CHAPTER 2

LITERATURE REVIEW

2.1 Noni fruit

Noni fruits have a distinctive and not altogether pleasant aroma. Noni fruits were traditional eaten by native cultures in Samoa, Fiji, South East Asia, Polynesia, India, South Pacific and the Caribbean. Noni was a feminine food and also fed for the livestock. The root and bark of the noni tree were sources of fabric dyes in Polynesia, Asia and Europe until the 1950s. Noni dye was used to produce yellow, red and purple colors. From Italy to India, noni dye colored carpet, sweaters and turbans (AGIS Phytochemical Database, 1998). Noni various vernacular names are: "Indian mulberry", "nuna", or "ach" on the Indian subcontinent, "yohban" in Thailand "mengkudu" in Malaysia, "nhau" in Southeast Asia, "painkiller bush" in the Caribbean, or "cheese fruit" in Australia (Morton, 1992; Nelson, 2001; Wang *et al.*, 2002; Cardon, 2003). Noni is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and Northern South America (Dixon *et al.*, 1999).

The Polynesians have been using the noni plant (Figure 2.1) for food and medicinal purposes for more than 2000 years. In traditional pharmacopoeia, the fruit is claimed to prevent and cure several diseases. It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections; it is also used to prevent the formation and proliferation of tumors, including malignant ones (Dixon *et al.*, 1999). Noni juice is also claimed to relieve inflammation. Most noni is consumed as juice, although leaves, flowers, bark and roots can also be used (Dixon *et al.*, 1999; McClatchey, 2002).

2.1.1 Botany

Noni [Figure 2.2 (left)] is a member of the Rubiaceae plant family. About 80 species are primarily of old world tropical regions. Name derived from the Latin morus, mulberry, and indicus, Indian, in reference to the similarity of the fruit of Indian mulberry to true mulberry. This species was commonly cultivated as a dye plant; the bark contains a red pigment and the roots a yellow pigment used in dying *kapa*. Foetid oil was extracted from the syncarp and used in the hair as an insecticide. The ripe fruit [Figure 2.2 (right)] was used as a poultice. Juice from the fruit was also used to make a medicinal drink, *aumiki 'awa*, as a remedy for tuberculosis, and another drink, *aumiki noni*, used to counter any unpleasant effects of 'awa. The rip fruit reportedly was used either raw or cooked for famine food (Anonymous, 2002).

Figure 2.1 Mature noni (*Morinda citrifolia*) plant (Source: Anonymous, 2002).

Figure 2.2 Noni (*Morinda citrifolia*) fruits in various stages of development, from flowering (foreground) to ripe (background) (left) and ripe noni fruit with seeds (right) (Source: Anonymous, 2002).

2.1.2 Noni's natural habitats

Noni is believed to be among the original "canoe plants" that Hawaii's Polynesian colonizers brought with them in their voyaging canoes. The voyagers valued the plant for its medicine and dyes. Since the early days of the colonizers noni has become naturalized on the main Hawaiian Islands. It grows naturally where it is relatively wet to moderately wet, from sea level to about 1500 feet elevation. It can be found near the coast, in open lowlands and grasslands, in gulches, as an early colonizing plant specie in recent lava flows, and in disturbed forests of the dryer areas, such as the lowland forests in which hala (*Pandanus odoratissimus*) and kukui nut (*Aleurites moluccana*) trees grow. It tolerates salinity and thrives within solution pits, or inland tide pools in which brackish water (ocean water mixed with fresh water) is found (Anonymous, 2002).

2.1.3 Benefits

Beneficial components in noni fruit include:

1) Anthraquinones

Some of the biological activity of anthraquinones includes fighting inflammation, bacteria, parasites and tumors. Some of these compounds are also considered analgesic (pain relievers). Anthraquinones are also used to fight fungal infections in addition to improving the immunity of the body (Duke, 1998). These compounds aid in metabolism, cellular respiration and Damnacanthal is an anthraquinone that has been characterized recently and has some important functional properties (mainly anti-carcinogenic) (Solomon, 1999). It is believed that some anthraquinones cut off the blood supply to tumors, depriving them of their nutrients slowing the growth of tumors by inhibiting important enzymes needed to form them (Hiramatsu *et al*, 1993). Alizarin, another anthraquinone, also slows tumor growth as well as fighting leukemia and inhibits the human immunodeficiency virus (HIV). Some also were found to significantly "tie-up" the mineral calcium and reduce the growth rate of urinary crystals, which benefits those afflicted with gout and kidney stones (Stalman *et al.*, 2003).

2) Glycosides

Glycosides are found in abundance in the plant kingdom. These simple sugars are glucose, xylose, fructose, or any other sugar. Some glycosides are valuable heart treatments, and include derivatives of digitalis for the treatment of heart ailments. One important glycoside found in noni juice is Asperuloside and used for the treatment of diuresis (reducing water retention), inflammation, varicose veins and phlebitis. It also helps in preventing chromosome breakage and mutation (Duke, 1998).

3) Sterols

Sterols include cholesterol found in animals and ergosterol found in plants. The plant sterols, called phytosterols alleviate problems associated with high levels of

low density lipoprotein (LDL) in our bodies that can cause cardiovascular disease. Noni slows down the intestinal absorption of cholesterol and lowers total plasma and LDL cholesterol levels (Duke, 1998). Noni juice is an important sources of (β) sitosterols, stigmasterol and campesterol the three most nutritionally important phytosterols. Sitosterols have been showed antiinflammatory, anticancer and lower fever, helping to balance the immune system. A balanced immune system will help improve rheumatoid arthritis, allergies, cancer, autoimmune diseases and chronicviral infections.

Stigmasterol and sitosterols are antiinflammatory when applied topically. These compounds also reduce the tendency of blood clotting conditions (thrombosis) and help to repair slightly damaged blood vessels (Solomon, 1999).

4) Terpenes

Terpenes are the major components in the oils of citrus fruits and they include the bioflavanoids and carotenoids. These substances combat fungal and bacterial infections, and are helpful in the treatment of glaucoma, spastic symptoms of multiple sclerosis, spinal lesions and in reducing the severity and side effects of chemotherapy. Eugenol, a terpene, acts as an active germicide as well as induces anesthesia of the trigeminal nerve associated with Trigeminal Neuralgia or Tic Doulureax. Eugenol relaxes smooth muscle by interfering with the muscle's contraction, and is found in a variety of aromatherapy oils, which are used for their soothing, calming and comforting effect. β-carotene, another terpene, is associated with a reduced rate of cancers of the cells of the lung, skin, cervix, respiratory tract and the gastro-intestinal tract. This is attributed to the compound's ability to "mop up" free radicals, thus reducing the oxidative damage. The thymus gland, which helps distribute T cells that destroy invading microbes, gradually deteriorates with age and stress, mostly from oxidative damage. Supplementing the diet with β-carotene will offer antioxidizing benefits to the immune system and offers protection to the large bacteria-eating immune cells called phagocytes and other immune cells. It also significantly reduces cholesterol levels, and supplies about two-thirds of the vitamin A needed for our bodies. Vitamin A helps our night sight and contributes to healthy skin and mucous membranes the body's first defense against

infection and injury. This important vitamin also cares for our gastro-intestinal tract, respiratory system and the genitourinary tract. Daily intake of noni juice is a good source of this vitamin. Limonene, another terpene, has been found to limit tumor formation and antimicrobial activity and is involved in Alzheimer studies (Anonymous, 2002). Ursolic acid, also known as urson and prunol, has medicinal value when applied topically or taken internally for relieving symptoms of inflammatory and fungal infections of the skin (Hirazumi, 1999). Taken internally it inhibits the formation and growth of tumors of the skin and acts as an antiinflammatory. It also has alopecia (loss of hair) and dandruff-preventing properties.

5) Okadaic acid

In noni fruit has also been increase synthesis of tumor necrosis factor (TNF-a) (Asahina *et al.*, 1990). Studies conducted on noni fruit demonstrated an antimicrobial activity, and inhibition both in the albicans, virus and *Cryptococcus*, a cause of fungal pneumonia. Sedative and analgesic effects have also been noted. Noni fruit appears to stimulate the production of T-cell, macrophages and thymocytes thereby enhancing immune function (Ganal and Hokama, 1987).

6) Pectin

This fiber is a non-cellulose complex sugar and performs important functions within the gastrointestinal tract. It absorbs water and slows the emptying of the stomach contents into the small intestine. It also binds bile acids and cholesterol and regulates glucose absorption through the gut wall, helping those with blood sugar conditions. Pectin in the diet will increase the volume of material moving through the bowel, reducing concentration of feces and carcinogenic substances in the bowel (Anonymous, 2003).

7) Scopoletin

Scopoletin belongs to a group of compounds called coumarins, which cares for the liver, inhibits the growth of *Escherichia coli* in the gut and is antibacterial against many other agents that cause viruses. It has also shown to be five times more

effective as aspirin as an anti-inflammatory effect for bronchial illnesses and asthma. Scopoletin that is found in noni fruit helps vasodilatation and improved the problem of hypertension and cardiovascular diseases (Abbott, 1992; Solomon, 1999). Scopoletin is a coumarin that was isolated in 1993 at the University of Hawaii and has been found to have analgesic properties as well as a significant ability to control serotonin levels in the body (Levand and Larson, 1979). Other researchers have shown that scopoletin may also have anti-microbial activity (Duncan *et al.,* 1998).

8) Xeronine

A novel substance that is found in noni fruit has been called xeronine. Xeronine is an alkaloid. Alkaloids are colorless, complex and bitter organic bases. They are essential to maintain healthy condition in the body. The body produces xeronine in order to activate enzymes and to regulate and give structure to proteins. However, extracting xeronine from the human body has been impossible until now. The body's protein molecules consume the alkaloid immediately after it is created. Therefore, there is never an appreciable insoluble amount in the body (Heinicke, 1985; Solomon, 1999). Eventhough the noni fruit has only a negligible amount of xeronine, the noni juice does contain a very large amount of a precursor to this essential alkaloid called proxeronine. Proxeronine is a colloid that unlike most colloids contains sugars, amino acids and nucleic acids and has been studied extensively. This compound initiates the release of xeronine in the intestinal tract after it comes in contact with a specific enzyme, which is also contained in the noni juice (proxeronase). This particular chemical combination is believed to be significantly affected cellular function, which can determine a whole host of physiological reactions. The enzymatic reactions can occur when taking noni juice on an empty stomach because hydrochloric acid and pepsin enzyme can destroy proxeronine and proxeronase (Heienicke, 1985).

Xeronine is a small alkaloid that is required in picrogram (trillionth of gram) amounts and is essential to the correct functioning of the body. Xeronine, formed in the large intestines, may encourage proper cell function and growth in the human body. Large amounts are used in times of physical or mental stress. Xeronine is

produced in the body from proxeronine and the enzyme proxeronase in the small intestine. All healthy cells require xeronine to function correctly (Heienicke, 1985).

Xeronine is required to regulate the shape and rigidity of certain proteins. Noni juice, the richest source of this nutrient, appears to improve the function and structure of hormones, antibodies, connective tissue, enzymes and neurotransmitter. Xeronine stimulates the production of a specific protease (an enzyme which dissolves protein) and quickly removes dead tissue from burns and promotes quick healing. Xeronine may help enlarge the pores in the walls of human cells and enable nutrients to enter the cells more easily. Proxeronine and proxeronase are generally regarded as the key of effective ingredients in noni. There are actually dozens of other neutraceuticals (natural properties) found in noni fruit (Heienicke, 1985).

Other proteins in the central nervous system become potential receptor sites and bind with endorphins that allow the individual to have the normal feeling. Once xeronine floods the body's systems then the xeronine displaces the chemicals and addiction to these other alkaloids is overcome with no physical withdrawal symptoms. Vitamins also require xeronine to properly function within the body. It allows the formation of pores through membranes in blood vessels, body organs and in the gastrointestinal tract. This facilitates digestion and improves the action of other medicines and herbs (Anonymous, 2003).

9) Amino acids

Of the 20 amino acids, including the 9 essential amino acids for our health, 17 amino acids are found in noni juice. If an individual does not get a complete protein balance, then production and maintenance of muscles, connective tissue, blood-clotting factors, blood transport systems, visual pigments and the protein matrix inside bones would be at risk (Anonymous, 2003).

10) Fatty acids

Many important fatty acids found in noni are important to the overall complex metabolic processes of the body. Lactic acid is the main acid and all fatty acids are made from caprylic acid, inhibits yeast and fungal overgrowth in the body (Dittmar, 1993). Linoleic acid, which cannot be manufactured in the body, serves

these important functions: strengthens capillary and cell membranes and increases skin strength, combine with cholesterol to form important compounds, helps lower serum cholesterol levels, aids in the transport and metabolism of cholesterol and build essential compounds that regulate bodily function (Anonymous, 2003).

Some vitamins and mineral contents of noni juice in one serving are displayed in Table 2.1

Table 2.1 Vitamin and mineral contents for one serving of noni juice (1 ounce)

Nutrient	Amount		%
Vitamin A	5.88	IU	0.117
Vitamin C	6.029	mg	10
Calcium	6.76	mg	0.67
Iron	0.1088	mg	0.6
Vitamin E	0.235	IU	0.78
Vitamin B1	0.0029	mg	0.196
Vitamin B2	0.0029	mg	0.17
Niacin	0.147	mg	0.735
Vitamin B6	0.038	mg	1.91
Folic acid	7.35	µg	1.84
Vitamin B12	0.097	µg	1.62
Biotin	1.47	µg	0.49
Pantothenic acid	0.147	µg	1.47
Phosphorus	2.058	mg	0.205
Magnesium	3.088	mg	0.772
Zinc	0.047	mg	0.313
Copper	0.006	mg	0.294
Other minerals			
Chromium	0.147	mg	-
Manganese	0.25	mg	-
Molybdenum	0.294	mg	-
Sodium	12.35	mg	-
Potassium	28.52	mg	-
Carbohydrate			
Fructose	1.2	g	-
Glucose	1.1	g	-
Fiber	0.7	g	-

Source: Morinda, Inc (2002)

2.1.4 Scientific research on noni fruit

The immunomodulatory properties (capacity to enhance the host immune system) of noni juice have recently been studied by a Japanese research team (Hirazumi *et al.*, 1996; Hirazumi and Furusawa, 1999). The ethanol precipitable fraction of noni juice, corresponding to a polysaccharide-rich substance composed of glucuronic acid, galactose, arabinose and rhamnose, has been found to have immunomodulatory and anti-tumor effects against Lewis lung carcinoma (LLC). On cell models, noni-ppt seems to stimulate the production of T-cells, thymocytes and macrophages that produce cytokines, which are important mediators of tumor cytostasis and tumor cytotoxicity. Noni-ppt also appears to stimulate the release of several mediators from murine effector cells such as cytokines, which slow down the cell cycle in tumors, increase the response of cells to other immunized cells that fight tumor growth and have a potent macrophage activator activity, suspected of playing a role in the death of tumors (Hirazumi *et al.*, 1996; Hirazumi and Furusawa, 1999).

Immunomodulating effects are also reported for some other safe foods and their components, such as kefir (Vinderola *et al.*, 2005), wakame, a seaweed widely consumed in Japan, beta-glucan, a major component of oats and some edible fungi (Kobayashi *et al.*, 2005) and even chocolate (Sanbongi *et al.*, 1997). The activity of noni juice against LLC was not due to cytotoxicity, but may be partially due to dietary antioxidant compounds, as reported for other foods (Sazuka *et al.*, 1995; Chen *et al.*, 2005). Similar activity has also been reported for several other fruit juices, including cranberry and apple (Sun *et al.*, 2002). The same research inoculated mice with LLC, those ingesting a daily dose of 15 mg of noni juice had a significant increase (119%) in life span. Nine out of 22 mice with terminal cancer survived for more than 50 days. In addition, the ingestion of noni-ppt, combined with conventional chemotherapy in the treatment of mice with cancer, proved to increase life spans (Hirazumi *et al.*, 1994).

Another Japanese team studied more specifically the influence of damnacanthal, an anthraquinone extracted from a chloroform extract of noni roots. Surprisingly, the researchers found that damnacanthal induced the normal

morphology of a particular type of cells found in human neoplasias (K-RAS-NKR cells) that multiply uncontrollably and are highly malignant (Hiramatsu *et al.,* 1993).

Recent research has demonstrated the effects of noni fruit on preventing arteriosclerosis, a disease related to the oxidation of low density lipoproteins (LDL). Methanol and ethyl acetate extracts showed with the thiobarbituric acid reactive substance method 88 and 96% inhibition, respectively, of copper-induced LDL. Another study tested the analgesic and sedative effects of extracts from the *M. citrifolia* plant. The extract was shown to be non-toxic and did show significant, dose-related, central analgesic activity in the treated mice. The conclusion of these researchers was that the extract did in fact demonstrate analgesic effects consistently in each experiment (Younos *et al.*, 1990).

The anti-microbial effect of noni may have been the first observed property: indeed, the fruit contains relatively large amounts of sugars that are not fermented when fruits are stored in closed containers at ambient temperature. This property is used to transport the fruit by boat from the scattered Pacific Islands to processing plants without specific treatment. It has been reported that noni inhibits the growth of certain bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus morgaii, Bacillus subtilis, E. coli, Helicobacter pylori, Salmonella* spp. and *Shigella* spp. (Atkinson, 1956). The same author claimed that the antimicrobial effect observed may be due to the presence of phenolic compounds such as acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones. Another study showed that an acetonitrile extract of the dried fruit inhibited the growth of *P. aeruginosa, B. subtilis, E. coli*, and *Streptococcus pyrogene* (Locher *et al.,* 1995). It has also been found that ethanol and hexane extracts of noni have an antitubercular effect since they inhibit by 89–95% the growth of *Mycobacterium tuberculosis*. The major components identified in the hexane extract were E-phytol, cycloartenol, stigmasterol, β-sitosterol, campesta-5, 7, 22-trien-3-β-ol, and the ketosteroids, stigmasta-4-en-3-one and stigmasta-4-22-dien-3-one (Saludes *et al.,* 2002).

2.2 Heat treatment

The object of microbial control can range from complete eradication of microorganisms to the mere inhibition of their growth, and the goal of the treatment will influence the control method chosen. Sterilization is the complete removal of all life forms from a given area. Treatments causing sterilization tend to be drastic and can sometimes alter the chemistry of the object being treated. Contamination of sterilized items with any type of organism would be deleterious. The next level down from sterilization is the selective removal of a subset of microbes. In many cases only certain microbes are damaging to an item and more gentle treatments can be used to eliminate only these harmful microbes without killing everything (Lewis and Heppell, 2000). Pasteurization is the name given to a moderate heating process that is intended to kill some types of microbe in food but not endospores or some other particularly resistant types. Pasteurization may be applied to reduce the risk from pathogens in milk or other drinking products, or to reduce the risk of spoilage in beer, fruit juices or vinegar. The pasteurization time and temperature for fruit juices can be given at 63°C for 30 min or at 77°C for 1 min or at 88°C for 15 s (Fellow, 1997). These pasteurization conditions were chosen because they were sufficient to have a high degree of expectation in killing yeast, moulds and enzymes that were present in fruit juices. The enzymes that would be destroyed were pectin esterase and polygalacturonase (Fellow, 1997).

2.2.1 High temperature

High temperature kills by causing lysis of the membrane or denaturation of critical enzymes. Two methods of providing heat are generally used. Dry heat involves incubation in an oven-like environment, while moist heat utilizes steam under pressure, and the latter is more effective. Water has a very high heat capacity (ability to carry heat) and moist air is capable of holding more heat than dry air. Moist heat is therefore more effective because it increases the rate of heat penetration into a substance. With dry heat a higher temperature or longer time of exposure is necessary to obtain the same amount of killing as that seen with moist heat. In either case, at temperatures above the lethal limit for a bacterial strain, cell population

decrease from heating follows a first-order exponential pattern. Because of this experimental pattern, the initial number of bacteria in a sample will affect the time necessary to eliminate that population from the sample. The goal of a heat treatment is to bring the target population down to some acceptable level. In addition, each species of microbe also has its own characteristic resistance to heat, with some bacteria being much more heat tolerant than others (Lewis and Heppell, 2000).

A final factor influencing the effectiveness of a heat treatment is the composition of the environment surrounding the microbe. High salt and acidic environments increase the rate of killing at a given temperature due to the damaging effects salt and acid on the cell. Conversely, fats and proteins in a solution have a protective effect. Because of all these mitigating factors on the effectiveness of heat treatment, determining the success of a given treatment on a given type of sample must be done empirically, that is by experimentation (Lewis and Heppell, 2000).

2.2.2 Types of heat treatments

The most common method of sterilization currently used in laboratories and hospitals is autoclaving. This employs steam under pressure to raise the temperature to 121°C at 15-17 psi for at least 15 minutes. At this elevated temperature all living cells, including endospores and viruses are killed. Large liquid volumes and some types of medium need longer autoclaving times to insure complete elimination of microbes. One of heat resistant endospore-forming microorganisms is *Bacillus stearothermophilus,* which contains spores that can maintain viability for about 13 minutes at 121°C. It can therefore be used as a standard bioassay to verify that an autoclave is working properly (Lewis and Heppell, 2000).

Dry heat in an oven can also be used to kill microbes. Obviously this treatment will not work for liquid items since they will reach a maximum of 100°C, but for non-liquid items that do not melt at the oven temperature (160-170°C or above), it can be effective. Items such as metal and glass can be sterilized in this manner however these treatments can ruin some items. For example surgical instruments loose sharpness when treated in this manner (Lewis and Heppell, 2000).

One problem with all of these high temperature methods is that they can drastically alter the composition of the sample due to the breakdown of heat labile components. In some cases alternatives to high temperature must be found to preserve the integrity of the item. For foods this often means treatment at lower temperatures since most food does not need to be sterile. The first and still most common method is pasteurization, named after the great microbiologist Louis Pasteur. Originally developed to prevent the spoilage of wine, it is commonly used for milk to eliminate the transmission of *Coxiella burnetti, M. tuberculosis*, *Brucella, Staphylococcus, Salmonella* and *E. coli* strain O157:H7. (*C. burnetti* is the cause a Q fever, an illness that can be transmitted through contaminated milk). In the original batch pasteurization method, the food was heated at 66°C for 30 min, but most modern applications use flash pasteurization, which is a treatment at 71°C for 15 seconds. The shorter heating time allows for easier automation and less damage to food during processing. Also, the higher temperature is more effective at killing *C. burnetti*. Pasteurization not only eliminates pathogens, but also greatly decreases the number of spoilage organisms. Pasteurization is used extensively in treating many other food products including beer, wine, yogurt, juices and cheese (Lewis and Heppell, 2000).

In commercial practice, juices that are heated by either steam or hot water are flash pasteurized in tubular or plate-type heat exchangers. The tubular type consists of a tube of sufficient length surrounded by a low pressure steam or hot water jacket. The plate type is constructed of successive stainless steel plates separated by compression gaskets of neoprene or Buna, and arranged so that steam-heated hot water circulates on one side of a plate while juice is flowing on the other side. Within the pasteurizer, juice flows by turbulence and may take from 1 to about 40 s to pass through. Modern heat exchangers are designed to prevent scorching or overheating while holding juice (Carter, 1983).

2.3 Fermentation

Fermentation (formerly called zymnosis) is an anaerobic metabolic breakdown of a nutrient molecule, such as glucose, without net oxidation. Fermentation does not release all the available energy in a molecule; it merely allows glycolysis (a process that yields two ATP per glucose) to continue by replenishing reduced coenzymes. Fermentation yields lactate, acetic acid, ethanol, or other reduced metabolites. Fermentation is also used much more broadly to refer to the bulk growth of microorganisms on a growth medium. This process is often used to produce or preserve food. Fermentation typically refers to the fermentation of sugar to alcohol using yeast, but other fermentation processes include the making of yogurt. The science of fermentation is known as zymology. Fermentation usually implies that the action of the microorganisms is desirable. Occasionally wines are enhanced through the process of cofermentation (Anonymous, 2001b).

2.3.1 Biochemistry of fermentation

Fermentation is a process that is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP by glycolysis. During fermentation pyruvate is metabolised to various different compounds. Examples of fermentation products are ethanol (drinkable alcohol), lactic acid and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Although the final step of fermentation (conversion of pyruvate to fermentation end-products) does not produce energy, it is critical for an anaerobic cell since it regenerates nicotinamide adenine dinucleotide (NAD^+), which is required for glycolysis. This is important for normal cellular function, as glycolysis is the only source of ATP in anaerobic conditions (Anonymous, 2001b).

Fermentation products contain chemical energy (they are not fully oxidised) but are considered waste products since they cannot be metabolised further without the use of oxygen (or other more highly-oxidised electron acceptors). A consequence is that the production of ATP by fermentation is less efficient than oxidative

phosphorylation, where pyruvate is fully oxidised to carbon dioxide. Fermentation produces two ATP molecules per molecule of glucose compared to approximately 36 by aerobic respiration (Anonymous, 2001b).

2.3.2 Products of fermentation

Products produced by fermentation are actually waste products produced during the reduction of pyruvate to regenerate NAD+ in the absence oxygen. Ethanol fermentation (done by yeast and some types of bacteria) breaks the pyruvate down into ethanol and carbon dioxide. It is important in bread-making, brewing, and wine-making. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced. Usually only one of the products is desired; in bread the alcohol is baked out, and in alcohol production the carbon dioxide is released into the atmosphere. Lactic acid fermentation breaks down the pyruvate into lactic acid. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some bacteria and some fungi. It is this type of bacteria that convert lactose into lactic acid in yogurt, giving it its sour taste. Bacteria generally produce acids. Vinegar (acetic acid) is the direct result of bacterial fermentation. In milk, the acid coagulates the casein, producing curds. In pickling, the acid preserves the food from pathogenic and putrefactive bacteria (Anonymous, 2001b).

2.3.3 Benefits of fermentation

The primary benefit of fermentation is the conversion, e.g., converting juice into wine, grains into beer and carbohydrates into carbon dioxide to leaven bread. According to Steinkraus (1995), food fermentation serves five main purposes:

1. Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates
2. Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid and alkaline fermentations

3. Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids and vitamins
4. Detoxification during food fermentation processing
5. A decrease in cooking times and fuel requirements

2.4 Noni juice and juice products in commercial scale (Anonymous, 2002)

Noni fruit juice and juice products are processed and prepared in Hawaii by a variety of methods. For example, noni juice may be fermented versus unfermented, or fresh-squeezed versus drip-extracted. The "traditional" juice is both drip-extracted and fermented/aged for at least two months. The "non-traditional" method of juice extraction is by pressing or squeezing the juice from ripe fruits. Noni juices may be amended with other additives or diluted, or bottled in its pure state. It may be bottled with or without pasteurization.

2.4.1 Traditional noni juice: Drip-extracted, fermented & aged, unadulterated (Anonymous, 2002)

1). Ripe noni fruits arrive at a juicing facility. Freshly picked, ripening noni fruits arrive at the juice processing facility in a variety of containers (Figure 2.3). The harvested noni fruits are mainly whitish in color with tinges of green.

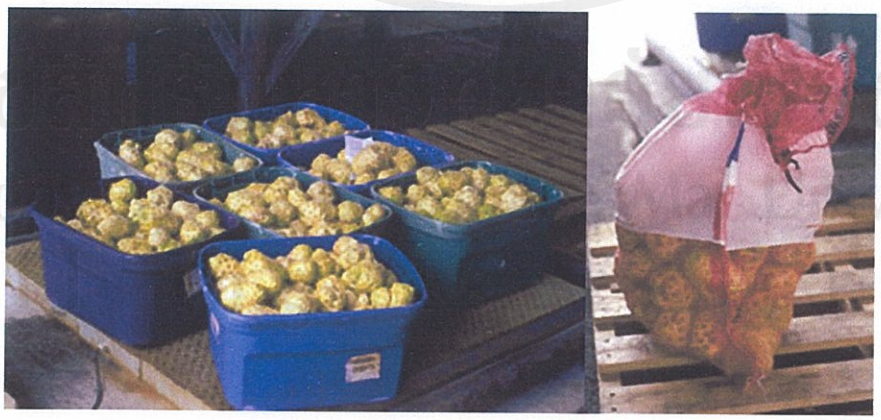

Figure 2.3 Noni fruits are being weighed in tubs (left). An onion bag full of freshly picked, nearly ripe noni fruits (right) (Anonymous, 2002).

Young noni fruits are very hard-skinned and durable, therefore resistant to superficial damage and bruising during shipping and handling. They require no special handling. Noni fruits at this stage of development will ripen overnight or in a few days at room temperature and can be processed for juice immediately thereafter.

2). Noni fruits are washed and air-dried.

An automatic noni fruit washer can be seen in Figure 2.4 and the air-dried process for the washed noni fruit is displayed in Figure 2.5.

Figure 2.4 An automatic noni fruit washer, adapted for use with noni from a vegetable operation (Anonymous, 2002).

Figure 2.5 Freshly picked noni fruits after washing are allowed to air-dry on raised tables before they are processed for juice (Anonymous, 2002).

Fully ripe fruits contain and release more juice than do under-ripe fruits. The most efficient noni juice extraction by weight is obtained when ripe, soft, translucent fruits are placed into the juice collection vessels. When green or hard noni fruits are placed into a juice collection vessel, the fruits release significantly less juice than soft, ripened fruits. Furthermore, a light-colored juice product is obtained from fully ripe, translucent fruits as opposed to the significantly darker-colored juice that is obtained when unripe or green fruits are placed into juice collection vessels.

3). Ripe noni fruits are placed into a juice collection vessel for 2 months or longer (Figure 2.6). During this time, the noni juice separates (drips) gradually from the pulp. The juice collection and fermentation vessels should be made of glass, stainless steel or food-grade plastic.

Figure 2.6 Plastic noni juices collection and fermentation vessels (left). Juices collection and fermentation vessels (approximately full of noni fruits and juices) (right) (Anonymous, 2002).

The noni juice collects inside the containers and ferments as it gradually seeps and sweats from the fruits. The juice appearance is initially an amber or golden colored liquid that gradually darkens with age (Figure 2.7). After the collection and fermentation process is complete, the juice is drained from spigots at the base of containers (and filtered). Fresh air is excluded from these containers, and contact between the juice and fresh air is minimized throughout the process. The final noni juice product is decanted, filtered and bottled.

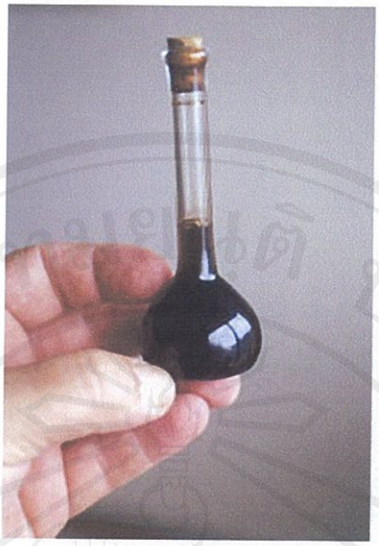

Figure 2.7 Fermented noni juice is a dark brown liquid, which is similar in appearance and texture to soy sauce. The pH is relatively low (approximately 3.5), lending a characteristically sour taste to aged noni juice (Anonymous, 2002).

After approximately 3 months, most of the noni juice separates naturally from the fruit pulp and may be drained from the container and filtered. The recovery of juice by this traditional method is approximately 40-50% of the original fruit weight. Therefore, using this method, 100 pounds of fruit may yield about 40-50 pounds of juice, or about 4.5 to 5.0 gallons of juice left over pulp. After all of the noni juice is drained from the collection and fermentation vessel, the residual pulp may be pressed to express the remaining juice fluids. The leftover pulp and seeds may be discarded, or they may be dehydrated and used in other noni products.

A variation of the traditional, drip-extraction method produces a non-fermented, sweeter juice. Some noni juice producers and consumers prefer a lighter-colored, sweeter-tasting product. If the noni juice is not allowed to ferment in the collection vessel, the juice will retain a relatively fruity, sweet taste, rather than the bitter, sour taste associated with fermented noni juice. To obtain sweeter, fruiter juice, the juice is drawn off from the collection vessel every couple of days, and not allowed to ferment. Rather, the juice is bottled and refrigerated (or frozen) immediately until it is marketed or consumed.

2.4.2 Non-traditional noni juice: fresh-squeezed, filtered and non-fermented (Anonymous, 2002)

Fresh-squeezed noni juice (Figure 2.8) has a sweeter (less acidic), fruitier flavor than aged, fermented noni juice.

Figure 2.8 Fresh-squeezed noni juice has a golden amber color and has significantly less sediment than fermented noni juice collected by the traditional method (Anonymous, 2002).

Conversely, fermented noni juice produced by the traditional method is very dark brown, resembling the color and texture of soy sauce. When noni fruits are ripe, the juice is separated from the pulp and seeds using a fruit press (Figure 2.9).

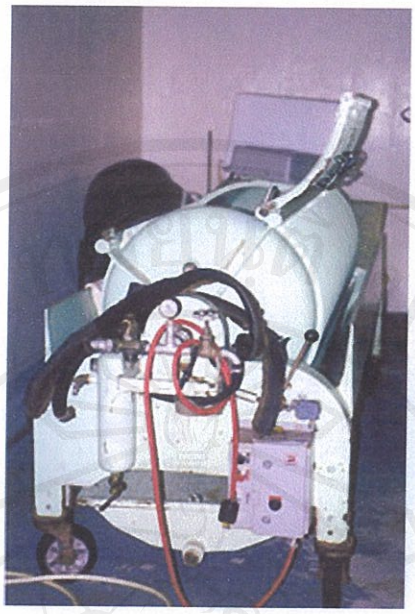

Figure 2.9 A hydraulic fruit press for making fresh-squeezed noni juice. Ripe fruits are loaded into the press through the top door. Juice is pressed from the pulp and bottled immediately (Anonymous, 2002).

Up to 65% juice recovery by weight is possible using this method of juice extraction. Home producers of noni juice use a wide range of fruit pressing methods, from squeezing by hand through cheesecloth, paint strainers, to more elaborate home-made pressing devices. Fermentation of fresh-squeezed juice can be arrested by refrigeration or by pasteurization. This will preserve the fruity, sweet taste of the non-fermented juice. Or, the fresh-squeezed juice may be allowed to ferment naturally in bottles or containers for a period of weeks or months prior to marketing or consumption. Drying noni fruit yields a material that can be powdered and put into dietary supplements. Bottled noni juices undergo pasteurization to eliminate microbial contamination but at the same time, it reduces volatile constituents. At present, a good method that is most likely to yield a beneficial noni fruit product is lyophilization or freeze-drying. Lyophilization is widely employed in the pharmaceutical industry to stabilize drugs and extend the lifetime of their potencies. The lyophilization process in a stabilizing procedure in which a substance is first frozen and dried by sublimation and desorption in order to destroy any chemical reaction. This process avoids the five destructive factors and produces a stable

material that retains a greater concentration of active compounds and volatile constituents.

2.4.3 Siam noni production (Suprederm, 2003)

The production of Siam noni fruit juice (Figure 2.10) is slightly different than the production of Hawii noni juice that was explained in the previous section. The result of Siam noni fruit juice can be seen in Figure 2.11. The production of Siam noni fruit juices is done without any addition of sugar and the process can be completed within 24 h under a closed system.

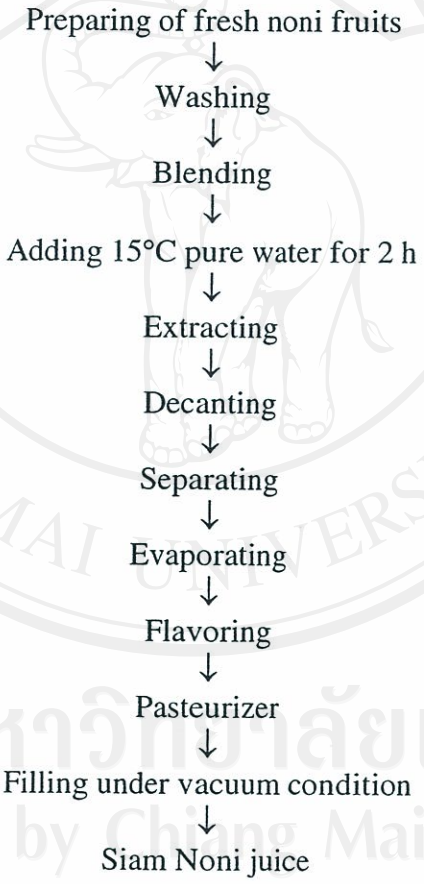

Figure 2.10 Diagram of Siam noni Processing (Suprederm, 2003)

Figure 2.11 Siam Noni™ (Suprederm, 2003)

2.5 Type of microorganisms

The term 'microorganism' is generally applied to any single-cell organism, or organisms consisting of cells that show little or no differentiation. In practice, this means that the groups of microorganisms are: the prokaryotes bacteria and cyanobacteria; the protista fungi, protozoa and algae; viruses and prions. Though all microbes are small, the range of sizes is enormous. The largest protozoan is more than 10,000 times the length of the smallest virus a size ratio similar to that of a 10-storey building to an ant (Harrigan and McCane, 1996).

2.5.1 Yeasts and moulds

The yeast and moulds, which are the most important fungi in the context of food hygiene, are larger than bacteria and structurally more complex, being typical eukaryotes in having mitochondria, golgi apparatus, distinct nuclei with true chromosomes and other cell constituents. Yeast exists as single cells or short chains and reproduces by budding or fission. They have a diameter of 4 μm or more. Thus one yeast cell size is bigger than bacteria cell. Moulds grow as a mycelium consisting of a complex array of tubes called hyphae, of indefinite length and from about 4 to 20 μm in diameter. Their spreading throughout the environment is principally achieved by the production of large numbers of spores or conidia. Thus, whereas for bacteria and yeasts, a cell number gives a reasonable indication of the amount of contamination of these organisms in food, the number of mould colony-forming unit has a very little relationship to the amount of contamination by moulds (Harrigan and Park, 1991).

Yeasts are fungi that grow as single cells, producing daughter cells either by budding (the budding yeasts) or by binary fission (the fission yeasts). They differ from most fungi, which grow as thread-like hyphae. But this distinction is not a fundamental one, because some fungi can alternate between a yeast phase and a hyphal phase, depending on environmental conditions. Such fungi are termed dimorphic (with two shapes) and they include several that cause disease of humans (Anonymous, 2000a).

Yeasts grow typically in moist environments where there is a plentiful supply of simple, soluble nutrients such as sugars and amino acids. For this reason they are common on leaf and fruit surfaces, on roots and in various types of food. With few exceptions, they are unable to degrade polymers, such as starch and cellulose, which are used by many hyphal fungi. Yeast species can have either obligately aerobic or facultatively anaerobic physiology. There is no known obligately anaerobic yeast. In the absence of oxygen, fermentative yeasts produce their energy by converting sugars into carbon dioxide and ethanol. In brewing, the

ethanol is bottled, while in baking the carbon dioxide raises the bread, and the ethanol evaporates (Anonymous, 2000a).

An example with glucose as the substrate is

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2C_2H_5OH \text{ (ethanol)} + 2CO_2$$

Yeasts can reproduce asexually through budding or sexually through the formation of ascospores. During asexual reproduction, a new bud grows out of the parent yeast when the condition is right, then, after the bud reaches an adult size, it separates from the parent yeast. Under low nutrient conditions yeasts that are capable of sexual reproduction will form ascospores. Yeasts that are not capable of going through the full sexual cycle are classified in the genus *Candida*. Many yeasts can be isolated from sugar-rich environmental samples. Some good examples include fruits and berries (such as grapes, apples or peaches), exudates from plants (such as plant saps or cacti). Some yeasts are found in association with insects (Anonymous, 2001c). Yeast fermentations comprise the oldest and largest application of microbial technology. Baker's yeast is used for bread production, brewer's yeast is used for beer fermentation and yeast is also used for wine fermentation. *Saccharomyces cerevisiae* is extensively used as a model organism by biologists studying genetics and molecular biology (Anonymous, 2000a).

Moulds able to cause spoilage of fruit juices and soft drinks include *Aspergillus ochraceus, A. tamarii, A. flavus, Byssoclamys nivea, B. fulva, Paecilomyces variotii, Neosartorya fischeri, Eupenicillium brefeldianum, Phialophora mustea, Talaromyces flavus, T. trachyspermus* and *Thermoascus aurantiacum.* Other include *Penicillium notatum, P. roquefortii* and *Cladosporum* spp. Mould spoilage within the factory is associated with poor hygiene. Various types of heat-resistant spores can be produced: ascospores, clamydospores and sclerotia (Wareing and Darvenport, 2000).

Mould problems can be devided into 2 types: growth of a variety of moulds due to poor hygiene within the factory or field environment and growth of heat-

resistant moulds within heat-processed juices. The former type can cause tainting, discoloration and other general problems associated with gross mould growth. The latter type can result in slow growth of the mould within the processed product. There is some overlap between the 2 groups. Xerophilic (highly sugar-tolerant) fungi are likely contaminants if hygiene is poor (Wareing and Darvenport, 2000).

Many of the above moulds are found on fruits pre- and post-harvest. Most moulds require oxygen to grow. Growth is exhibited as surface mats, sometimes producing copious spores. Some moulds produce extracellular degradative enzymes, such as pectinase. Detection of heat-tolerant moulds is usually carried out by plating out a sample of heat-shocked juice. (Wareing and Darvenport, 2000).

2.5.2 Lactic acid bacteria

This group is comprised of 11 genera of Gram positive bacteria at this time: *Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Vagococcus* and *Weissella.* Details and some species of each genus can be seen in Table 2.2. The most important member of streptococci is *Streptococcus thermophilus. Streptococcus diacetilactis* is classified as a citrate utilizing strain of *Lactobacillus lactis* subsp. *lactis.* Although, *Lactococcus cremoris* has been reduced to a subspecies of *Lactobacillus lactis*, this biovar is important in cheddar cheese production. Those that produce lactic acid as the major or sole product of glucose fermentation are designated homofermentative. The homolactics are able to extract about twice as much as energy from a given quantity of glucose as the heterolactics. Those lactics that produce equal molar amounts of lactate, carbon dioxide and ethanol from hexoses are designated heterofermentative. All members of the genera *Pediococcus, Streptococcus, Lactococcus* and *Vagococcus* are homofermenters, along with some of the lactobacilli, while all *Leuconostoc* spp., as well as some lactobacilli, are heterofermenters. The heterolactics are important than the homolactics in producing flavor and aroma components such as acetylaldehyde and diacetyl.

Table 2.2 Homofermentative and heterofermentative lactic acid bacteria

Homofermentative			**Heterofermentative**		
Organisms	Lactate Config-uration	%G + C	Organisms	Lactate Config-uration	%G + C
Lactobacillus			***Lactobacillus***		
L. acidophilus	DL	36.7	*L. bervis*	DL	42.7-46.4
L. bulgaricus	D(-)	50.3	*L. buchneri*	DL	44.8
L. casei	L(+)	46.4	*L. cellobiosus*	DL	53
L. coryniformis	DL	45	*L. confusus*	DL	44.5-45.0
L. curvatus	DL	43.9	*L. coprophilus*	DL	41.0
L. delbrueckii	D(-)	50	*L. fermentum*	DL	53.4
L. helveticus	DL	39.3	*L. hilgardii*	DL	40.3
L. jugurti	DL	36.5-39.0	*L. sanfrancisco*	DL	38.1-39.7
L. jensenii	D(-)	36.1	*L. trchodes*	DL	42.7
L. lactis	D(-)	50.3	*L. viridescens*	DL	35.7-42.7
L. leichmannii	D(-)	50.8	***Leuconostoc***		
L. plantarum	DL	45	*L. cremoris*	D(-)	39-42
L. salivarius	L(+)	34.7	*L. dextranicum*	D(-)	38-39
Pediococcus			*L. lactis*	D(-)	43-44
P. acidilactici	DL	44.0	*L. mesenteroides*	D(-)	39-42
P. cerevisiae	DL		*L. oenos*	D(-)	39-40
P. pentosaceus	DL	38	*L. paramesenteroides*	D(-)	38-39
Streptococcus			*L. gelidum*	D(-)	37
S. bovis	D(-)	38-42	*L. carnosum*	D(-)	39
S. thermophilus	D(-)	40	***Carnobacterium***		
Lactococcus			*C. divergens*		33.0-36.4
L. lactis subsp. *lactis*	D(-)	38.4-38.6	*C. mobile*		35.5-37.2
L. lactis subsp. *cremoris*	D(-)	38.0-40.0	*C. gallinarum*		34.3-36.4
L. lactis subsp. *hordniae*		35.2	*C. piscicola*		33.7-36.4
L. garvieae		38.3-38.7			
L. plantarum		36.9-38.1			
L. raffinolactis		40.0-43			
Vagococcus					
V. fluvialis		33.6			
V. salmoninarum		36.0-36.5			

Source: Jay (1996)

The genus *Lactobacillus* has been subdivided classically into three subgenera: *Betabacterium*, *Streptobacterium* and *Thermobacterium*. All of the heterolactic lactobacilli in Table 2.2 are betabacteria. The streptobacteria (for example, *L. casei* and *L. plantarum*) produce up to 1.5% lactic acid with an optimal growth temperature of 30°C, while the thermobacteria (such as *L. acidophilus* and *L. bulgaricus*) can produce up to 3% lactic acid and have an optimal temperature of 40°C (Jay, 1996).

In term of their growth requirements, the lactic acid bacteria require performed amino acids, B vitamins and pyrimidine purine bases. Although there are mesophilic, some can grow below 5°C and some as high as 45°C. With respect to growth pH values, some can grow as low as 3.2 and some can grow at 9.6, although most of them can grow in the pH range of 4.0 to 4.5. The lactic acid bacteria are only weakly proteolytic and lipolytic activity (Jay, 1996).

2.5.3 Gram negative bacteria

The cell wall of Gram negative bacteria is a thinner structure with distinct layers. There is an outer layer, which is more like a cytoplasmic membrane in composition with the typical trilaminar structure. The main component of the Gram negative cell wall is lipopolysaccharide. Additionally there is present phospholipid, protein, lipoprotein and a small amount of peptidoglycan. The lipopolysaccharide consists of a core region to which are attached repeating units of polysaccharide moieties. A component of the cell wall of most Gram negative bacteria is associated with endotoxic activity, which are associated the pyrogenic effects of Gram negative infections. On the side chains are carried the bases for the somatic antigen specifity of these organisms. The chemical composition of these side chains both with respect to components as well as arrangement of the different sugars determines the nature of the somatic or O antigen determinants, which are such important means of serologically classifying many Gram negative species. In many cases it has been shown that the reason for certain organisms belonging to quite different species, giving strong serological cross-reactivity is due their having chemically similar carbohydrate moieties as part of their lipopolysaccharide side chains, which generally

have about 30 repeating units. This group of 15 classes of Gram negative bacteria at this time: *Rickettsiaceae, Spirochaetaceae, Vibrionaceae, Acetobacteriaceae, Alcaligenaceae, Bacteroidaceae, Brucella, Chromatiaceae, Chromobacterium, Enterobacteriaceae, Legionellaceae, Neisseriaceae, Nitrobacteriaceae, Pseudomonadaceae* and *Rhizobiaceae* (Anonymous, 2000b).

2.5.4 Spore-forming bacteria

These are Gram positive, sporing non-acid fast straight rods. If motile they have peritrichous flagellae. They include aerobes, facultative anaerobes and strict anaerobes and are generally nonhalophylic with a wide growth range depending on the group. Many will grow on most simple bacteriological media. A variety of biochemical activities are noted in this family, including fermentative, proteolytic activities and the ability to grow on minimal media. Some species can fix nitrogen. A number of species are also characterised by producing specific toxins. The two main genera *Bacillus* and *Clostridium* are distiguished by the former being aerobic, while the latter is anaerobic. Most of these include a wide variety of species. Some characteristic of the *Bacillus* and related groups include they are able to survive in air. These are peritrichoulsly flagellated, form ellipsoidal or spherical, endospores, which may or may not swell the sporangium. They are aerobic to facultatively anaerobic and generally catalase positive. There are currently very many species in this genus. *Bacillus* spp. is a group of Gram positive, spore-forming, generally motile, aerobic rod-shaped bacteria, which also grow well anaerobically. They produce ellipsoidal or cylindrical spores either centrally or subterminally. The spores do not distend the cells. They all form spores readily on most media. Due to its highly virulent pathogenicity *B. anthracis* has been maintained as a separate species, as has *B. thuringiensis*, whose strains form the crystalline inclusion (Cry protein) or d-endotoxin, which is highly toxic for certain types of insect. *B. anthracis* and *B. cereus* are the only members of the *Bacillus* genus, which are human pathogens (Anonymous, 2000c).

The characteristic of the *Clostridium* and related groups are generally characterised by an inability to grow in air, although some may tolerate it. They

include many species, which can be psychrophilic, mesophilic or thermophilic. They are generally Gram positive with peritrichous flagellation. They degrade organic materials to acids, alcohols, CO_2, H_2 and minerals. Acids, particularly butyric acid, are a frequent product of clostridial fermentation. They form ellipsoidal or spherical, endospores, which may or may not swell the sporangium. They tend to be grouped into saccharolytic, proteolytic species but some are both and there are also some species, which are specialised in being limited in their biochemical activities. *Cl. botulinum* is subdivided into a number of types according to the serological specificities of the toxins produced. These specificities are based on neutralisation studies. Other *Clostridium* species can also produce botulinum toxins (Anonymous, 2000c).

2.5.5 Coliform bacteria

The term 'indicator organisms' can be applied to any taxonomic, physiological or ecological group of organisms, whose presence or absence provides indirect evidence concerned a particular feature in the past history of the sample. It is often associated with organisms of intestinal origin but other groups may act as indicators for other situations. For example, the presence of members of the set 'all Gram negative bacteria' in heat-treated foodstuffs is indicative of inadequate heat-treatment (relative to the initial numbers of these organisms) or of any contamination in the subsequent process after the heating. Coliform is a saprophytic lactose-fermenting members of the *Enterobacteriaceae* that are unable to grow (or unable to ferment lactose) at higher temperatures. Coliform counts, although the organisms represent only a subset of 'all Gram negative bacteria', provide a much less sensitive indicator of problems associated with heat treatment, but are still frequently used in the examination of heat-treated foodstuffs (Jay, 1996).

2.5.5.1 Growth of coliform bacteria

Like most other nonpathogenic Gram negative bacteria, coliforms grow well on a large number of media and in many foods. They have been reported to grow at temperatures as low as -2°C and as high as 50°C. In foods, growth is poor or very

slow at 5°C, although several authors have reported the growth of coliforms at 3-6°C. Coliforms have been reported to grow over a pH range of 4.4-9.0. *E. coli* can be grown in a minimal medium containing only an organic carbon source such as glucose and a source of nitrogen such as $(NH_4)_2SO_4$ and other minerals. Coliforms grow well on nutrient agar and produce visible colonies within 12-16 h at 37°C. They can be expected to grow in a large number of foods under the proper conditions (Jay, 1996).

Coliforms are capable of growth in the presence of bile salts, which inhibit the growth of Gram positive bacteria. Advantage is taken of this fact in their selective isolation from various sources. Unlike most other bacteria, they have the capacity to ferment lactose with the production of gas, and this characteristic alone is sufficient to make presumptive determinations. The general ease with coliforms which can be cultivated and differentiated makes them nearly ideal as indicators except that their identification may be complicated by the presence of atypical strains. The aberrant lactose fermenters, however, appear to be of questionable sanitary significance (Jay, 1996). One of the attractive properties of *E. coli* as a fecal indicator for water is its period of survival. It generally dies off about the same time as the more common intestinal bacterial pathogens, although some reports indicate that some bacterial pathogens are more resistant in water. It is not, however, as resistant as intestinal viruses. It was concluded that various pathogens persist after *E. coli* is destroyed in foods that are frozen, refrigerated, or irradiated. Similarly, pathogens may persist in treated waters after *E. coli* destruction. Only in acid food does *E. coli* have a particular value as an indicator organism due to its relative resistance to low pH (Jay, 1996).

2.5.5.2 Fecal coliforms

Fecal coliforms (FC) are pretty specialized types of bacteria and are dominated by *E. coli.* This bacterium thrives in the healthy human intestine and passes out in high numbers in the fecal material. These can be counted in water by using the fecal coliform tests and the counts are usually given as FC cells in 100 ml of water. Counting the FC is done by growing the bacteria under very specialized

conditions, at higher temperatures (44.5°C) which discourage the total coliforms from growing at all or, from growing in a peculiar (atypical) way which the technician will ignore when counting the typical colonies. Human feces tend to have much more FC than Fecal Streptococci (FS), which some people look for as well as or instead of FC. These FS tend to be more common in animal feces and so comparing the numbers of FC to FS (FC: FS ratio) is handy for getting an ideal as to whether the water has been polluted with human fecal wastes (>2:1 ratio) or animal wastes (<1:1) (Jay, 1996).

E. coli is commoned in the human intestine, but it has not usually harmful. (However, there are some strains, which can cause infections.) What is important to remember is that the presence of FC is a widely accepted indicator of the potential pollution of water with fecal. If that material is present, then there is a much greater risk of infectious microorganisms occurring in numbers large enough to cause an infection to break out if the water is consumed. These organisms include viruses, other bacteria, protozoa and a variety of worms (Jay, 1996).

2.5.6 Proteolytic bacteria

The nitrogen in proteins (as well as nucleic acids) may by regarded as the end of the line as far as synthesis of nitrogenous compounds is concerned, for the nitrogen in proteins is "locked" and is not available as a nutrient to plants. In order to set this organically bound nitrogen free for recirculation, the first process that must take place is the enzymatic hydrolysis of proteins (proteolysis). These accomplished by microorganisms capable of elaborating extracellular proteinases that convert the protein to smaller units (peptides). The peptides are then attacked by peptidases, resulting ultimately in the release of individual amino acids (Pelczar and Reid, 1972). The overall reactions may be summarized:

$$\text{Proteins} \xrightarrow{\text{proteinases}} \text{peptides} \xrightarrow{\text{peptidases}} \text{amino acids}$$

Relatively few bacterial species elaborate large amounts of proteolytic enzymes. Among the most active in this respect are some of clostridia, e.g.,

Cl. histolyticum and *Cl. sporogenes*; a lesser degree of activity is found in species of the genera *Proteus, Pseudomonas* and *Bacillus* spp. Many fungi and soil actinomycetes are extremely proteolytic. Peptidases, however, occur widely in microorganisms as demonstrated by the fact that peptones (partially hydrolyzed proteins) are a common constituent of bacteriological media and provide a readily available source of nitrogen. The ultimate products of proteolysis are amino acids. Their fate in the soil may be utilized as nutrients by microorganisms or degradation by microbial attack. Amino acids are subjects to a variety of pathways for microbial decomposition. The liberation of nitrogen from these compounds, which is accomplished by deamination, i.e., removal of the amino group will produce products that affect the food products. Although several variation of deamination is exhibited by microorganisms, one of the end products is always ammonia, NH_3. An example of a specific deamination reaction is shown below (Pelczar and Reid, 1972).

$$\underset{\text{Alanine}}{CH_3CHNH_2COOH} + 1/2\ O_2 \xrightarrow{\text{alanine deaminase}} \underset{\text{Pyruvic acid}}{CH_3COCOOH} + \underset{\text{Ammonia}}{NH_3}$$

This reaction is classified as an oxidative deamination. Many microorganisms can deaminate amino acids. The production of ammonia is referred to as ammonification. The fate of the ammonia thus produced varies, depending upon conditons in the soil. Some of the possibilities include accumulation and utilization by plants and microorganisms, and, under favorable conditions, oxidation to nitrates (Pelczar and Reid, 1972).