<i>Enterococcus faecium</i>	<i>Escherichia coli</i> strain nissle
<i>L. casei</i>	<i>B. animalis</i>	<i>Leuconostoc mesenteroides</i>	<i>Propionibacterium freudenreichii</i> ^{1,2}
<i>L. crispatus</i>	<i>B. bifidum</i>	<i>Pediococcus acidilactici</i>	<i>Saccharomyces cerevisiae</i> ²
<i>L. galinarum</i> ¹	<i>B. breve</i>	<i>Sporolactobacillus inulinus</i> ¹	<i>Saccharomyces boulardii</i> ²
<i>L. gasseri</i>	<i>B. lactis</i> ³	<i>Streptococcus thermophilus</i>	
<i>L. johnsonii</i>	<i>B. longum</i>	<i>Lactococcus lactis</i>	
<i>L. paracasei</i>			
<i>L. plantarum</i>			
<i>L. reuteri</i>			
<i>L. rhamnosus</i>			

¹ Main application for animals.

² Applied mainly as pharmaceutical preparations.

³ Probably synonymous with *B. animalis*.

4. Treating overgrowth of "bad" organisms in the gastrointestinal tract (a condition that tends to cause diarrhea and may occur from use of antibiotics).
5. Alleviating symptoms of irritable bowel syndrome and, possibly, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis).
6. Preventing and/or reducing the recurrence of vaginal yeast infections, urinary tract infections and cystitis (bladder inflammation). The best scientific evidence exists for vaginal infections.
7. Improving lactose absorption digestion in people who are lactose intolerant.
8. Enhancing immune response. Studies have suggested that consumption of yogurt or milk that contains specific strains of *Lactobacillus* or supplements with *Lactobacillus* or *Bifidobacterium* may improve the natural immune response. Further research is needed to confirm these early findings and to

understand how the improved immune function may or may not help in warding off infections.

9. Aiding the treatment of respiratory infections such as sinusitis, bronchitis and pneumonia. More research is needed in this area.
10. Lowering risk of allergies. Examples include asthma, hay fever, food allergies to milk and skin reactions such as eczema.
11. Helping to treat high cholesterol. More research is needed.
12. Reducing the risk of recurring bladder tumors once this cancer has been treated. Much more research is needed in this area.

2. Prebiotics

Prebiotics are any food component that cannot be hydrolyzed by human digestive enzymes or absorbed in upper gastrointestinal tract. When entering colons, it may also serve as a growth substrate for intestinal microflora. The term prebiotics come from the observation that inulin (long chain oligosaccharides, ranging from 2-60 sugar units) and fructo-oligosaccharides (FOS, short chain oligosaccharides with 2-7 sugar units) selectively stimulate growth of bifidobacteria which are considered to be beneficial for human health (Gibson and Roberfroid, 1995). Nowadays, researches have been done on many other non-digestible oligosaccharides including xylo-oligosaccharides, lactulose, lactitol, lactosucrose, pyrodextrins and a range of oligosaccharides that supply as a source of fermentable carbohydrate that can give a beneficial effect on host health by selectively stimulate growth and/or activity of one or limited numbers of colon bacteria (Anonymous, 2005f).

3. Synbiotics

Synbiotic refers to a product containing both probiotic and prebiotic. The effect of synbiotic may directly toward two different target regions of the gastrointestinal tract such as small and large intestines. In addition, if the prebiotic carbohydrate is utilized by a probiotic strain, its growth and proliferation in the gut will be selectively promoted (Holzapfel and Schillinger, 2002).

4. *Lactobacillus acidophilus*

L. acidophilus is commonly used commercially together with *Streptococcus thermophilus* in production of yogurt. The scientific classification of the bacterium is

Domain	<i>Eubacteria</i>
Kingdom	<i>Monera</i>
Division	<i>Firmicutes</i>
Class	<i>Bacilli</i>
Order	<i>Lactobacillales</i>
Family	<i>Lactobacillaceae</i>
Genus	<i>Lactobacillus</i>
Species	<i>L. acidophilus</i>

L. acidophilus obtains its name from *lacto* (meaning milk), *bacillus* (meaning rod-like) and *acidophilus* (meaning acid loving). The bacterial shape is rod with round ends, generally 0.6 – 0.9 x 1.5 – 6 µm in size and occurring singly, in pairs or short chains (Figure 2.1). It thrives in acidic environments with pH ranging from 4-5 to lower and grow best at 45°C (Sneath *et al.*, 1986 and Anonymous, 2006h).

L. acidophilus occurs naturally in a variety of foods including dairy, grains, meat, fruits and vegetables. It is also present in human and animal intestines, mouths

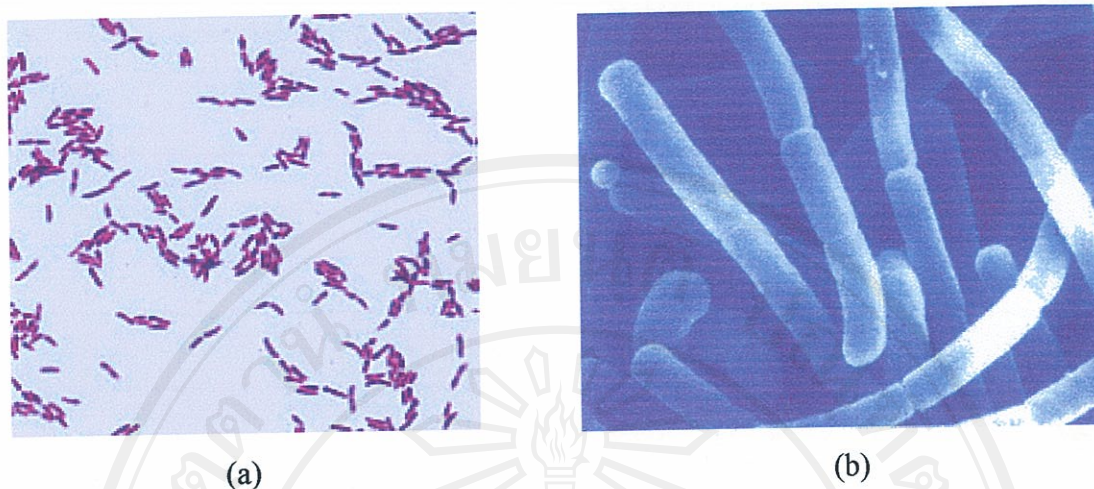


Figure 2.1 *Lactobacillus acidophilus* (a) Gram stain (Anonymous, 2006i) and (b) Scanning electron micrograph (Anonymous, 2006j).

and vaginas. Like other lactic acid bacteria, *L. acidophilus* can absorb and metabolize lactose into lactic acid. The bacterium effectively produces energy, ATP, by a substrate level phosphorylation via a homolactic acid fermentative pathway to yield almost solely lactic acid (Figure 2.2). Certain related species known as heterofermentative lactic acid bacteria can also produce ethanol, carbon dioxide and acetic acid as by-products (Figure 2.3) (Anonymous, 2006h).

Ninety percent or more strains of *L. acidophilus* can ferment various types of carbohydrate including amygdalins, cellobiose, esculin, fructose, galactose, glucose, lactose, maltose, mannose, salicin, sorbitol and sucrose. Other nutritional requirements are calcium pantothenate, folic acid, niacin and riboflavin while pyridoxal, thiamine, thymidine and vitamin B12 are not required (Sneath *et al.*, 1986). Srinivas *et al.* (1990) studied on utilization of various carbohydrates viz., glucose,

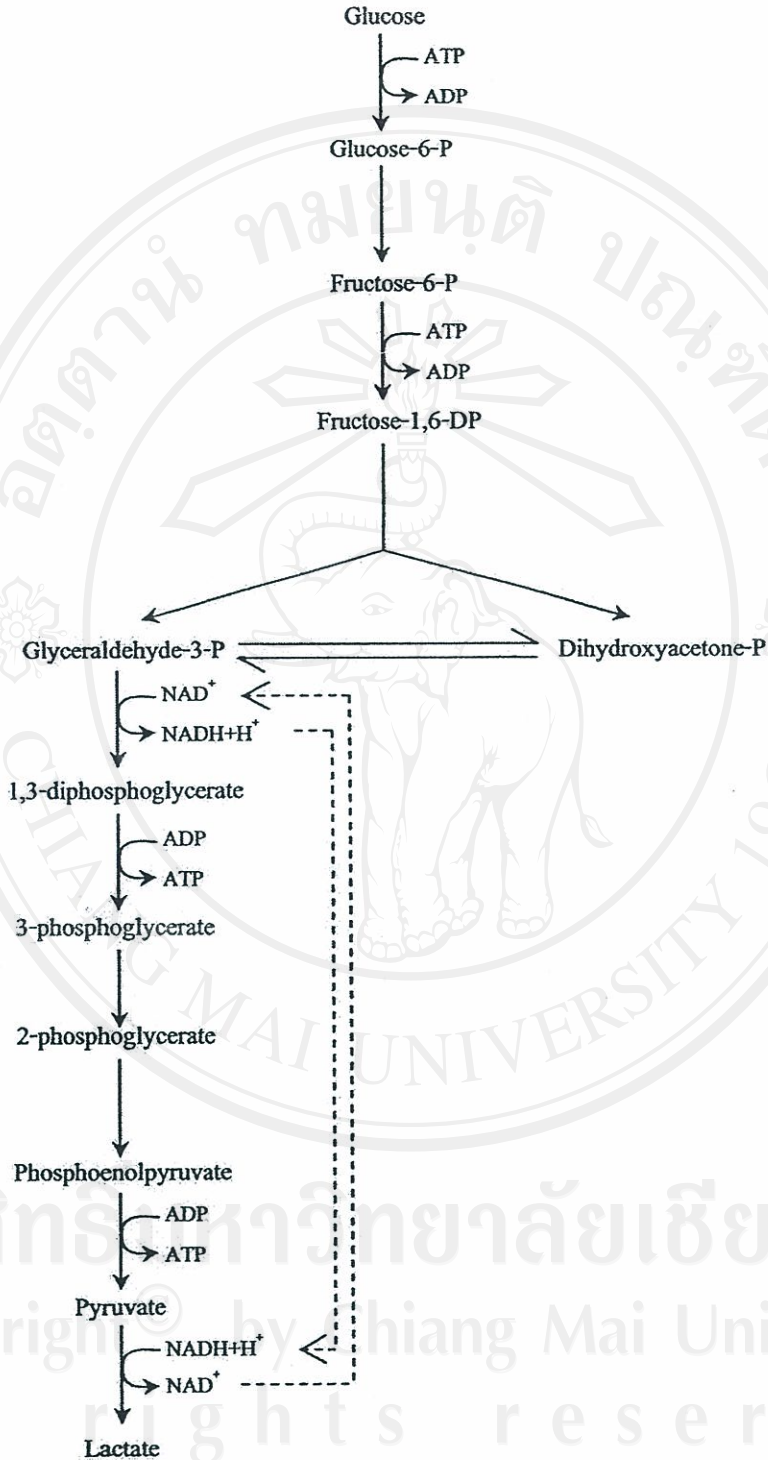


Figure 2.2 Homolactic acid fermentation (Axelson, 1993)

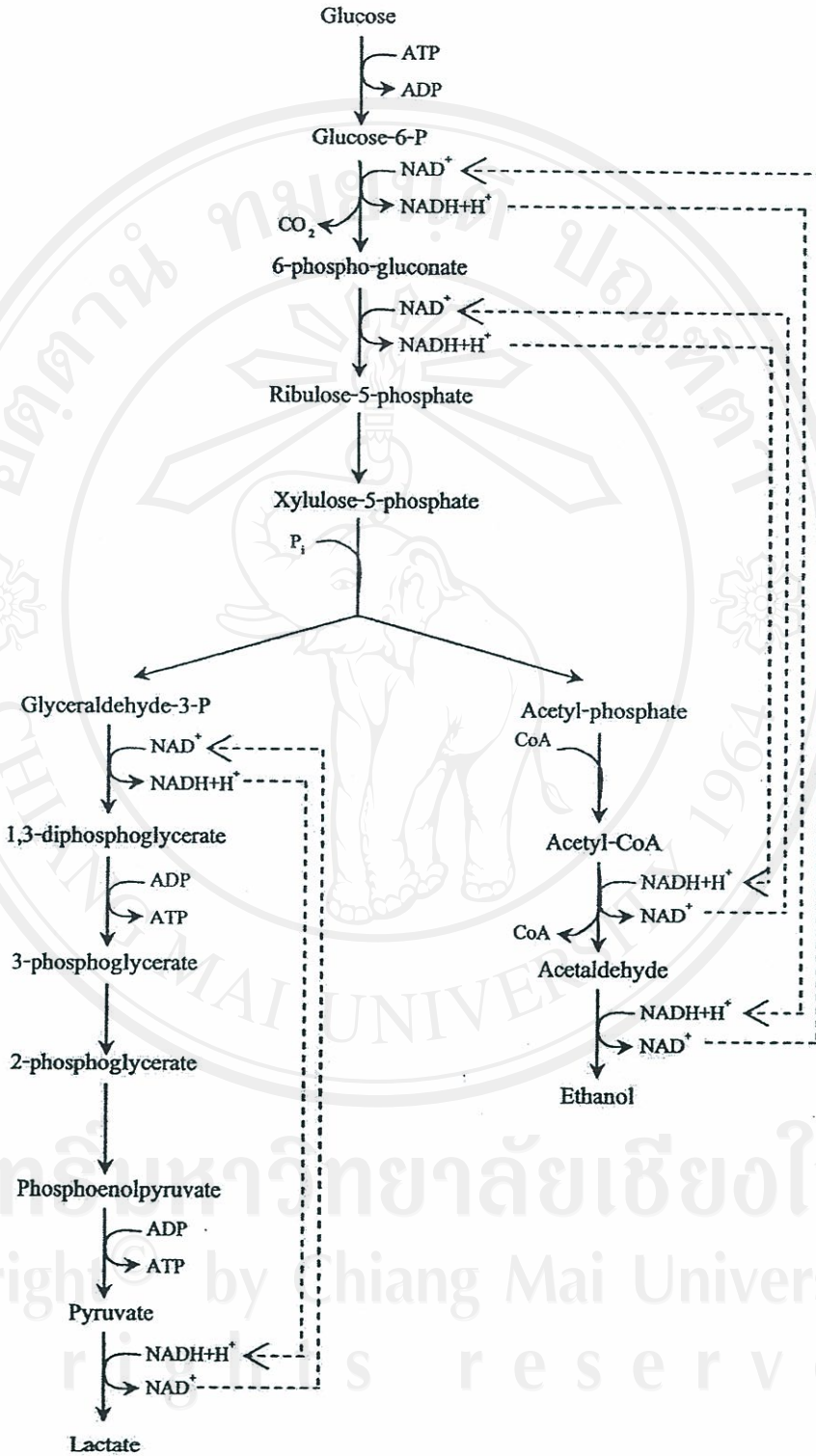
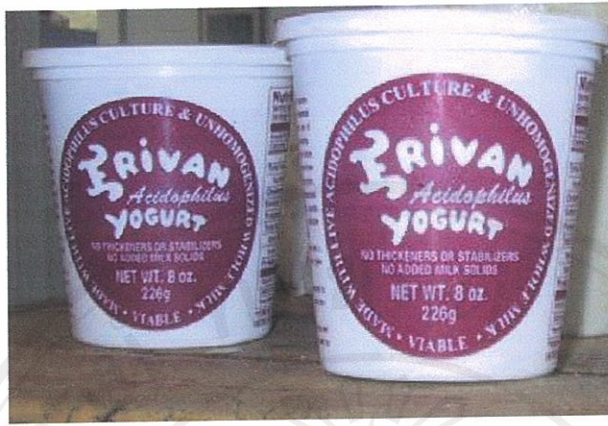


Figure 2.3 Heterolactic acid fermentation (Axelson, 1993)

fructose, sucrose, lactose and galactose by *L. acidophilus* strains that was investigated in Lactobacillus Selection Broth. Maximum viable counts, acid production and sugar utilization by different test strains were in the order: glucose \geq fructose $>$ sucrose \geq lactose $>$ galactose. The generation time of the tested strains was shorter in glucose medium as compared to sucrose or lactose medium.

The primary dietary sources of *L. acidophilus* are acidophilus milks, yogurts, acidophilus bifidus yogurt, miso and tempeh (Figure 2.4). The common use of *L. acidophilus* as a probiotic is due to its ability to inhabit human and animal intestines and vaginas, and to protect its host against entry and proliferation of pathogenic organisms especially bacteria. One of the mechanisms to inhibit growth of pathogens is the breakdown of foods by *L. acidophilus* leading to production of lactic acid, hydrogen peroxide and other by-products that make the gut environment hostile for undesired organisms. Also, *L. acidophilus* is able to produce lactase, an enzyme that breaks down lactose in milk into simpler sugars, glucose and galactose. Lactose intolerant consumers do not have this enzyme; hence, milk containing *L. acidophilus* will help reducing effect of lactose on their gastrointestinal tract. Probiotic properties of *L. acidophilus* include balance of intestinal microflora, reduction of faecal enzymes, antitumor, stimulation of immune response and prevention of diarrhea (Anonymous, 2005d, Chukeatirote, 2003 and Ziemer and Gibson, 1998).



(a)



(b)



(c)

Figure 2.4 Commercial products containing *L. acidophilus* (a) yogurts (Anonymous, 2006k), (b) tempeh (Anonymous, 2006l) and (c) miso (Anonymous, 2007f)

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5. Rice

Scientific classification of rice is (Anonymous, 2006d)

Kingdom	<i>Plantae</i>
Division	<i>Magnoliophyta</i>
Class	<i>Liliopsida</i>
Order	<i>Poales</i>
Family	<i>Poaceae</i>
Genus	<i>Oryza</i>
Species	<i>Oryza glaberrima</i> <i>Oryza sativa</i>

Rice refers to two species of grasses, *Oryza sativa* L. and *Oryza glaberrima*, native to tropical and subtropical southern and southeastern Asia and to Africa, which together provide more than one fifth of the calories consumed by human. It is an annual plant, growing to 1-1.8 m tall, occasionally more, with long slender leaves 50-100 cm long and 2-2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30-50 cm long. The seed is a grain (caryopsis) with 5-12 mm long and 2-3 mm thick (Figure 2.5) (Anonymous, 2006d).

O. glaberrima is an annual species originating in West Africa, covering a large region extending from the central Delta of the Niger River to Senegal.

Slight morphological differences separate the two species of rice, making them difficult to tell apart in the field. Generally speaking, *O. glaberrima* has small grains that are pear-shaped and have a red bran and an olive-to-black seedcoat, straight awnicles that are simply branched and short rounded ligules. Some *O. sativa* L. have pear-shaped grains with a red bran and some *O. glaberrima* have pointed ligules (Linares, 2002).

O. sativa L. is a short-lived plant related to the grass family, with a life cycle of 3-7 months. The various cycle spans depend on rice types and the growing environment. Rice cultivation requires an extensive irrigation system and properly leveled fields. A uniformly leveled field enables each rice kernel to absorb the same amount of moisture from the field. This uniform moisture level will maintain a consistency in the rice quality. If the moisture level runs too high, the rice may spoil faster (Anonymous, 2006a).

Rice grain, kernel or true fruit contains the followings (Anonymous, 2006a and Anonymous, 2007g):

1. Shell, husk or hull contains three protective layers called awn, palea and lemma to cover a kernel or grain. These structures are inedible, but used as fuel in power plants and rice mills.
2. Bran coat contains fiber, vitamin B1 (thiamine), niacin, vitamin B6, iron, phosphorus, magnesium, potassium, protein and fat. It is the most nutritious part of rice. It resides in the outermost part of brown rice; giving its color and nutty flavor.
3. Starchy endosperm lies below a bran coat. This layer makes up most of the rice grain. It consists of an embryo at the grain base. Amylose and amylopectin are main starches in the endosperm giving energy source used by a germinating seed. The mixture of these two starches determines the cooking texture.
4. Embryo is the innermost part of a rice kernel. Together, the starchy endosperm, bran coat and embryo are called brown rice (caryopsis).

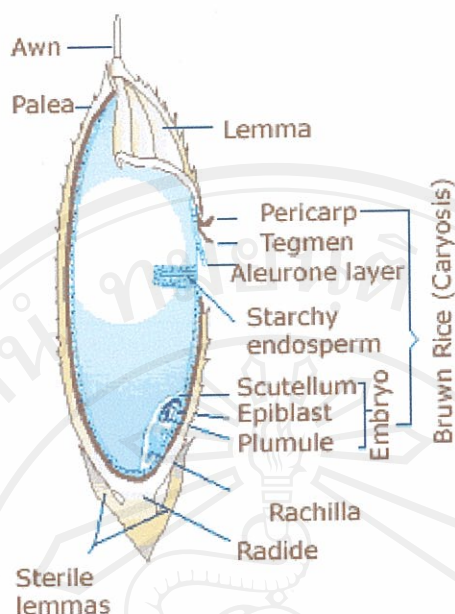


Figure 2.5 *Oryza sativa* L. structure (Anonymous, 2006a)

5.1 Rice varieties and cultivars

Generally, *O. sativa* L. can be classified into three varieties (Anonymous, 2006a):

1. ***Oryza sativa* L. var. Indica:** commonly grown in warm climate region including Thailand, India, Pakistan and southern United States of America. It has long grain.
2. ***Oryza sativa* L. var. Japonica:** having round grain. The cultivating areas are mostly in colder weather such as Japan, Korea, northern China and California.
3. ***Oryza sativa* L. var. Javanica:** having medium grain size comparing to the other two cultivars. The only cultivation place is Indonesia.

The cultivar of rice emerges rapidly due to attempts to develop high rice quality. In Thailand, there are approximately six popular rice for export including jasmine rice, white rice, white glutinous rice, black glutinous rice, brown rice and red cargo rice (Figure 2.6) (Anonymous, 2007a).

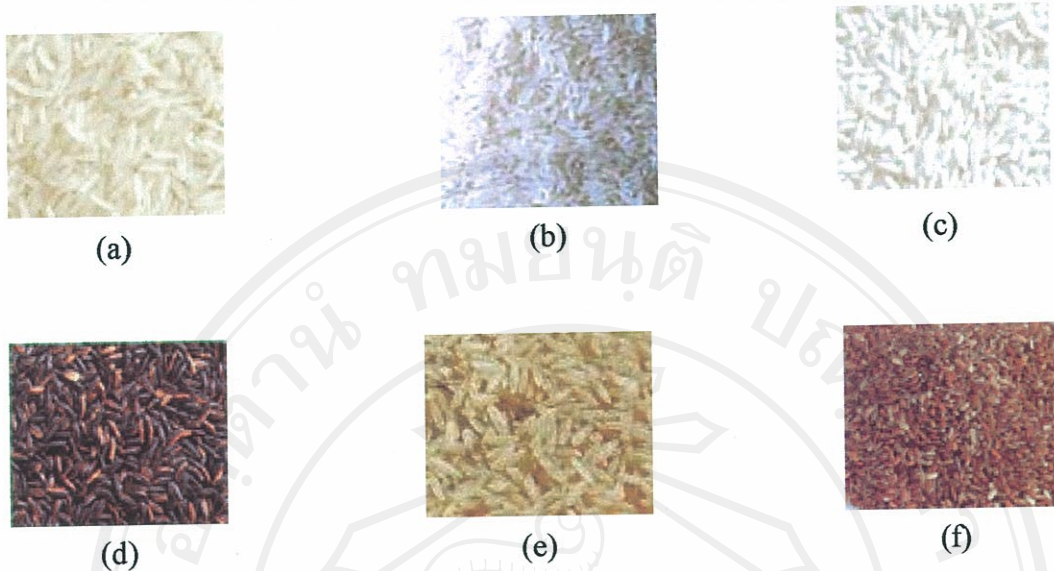


Figure 2.6 Thai rice cultivars (Anonymous, 2006a). (a) jasmine rice (b) white rice (c) white glutinous rice (d) black glutinous rice (e) brown rice (f) red cargo rice

Jasmine rice (*kao hom mali*) is a long-grain variety of rice that has a nutty aroma and subtle flavor. Most households in Thailand consume this type of rice and it is commonly found in supermarkets. The rice is mainly used for many types of rice dishes or only as plain white rice in Thailand (Anonymous, 2007a).

White rice is the name given to milled rice which has had its husk, bran and germ removed. This is done largely to prevent spoilage and to extend the storage life of the grain. After milling, the rice is polished, resulting in a seed with a bright, white and shiny appearance. Milled and polished kernel loses many of its nutrients when the outer layer (the husk and bran layer) is removed. It contains much less niacin, thiamin, magnesium, zinc, iron and fiber than the brown rice (Anonymous, 2007a and Anonymous, 2007b).

White glutinous rice (also called sticky rice, sweet rice, waxy rice, botan rice, mochi rice and pearl rice) is a type of short-grained Asian rice that is especially sticky

when cooked. It is called glutinous in the sense of being glue-like or sticky and not in the sense of containing gluten; on the other hand, it is called sticky but should not be confused with the other varieties of Asian rice that become sticky to one degree or another when cooked. Glutinous rice does not contain dietary gluten (i.e. does not contain glutenin and gliadin), and thus should be safe for gluten-free diets. What distinguishes it from other types of rice is having no (or negligible amounts of) amylose, and high amounts of amylopectin, the two components of starch. Amylopectin is responsible for the sticky quality of glutinous rice. The difference has been traced to a single mutation that was selected for by farmers. Glutinous rice can be used either milled or unmilled (that is, with the bran removed or not removed). The former is white and the latter is black or purple. Either can be cooked as grains or ground into flour and cooked as a paste (Anonymous, 2007c).

Black glutinous rice is glutinous rice which has a black color for the whole kernel, including kernels that are dark brown (Anonymous, 2007d).

Brown rice (or otherwise called "hulled rice") is a kind of whole grain that is unmilled or partly milled rice it comes from paddy which only the external and non-edible husk has been removed. The bran layer remains, making it more nutritive than white rice. In Europe, this type of rice is often called "cargo rice" because of the way it is transported by sea. It has a mild nutty flavor, is chewier than white rice, becomes rancid more quickly, but is far more nutritious. Any rice, including sticky rice, long-grain rice or short-grain rice, may be eaten with brown rice (Anonymous, 2007a and Anonymous, 2007b).

Red cargo rice is rice which has a red bran covering the kernel and only its husk has been removed (Anonymous, 2007b).

5.2 Rice types

Many types of rice are formed during milling process. In Thailand, there are brown (cargo), white and broken rice (Figure 2.7).

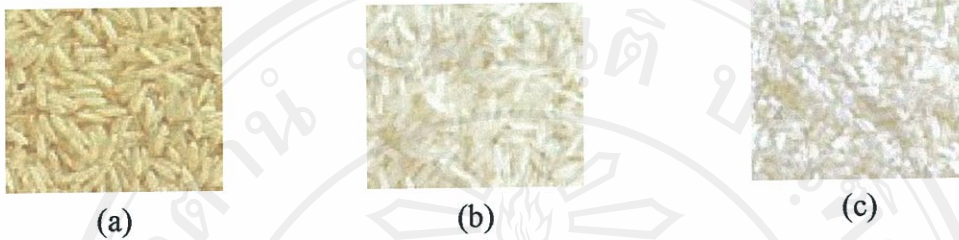


Figure 2.7 Thai rice forms (Anonymous, 2006a) (a) brown rice (b) white rice
(c) broken rice

Brown rice or cargo rice: When husk layer is removed after running through a milling process, the rice kernel is only covered by a bran coat rendering its brown color. The grain is called brown rice, which is nutritionally rich (Anonymous, 2007b).

White rice: White rice is rice which has had its husk, bran and germ removed. After milling, the rice is polished, resulting in a seed with a bright, white and shiny appearance (Anonymous, 2006a).

Broken rice: During the milling process, broken rice is separated from the rice, whose shape remains intact. In other words, broken rice is the damaged white rice and is normally used in animal feeding or other food and beverage processes, such as brewing and flour processing. A grain of broken rice gives a low fiber texture and low nutrient level, while retaining its high energy content (Anonymous, 2006a).

Brown rice and white rice have similar amounts of calories, carbohydrates, fat and protein. The difference between the two lies in processing and nutritional content. If the outermost layer of a grain of rice (the husk) is removed, the result is brown rice. If the husk and the bran layer underneath are removed, the result is white rice.

Several vitamins and dietary minerals are lost in this removal and the subsequent polishing process. A part of these missing nutrients, such as B1, B3 and iron are sometimes added back into the white rice making it "enriched", as food suppliers in the US are required to do by the Food and Drug Administration (FDA) (Anonymous, 2007a).

Analysis for some types of rice grain by คำเนิน *et al.* (2543) showed that rice grain contains many nutrients (Table 2.2).

Table 2.2 Nutritional values of brown, white and black glutinous rices
(คำเนิน *et al.*, 2543)

Nutrient	Brown rice	White rice	Black glutinous rice
Moisture (%)	12.0	12.0	12.0
Energy (Kcal/100 g)	360	363	360-363
Protein (%)	7.5	6.7	7.0-8.0
Fat (%)	1.9	0.4	1.5-2.5
Ash (%)	1.2	0.5	1.0-1.5
Fiber (%)	0.9	0.3	0.9-1.0
Carbohydrate (%)	77.4	80.4	75.0-80.0
β -carotene (mg/kg)	3.58	0.12	4.0-5.1
Thiamine (mg/100g)	0.34	0.07	NA

Research had indicated that rice grains contain phenolic compounds, such as ferulic acid, *p*-coumaric acid and diferulates, which are not significant present in fruits and vegetables. These phenolic compounds have activities of antioxidant, antimutagenic and anticancer, as well as other positive effects which play an important role in maintaining health. Most of these compounds are bound to cell wall

polysaccharides containing glucose, arabinose, xylose, galactose, rhamnose, and mannose residues (Tian *et al.*, 2005).

Zhou *et al.* (2004) studied the distribution of phenolic acids in rice. They quantified these acids in three cultivars of fresh and aged rice. High levels of ferulic acid (255-362 mg/kg grain) and *p*-coumaric acid (70-152 mg/kg grain) were found in brown rice with lower levels (e.g. ferulic acid 61-84 mg/kg grain) in milled rice. Bound phenolic acids comprised 80-90% of the total phenolic acids for brown rice and 53-74% for milled rice. Storage led to a decrease in the total and bound phenolic acid contents in both brown and milled rice and the decline was greater at 37°C than at 4°C storage.

In 1993, an antitumor activity was found in Thai rice (RD-7), which had potent cytotoxic effects on four out of seventeen transformed cell lines, but not on untransformed cell strains. The activity was very weak, though significant, in dormant rice seeds, but it increased remarkably from 3 days to 7 days after inoculation, decreasing again in the later stages of growth (Okai *et al.*, 1993).

A great deal of interest has been given to the association between the consumption of pigmented rice and the improvement of human health due to the great antioxidant potency of phenolic compounds that the rice contain. Pigmented rice has been reported to contain acetylated procyanidin, anthocyanins and other phenolic compounds with significant free radical scavenging activity (Yawadio *et al.*, 2006). Hu *et al.* (2003) reported that black rice (*O. sativa* L. Indica) contains pigments locating the aleurone layer. Known proportions of cyanidin 3-glucoside and peonidin 3-glucoside exhibited marked antioxidant activities

and free radical scavenging capacities in a battery of *in vitro* model systems. Prevention of supercoiled DNA strand scission induced by reactive oxygen species specifically peroxy radical and hydroxyl radicals and suppression of the oxidative modification of human low-density lipoprotein were obtained from black rice pigmented fraction. In addition, black rice pigmented fraction reduced the formation of nitric oxide by suppressing inducible nitric oxide synthase expression in murine macrophage RAW264.7 cells without introducing cell toxicity.

Chen *et al.* (2005) used two bioactive compounds, peonidin 3-glucoside and cyanidin 3-glucoside, from *O. sativa* L. Indica to treat various cancer cells. The results showed that among analyzed cell lines, HS578T was the most sensitive to peonidin 3-glucoside and cyanidin 3-glucoside. The strong inhibitory effect on cell growth was via G2/M arrest. Regarding cell cycle related proteins, peonidin 3-glucoside treatment resulted in down regulation of protein levels of cyclin-dependent kinase (CDK)-1, CDK-2, cyclin B1 and cyclin E, whereas cyanidin 3-glucoside could decrease the protein levels of CDK-1, CDK-2, cyclin B1 and cyclin D1. In addition, cyanidin 3-glucoside or peonidin 3-glucoside also induced caspase-3 activation, chromatin condensation and cell death. Furthermore, anthocyanins from *O. sativa* L. indica were reported by their inhibition effect on the growth of Lewis lung carcinoma cells *in vivo*.

The colors found in black rice are naturally occurring compounds belonging to the family of flavonoids. The health benefits of flavonoids are usually linked to two properties; inhibition of certain enzymes such as xanthine oxidase and aldose reductase, and antioxidant activity (Yawadio *et al.*, 2006). It was suggested that notable antioxidant and anti-inflammatory properties of

anthocyanin in black rice are promising to be used as nutraceuticals or functional food formulations.

6. Honey

According to the United States National Honey Board 2003 and other nations' food regulations, honey is a sweet and viscous fluid produced by honeybees and other insects from the nectar of flowers. The definition of honey stipulates a pure product that does not allow for addition of any other substances. This includes, but not limited to, water or other sweeteners. This definition refers exclusively to the honey produced by honeybees (genus *Apis*) while honey produced by other bees or insects has different properties and hence does not include in here (Anonymous, 2006b).

Honey is significantly sweeter than table sugar and has attractive chemical properties for baking. Honey has a distinctive flavor leading to its preference over sugar and other sweeteners by some consumers. Liquid honey does not spoil. Because of its high sugar concentration, honey kills bacteria by plasmolysis. Natural airborne yeasts cannot become active in honey because the moisture content is too low. Naturally, moisture content in raw honey varies from 14% to 18%. As long as the moisture content remains under 18%, virtually no organism can successfully multiply to significant amounts in honey (Anonymous, 2006b).

Existence of pollens and spores in raw honey are evaluated by using a melissopalynology technique which can determine floral sources of honey. Because bees carry an electrostatic charge, which attracts other particles, the melissopalynology can be used for environmental studies of radioactive particles,

dust, or particulate pollution. A main effect of bees collecting nectar to make honey is pollination, which is crucial for flowering plants (Anonymous, 2006b).

6.1 Honey composition

Honey is a mixture of sugars and other compounds (Table 2.3). The specific composition of any batch of honey will depend largely on the mix of flowers consumed by the bees that produced the honey, for example honey from longan flowers (Table 2.4). Honey has a density of about 1500 kg/m³ (50% denser than water) which means 12-13 pounds per gallon (Anonymous, 2006b).

Often honey is recommended because of its content of other nutrient like vitamins and mineral, but their quantity are so low that it is unrealistic to think they can provide any significant supplement in a deficient diet (Table 2.5) (Anonymous, 2005b).

Table 2.3 Composition of U.S. honey (Anonymous, 2005b)

Component (% except pH and diastase value)	U.S honey
	Value (%)
Water	17.20
Fructose	38.20
Glucose	31.30
Sucrose	1.30
Maltose (reducing disaccharides calculated as maltose)	7.30
Higher sugar	1.50
Free acids (as gluconic acid)	0.43
Lactone (as glucolactone)	0.14
Total acid (as gluconic acid)	0.57

Table 2.3 (Continued)

Component (% except pH and diastase value)	U.S honey
	Value (%)
Nitrogen	0.04
pH	3.91
Diastase value	20.80

Table 2.4 Composition of honey from longan flower (Deeponpuk, 2005)

Component (% except pH, a_w and hydrogen peroxide)	Longan flower honey
	Value (%)
Water	21.20
Fructose	39.69
Glucose	32.12
Solid	78.80
Reducing sugar	72.23
Water activity (a_w)	0.59
Total acid	14.55
Ash	0.15
Nitrogen	0.04
pH	4.16
Hydrogen peroxide (mg/ml)	2.50

Table 2.5 Nutrients in honey (Anonymous, 2005b)

Nutrient	Average amount in 100 g honey	Unit
Energy equivalent	304	kcal
Vitamins		
B1 (thiamin)	0.004-0.006	mg

Table 2.5 (Continued)

Nutrient	Average amount in 100 g honey	Unit
B2 (riboflavin)	0.002-0.06	mg
B6 (pyridoxine)	0.008-0.32	mg
C (ascorbic acid)	2.2-2.4	µg
Nicotinic acid (niacin)	0.11-0.36	mg
Pantothenic acid	0.02-0.11	mg
Minerals		
Calcium	4-30	mg
Copper	0.01-0.1	mg
Iron	1-3.4	mg
Magnesium	0.7-13	mg
Phosphorous	2-6	mg
Potassium	10-470	mg
Sodium	0.6-40	mg
Zinc	0.2-0.5	mg

6.2 Honey properties

6.2.1 Antimicrobial activity

Explanation for antibacterial activities of honey

6.2.1.1 Osmotic effect

Honey is a saturated or super-saturated solution of sugars, 84% being a mixture of fructose and glucose. The water content is usually only 15-21% by weight. The strong interaction of these sugar molecules with water molecules leaves very few of the water molecules available for microorganisms. This "free" water is what is

measured as the water activity (a_w); mean values for honey have been reported from 0.56 to 0.62. Although some yeasts can live in honeys that have a high water content and cause honey spoilage, the a_w of ripened honey is too low to support growth of any other species. It has been reported that no fermentation occurring if the water content is below 17.1%. Many species of bacteria have their growth completely inhibited if the a_w is shown in the range of 0.94–0.99. These values correspond to solutions of a typical honey (a_w of 0.6 undiluted) of concentrations from 12% down to 2% (v/v). On the other hand, some species have their maximum rate of growth when the a_w is 0.99 thus, inhibition by the osmotic (water-withdrawing) effect of dilute solutions of honey obviously depends on the species of bacteria (Anonymous, 2006c).

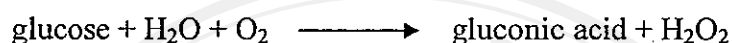
6.2.1.2 Acidity

Honey is characteristically quite acidic, its pH being between 3.2 and 4.5, which is low enough to inhibit many animal pathogens. The optimum pH for growth of these species normally falls between 7.2 and 7.4. The minimum pH values for growth of some common wound-infecting species are 4.3, 4.0, 4.4 and 4.5 for *Escherichia coli*, *Salmonella sp.*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, respectively. Thus in undiluted honey the acidity is a significant antibacterial factor. But if honey is diluted, especially by body fluids which are well buffered, the pH will not be so low and the acidity of honey may not be an effective inhibitor of many bacteria species (Anonymous, 2006c).

6.2.1.3 Hydrogen peroxide

The major antibacterial activity in honey has been found to be due to hydrogen peroxide produced enzymically in the honey. The glucose oxidase enzyme is secreted

from the hypo pharyngeal gland of the bee into the nectar to assist formation of honey from the nectar. The reaction to produce hydrogen peroxide and acidity is



Both H_2O_2 and gluconic acid serve to preserve the honey. The hydrogen peroxide produced acts as a sterilizing agent only during the ripening of honey. Full-strength honey has a negligible level of hydrogen peroxide because this substance is short-lived in the presence of transition metal ions and ascorbic acid in honey which catalyze its decomposition to oxygen and water. The enzyme has been found to be practically inactive in full-strength honey, giving rise to hydrogen peroxide only when the honey is diluted. This is because the acidity produced in the action of the enzyme drops the honey pH to a point which is too low for the enzyme to work. Diluted honey increases the activity by a factor of 2,500 - 50,000, thus, giving a "slow-release" antiseptic activity at a level which is not tissue-damaging (Anonymous, 2006c).

6.2.1.4 Phytochemical factors

There have been some reports of isolation of antibacterial substances from honey that are not hydrogen peroxide. It has been found that heating honey, which inactivates glucose oxidase, causes loss of activity against some species whilst it is retained against others. The most direct evidence for the existence of non-peroxide antibacterial factors in honey was seen when antibacterial activity persisted in honeys treated with catalase to remove the hydrogen peroxide activity (Anonymous, 2006c).

Several chemicals in honey with antibacterial activity have been evaluated including pinocembrin, terpenes, benzyl alcohol, 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate),

3,4,5-trimethoxybenzoic acid, 2-hydroxy-3-phenylpropionic acid, 2-hydroxybenzoic acid and 1,4-dihydroxybenzene. However, the quantities of these compounds present were far too low to account for any significant activity (Anonymous, 2006c).

6.2.2 Benefit to digestive tract

Honey is mentioned to improve food assimilation and to be useful for chronic and infective intestinal problems such as constipation, duodenal ulcers and liver disturbances (Anonymous, 2005b).

6.2.3 Benefit to respiratory system

In temperature climates and places with considerable temperature fluctuations, honey is a well known remedy for treatment and prevention of colds, and mouth, throat or bronchial irritations and infections. The benefits, apart from antibacterial effects, are assumed to relate to the soothing and relaxing effect of fructose (Anonymous, 2005b).

6.2.4 Benefit to skin and wound healing

Honey is not only used in moisturizing and nourishing cosmetic creams, but also in pharmaceutical preparations applied directly on open wounds, sores, bad sores, ulcers, varicose ulcers and burns. It helps against infections, promotes tissue regeneration and reduces scarring by its pure, unprocessed form. If applied immediately, honey reduces blistering of burns and speeds regeneration of new tissue (Anonymous, 2005b).

6.2.5 Prebiotic properties of honey carbohydrate

Researcher at Michigan State University reported that honey enhanced growth, activity and viability of commercial bifidobacteria typically used in manufactures of fermented dairy products. It was noted that the growth of *Bifidobacterium bifidum* was actually more enhanced with honey than with fructo-oligosaccharides (FOS) or galacto-oligosaccharides (GOS), but not as well as with inulin. This result was accordance with reports from the University of Reading in UK and the Instituto de Fermentaciones Industriales in Madrid (Anonymous, 2005a).

7. Sugar

Generally, non-scientists refer "sugar" a white crystalline solid disaccharide to sucrose, or table sugar or saccharose. Humans commonly use sucrose as their sugar of choice for altering flavor and properties (such as mouthfeel, preservation and texture) of beverages and foods. Commercially produced table sugar comes either from sugar cane or from sugar beet. Sugar is also present in brown sugar, molasses, maple syrup, and to small extent in fruits (Anonymous, 2006f). The composition of white and brown sugar are shown in Table 2.6.

Sucrose is a disaccharide, composed of glucose and fructose, with molecular formula $C_{12}H_{22}O_{11}$. Its systematic name is β -D-fructofuranosyl- α -D-glucopyranoside. (Anonymous, 2006f and Williams and Caliendo, 1984). Sucrose is a non-reducing

Table 2.6 Composition of minerals and vitamins in white and brown sugar in 100 kcal portions (Williams and Caliendo, 1984).

Sugar	Calcium (mg)	Zinc (mg)	Iron (mg)	Thiamine (mg)	Riboflavin (mg)
White	0	0.01	0.03	0	0
Brown	22.6	U	0.91	0.002	0.009

U = unknown but thought to be present

sugar due to both of the hydrogen atoms removed in the dehydration reaction came from OH groups. Consequently, neither of the rings is able to open (Figure 2.8) (Anonymous, 2006e).

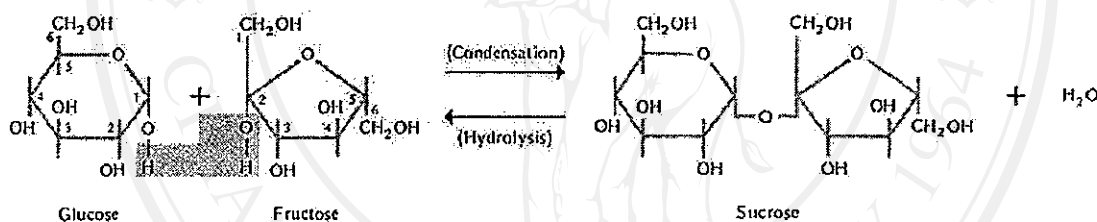


Figure 2.8 Formation of sucrose (Williams and Caliendo, 1984)

7.1 Hydrolysis of sucrose

In the hydrolysis of any di- or polysaccharide, a water molecule helps breaking the acetal bond. When acetal bond is broken, the hydrogen atom from water is added to oxygen on the glucose. The -OH group is then added to the carbon on the fructose molecule (Anonymous, 2006g).

When sucrose is hydrolyzed, it forms a 1:1 mixture of glucose and fructose. The monosaccharide produced is called invert sugar because the angle of specific rotation of plain polarized light changes from a positive to a negative value due to the presence of optical isomers of glucose and fructose (Anonymous, 2006g).

7.2 Brown sugar

Brown sugar derives from the late stages of sugar refining when sugar forms fine crystals with significant molasses content, or by coating white refined sugar with a cane molasses syrup. Their color and taste become stronger with increasing molasses content, as do their moisture retaining properties. Brown sugars also tend to harden if exposed to the atmosphere, although proper handling can reverse this (Anonymous, 2006f).

8. Cell encapsulation

Microencapsulation is a process in which the cells are retained within an encapsulating membrane to reduce cell injury or cell loss. Microencapsulation of various bacterial cultures including probiotics has been a common practice for extending their storage life and converting them into a powder form for ease of their use. Encapsulation in hydrocolloid beads entraps or immobilizes the cells within the bead matrix, which in turn provides protection in an adverse environment. The encapsulation techniques applied to probiotics for the use in fermented milk products or biomass production can be classified into 2 groups, depending on the method used to form the beads: extrusion (droplet method) and emulsion or two-phase system. Both extrusion and emulsion techniques increase the survival of probiotic bacteria by up to 80–95% (Krasaekoopt *et al.*, 2003).

8.1 Microencapsulation of bacterial cells in hydrocolloid beads

8.1.1 Extrusion technique

Extrusion is the oldest and most common approach to make capsules with hydrocolloids. It simply involves preparing a hydrocolloid solution, adding microorganisms to it and extruding the cell suspension through a syringe needle in the form of droplets to free-fall into a hardening solution or setting bath (Figure 2.9). The size and shape of the beads depend on the diameter of the needle and the distance of free-fall, respectively. This method is the most popular due to its ease, simplicity, low cost and gentle formulation conditions ensuring high retention of cell viability (Krasaekoopt *et al.*, 2003).

The supporting material used for extrusion is alginate, which is a linear heteropolysaccharide of D-mannuronic and L-guluronic acid extracted from various species of algae. Depending on the source, the composition and the sequence in L-guluronic acid and D-mannuronic acid vary widely. The functional properties of alginate as supporting material correlate strongly with the composition and sequence of L-guluronic acid and D-mannuronic acid. Divalent cations such as Ca^{2+} bind preferentially to the polymer of L-guluronic acid. The length of the polymer of D-mannuronic acid is, therefore, the main structural feature contributing to gel formation (Krasaekoopt *et al.*, 2003).

To form beads, a cell suspension is mixed with a sodium alginate solution, and the mixture is dripped into a solution containing a multivalent cation (usually Ca^{2+} in the form of CaCl_2). The droplets form gel spheres instantaneously, entrapping the cells in a three-dimensional lattice of ionically cross-linked

alginate. The success of the alginate gel encapsulation technique is due to the gentle environment it provides for the entrapped material, cheapness, simplicity and its biocompatibility (Krasaekoopt *et al.*, 2003).

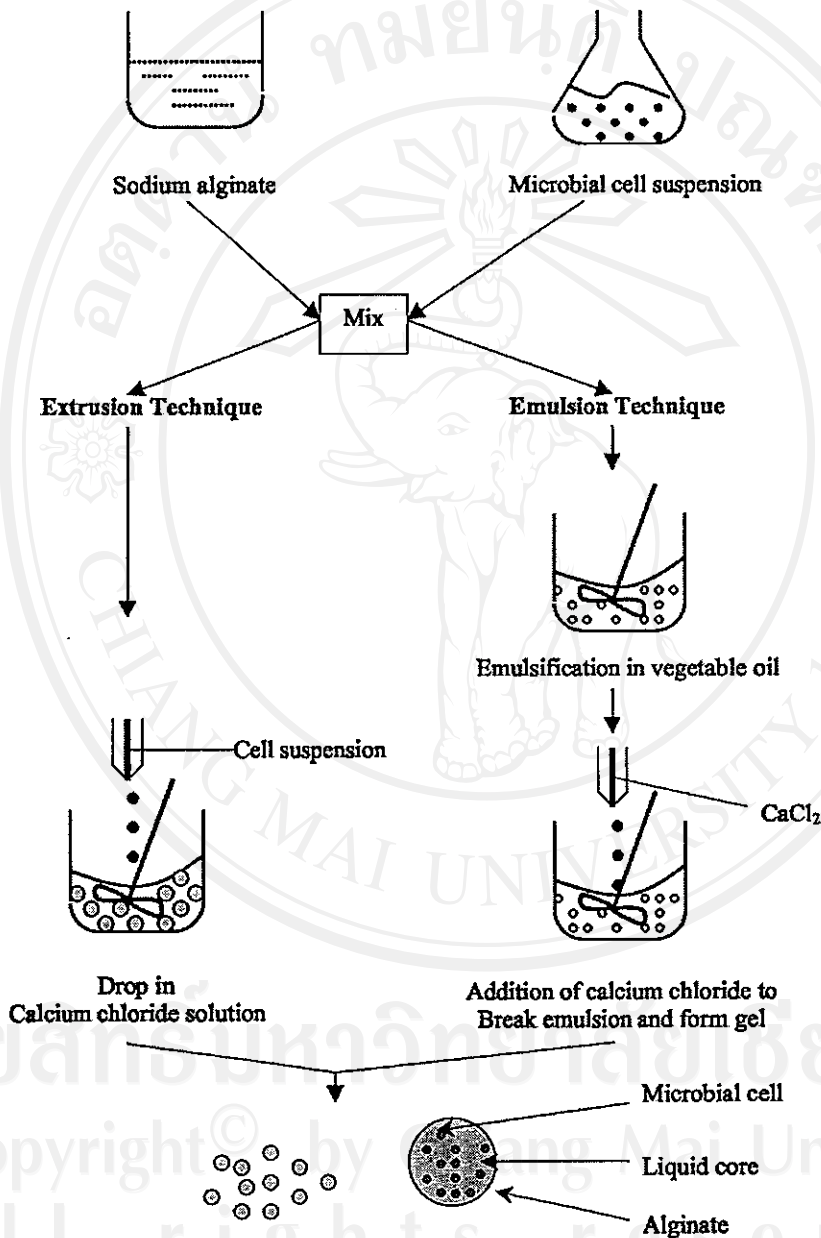


Figure 2.9 Flow diagram of encapsulation of bacteria by the extrusion and emulsion techniques (Krasaekoopt *et al.*, 2003)

The concentrations of alginate and CaCl_2 used to form the gel vary from 1–2% and 0.05–1.5 M, respectively. The size of the beads is approximately 2–3 mm in diameter. Moreover, the size and sphericity of the bead depend mainly on the viscosity of the sodium alginate solution and the distance between the syringe and the calcium chloride collecting solution. As the concentration, and hence viscosity, of sodium alginate increases, the size of the beads decreases. The extruder orifice diameter is another important factor, which regulates droplet size. Using a 0.27 mm syringe, it could obtain a bead size of 2–3 mm. The composition of the alginate also influences bead size. Small beads result from low guluronic alginates (Krasaekoopt *et al.*, 2003).

8.1.2 Emulsion technique

In this technique, a small volume of the cell-polymer suspension (discontinuous phase) is added to a large volume of continuous phase. The mixture is homogenized to form a water-in-oil emulsion. Once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilized (cross-linked) to form tiny gel particles within the oil phase (Figure 2.9). The smaller the internal phase particle size of the emulsion, the smaller the final microparticles will be. The insolubilization method of choice depends on the type of supporting material used. The beads are harvested later by filtration. The size of the beads is controlled by the speed of agitation, and can vary between 25 μm and 2 mm. This technique has been used successfully to encapsulate lactic acid bacteria for batch and continuous fermentation (Krasaekoopt *et al.*, 2003).

For food applications, vegetable oils are used as the continuous phase. Some studies have used white light paraffin oil and mineral oil. In some cases emulsifiers are added to form a better emulsion, because they can lower the surface tension, resulting in smaller spheres. The most common emulsifier used is Tween 80 at 0.2%. However, Tween 80 together with 0.5% sodium lauryl sulphate can produce a bead size of 25–35 μm (Krasaekoopt *et al.*, 2003).

There are many supporting materials used with the emulsion technique. These include a mixture of κ -carageenan and locust bean gum, cellulose acetate phthalate, alginate, chitosan and gelatin (Krasaekoopt *et al.*, 2003).

8.2 Special treatment

Despite the suitability of alginate as the entrapment matrix material, gel entrapment in alginate has some limitation due to low stability in the presence of chelating agents such as phosphate, lactate and citrate. The chelating agents share affinity for calcium and destabilize the gel. Thus, stability problems are encountered during lactic acid fermentation and cause cell release from the beads. In the case of other matrix material, such as chitosan, the entrapped cells can be released from the beads during fermentation and cause low initial loading for the next fermentation. Therefore, special treatments, such as coating the beads, are applied in order to improve the properties of encapsulated beads. Coated beads not only prevent cell release but also increase mechanical and chemical stability. Cross-linking with cationic polymers, coating with other polymers, mixing with starch and incorporating additives can improve stability of beads (Krasaekoopt *et al.*, 2003).

8.3 Advantages and limitations of the extrusion and emulsion techniques

For encapsulation of probiotics, both extrusion and emulsion techniques can be applied. Advantages and disadvantages of these techniques are shown in Table 2.7. Extrusion is a relatively simple technique. It usually produces entrapped, rather than encapsulated core material, although encapsulation can be achieved through co-extrusion devices or dropping into a bath of coating material which react at the droplet surface. This method can be difficult for large-scale production because of slow formation of beads compared with the emulsion technique (Krasaekoopt *et al.*, 2003).

On the other hand, the emulsion technique is relatively new to the food industry and easy to scale up for large-scale production. It provides both encapsulated and entrapped core materials. The size of the beads formed by this method is smaller (25 μm to 2 mm) than that of beads produced by the extrusion method (2–5 mm). The size of beads from the extrusion method depends mainly on the size of the needle used while the size of beads from the emulsion method depends on the speed of agitation and the type of emulsifier used. Due to the need for a vegetable oil, the operating cost of the emulsion technique may be higher than that of the extrusion technique (Krasaekoopt *et al.*, 2003).

Table 2.7 Positive and negative features of extrusion and emulsion techniques (Krasaekoopt *et al.*, 2003)

	Extrusion	Emulsion
Technological feasibility	Difficult to scale up	Easy to scale up
Cost	Low	High
Simplicity	High	Low

9. Related studies

The development of nondairy probiotic product is a challenge to the food industry in its effort to utilise the abundant natural resources by producing high quality functional products (Charalampopoulos *et al.*, 2002).

There are many research studies about the survival of probiotic bacteria in non-dairy products. In 2002, Mårtensson *et al.* reported a study about the survival of probiotic strains, including *L. reuteri* ATCC55730, *L. acidophilus* DSM20079 and *Bifidobacterium bifidum* DSM20456, all of human origin, in three different oat-based, non-dairy products. The products were fermented by the three probiotic strains with and without the presence of a commercial yoghurt culture. Samples were stored at 6°C for up to 30 days. The work showed that the oat-based products with different mono- and disaccharide composition could be used to support the growth of human intestinal bacteria and also maintained high cell viabilities during cold storage.

Yoon *et al.* (2005) evaluated red beets as a potential substrate for the production of probiotic fermented beet juice by adding four species of lactic acid bacteria, which were *L. acidophilus*, *L. casei*, *L. delbrueckii* and *L. plantarum*. All bacteria were capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. *L. acidophilus* and *L. plantarum* produced a greater amount of lactic acid than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 hours of fermentation at 30°C. Although the lactic acid cultures in fermented beet juice gradually lost their viability during cold storage, the viable cell count of these lactic acid bacteria except for *L. acidophilus* in the fermented beet juice still remained at $10^6 - 10^8$ CFU/ml after 4 weeks of cold storage at 4°C.

In recent year, there has been more focus on synbiotic, a combination of pre- and probiotic in a single product (Helland *et al.*, 2004). Honey is a natural sweet substance produced by honeybees, which has many kind of carbohydrates including oligosaccharides. There was a report that researchers from the University of Reading in UK and the Instituto de Fermentaciones Industriales in Madrid studied the effect of honey oligosaccharide on growth of faecal bacteria. The result showed that honey oligosaccharides had a potential prebiotic activity, increasing the populations of bifidobacteria and lactobacilli, although not to the level of fructooligosaccharide activity (Anonymous, 2005c). This report was consistent with a report from Shamala *et al.* (2000). The latter researcher team studied the effect of honey and sucrose on lactic acid bacteria *in vitro* to determine whether these organisms were affected differently by honey compared with sucrose. Under the *in vitro* condition, the number of *L. acidophilus* count increased 10-100 fold in the presence of honey compared with sucrose. Besides these reports that showed a potential capability of honey as a prebiotic compound, honey also has other good properties, including antimicrobial activity, benefit to the digestive apparatus, respiratory system, skin and wound healing (Anonymous, 2005b).

Encapsulation techniques are used to improve the viability of probiotic strains in functional foods. In 1999, some researchers investigated the possibility to use high amylase maize (amylomaize) starch as a delivery system for probiotic bacteria. In this case, *Bifidobacterium* strains isolated from a healthy human were used and adhered to amylomaize starch granules. *In vitro* studies showed that growth in the presence of amylomaize starch granules led to a better survival of the probiotic strains. Survival *in vivo* was also monitored by measuring the faecal level of

Bifidobacterium after oral administration of the strain to mice. A sixfold better recovery of the strains was noted for cells grown in amylase-containing medium compared with the control (Charalampopoulos *et al.*, 2002).

A modified method using calcium alginate for the microencapsulation of probiotic bacteria was studied by Sultana *et al.* (2000). Incorporation of maize starch (a prebiotic) with alginate improved the encapsulation of viable bacteria as compared to when the bacteria were encapsulated without the starch. The survival of encapsulated cultures was in all cases higher than with the free cells.

Tsen *et al.* (2004) used kappa-carrageenan to immobilize *L. acidophilus* in a fermentation of banana media while Krasaekoopt *et al.* (2005) applied chitosan-coated alginate beads to encapsulate *L. acidophilus* 547, *B. bifidum* ATCC 1994 and *L. casei* in yoghurt. In all cases, the encapsulation technique could be used to transmit probiotic bacteria via intestinal tracts or fermented products and maintained a good number of survival bacteria.