# CHAPTER II

# LITERATURE REVIEW

## 2.1 CHINESE STEAMED BUN

Chinese steamed bun is a round, white colored, bread-like item in Chinese cuisine. It has long been accustomed to Chinese and other Asians and has been brought to Thai culture probably by Chinese immigrants. The filling can be meat, such as minced pork, barbecued pork, or custard cream, etc. According to legend, it was invented by the scholar and military strategist Zhuge Liang in $2^{nd}$ century AD. It can be eaten at any meal in Chinese culture, and is often eaten for breakfast. It is typically eaten as a staple in many parts of China.

The main ingredients of this product were milled wheat flour, sugar, butter, yeast or baking powder. A crumb of Chinese steamed bun is soft and a filling must not split outside (Thai Industrial Standards Institute [TISI.], 2006). It is similar in nutritional and eating qualities to the white bread of the West. In size and texture, they range from 4 cm, soft and fluffy in the most elegant restaurants, to over 15 cm, firm and dense for the working man's lunch. The culinary practice of making Chinese steamed bun has been passing along from generations to generations. In Thailand, the popularity of the Chinese steamed bun has been rising and the need of mass production has been strongly emerging (Sotarat and Maisuttikul, 2008).

### 2.1.1 Chinese steam bun process

Chinese steamed bun dough processing starts from mixing all ingredients such as wheat flour, water, yeast, baking powder and sugar and finally forming into round shape. It can be made by various methods depending on mixing and proofing as follows: traditional process, straight dough process and sponge dough process (Saengmuang, 2000).

Traditional process, starter dough is prepared by combining sour dough and wheat flour dough. The starter dough is then proofed for a day. Right before using, the starter dough is mixed with wheat flour and water. Then it is proofed for 60 minutes before steaming (Rubenthaler, Huang, and Pomeranz, 1990).

In straight dough process, wheat flour, water, yeast, salt, and sugar are mixed and combined to form dough. The dough is kneaded until its texture is homogeneous and then formed into round shape before proofing for about 60 minutes and steaming. This method is simple and straightforward, hence having shorter processing time. However, the proofing time is quite difficult to control. The successful proofing process only depends on yeast activities. The appropriate proofed dough depends heavily on the proofing temperature, relative humidity and volume of dough. Especially, if the dough is proofed exceedingly, the entire structure may collapse (Jammek and Naivikul, 1982).

For the sponge-dough process, the mixing was performed in two steps (Figure 2.1). In the first step, wheat flour, water, yeast and sugar is mixed and kneaded to form the sponge. In the second step the sponge is mixed with remaining ingredients including wheat flour, water, sugar, salt, baking powder and shortening. This method is better than other methods as less amount of yeast utilized (Jammek and Naivikul, 1982). However, the sponge-dough process is a time-consuming because of the repetition of mixing process.

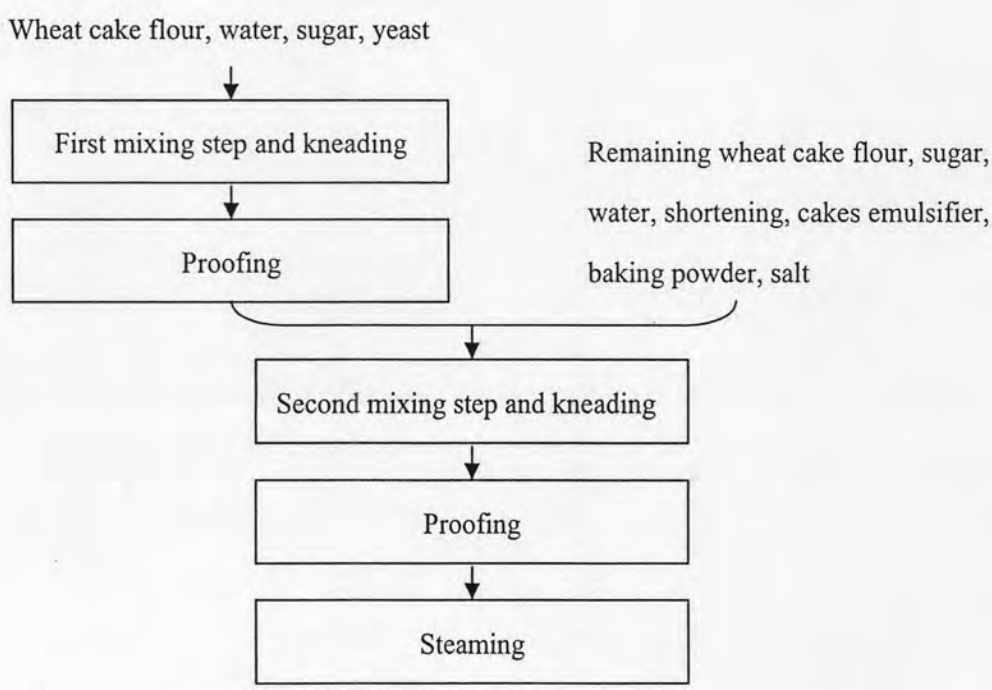


**Figure 2.1** Flow diagram of sponge-dough process of Chinese steamed bun

### 2.1.2 Proofing process

Proofing process is the important step of making Chinese steamed bun that determines the quality of final product. Yeast and baking powder are both used to make dough rise by providing the carbon dioxide gas that stays as bubbles in the dough. During proofing process, the dough texture is developed by forming the air gaps and increasing the porosity and volume of the dough texture (Linda and Stanley, 1999).

2.1.2.1 Yeast

The air gap is introduced mainly by the entrapment of carbon dioxide produced from yeast fermentation. During proofing process, biological leavening agent, *Saccharomyces cerevisiae*, plays a dominant role in developing a desirable dough texture (Eliasson and Larsson, 1993). The yeast and its by-products such as ethanol also contribute to flavour and aroma. The main reaction of yeast fermentation can be expressed Equation 2.1.

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad \text{---(2.1)}$$

2.1.2.2 Baking powder

Baking powder is made of sodium bicarbonate and leavening acid such as acetic acid, citric acid and lactic acid. These acids react with sodium bicarbonate suddenly when they are mixed (Hoseney, 1986). When water was added to the baking powder, the dry acid and base go into solution and start reacting to produce carbon dioxide bubbles. The carbon dioxide and water are produced as shown in Equation 2.2. When heat is applied, sodium bicarbonate is undergone the chemical reaction (Equation 2.3), which results in the production of carbon dioxide and water. In this process carbon dioxide gas is released, but without the release of aroma substances or flavour compounds. The taste of the product will therefore not be affected.

$$NaHCO_3 + H^+ \rightarrow Na^+ + CO_2 + H_2O \qquad \text{---(2.2)}$$

$$2\,NaHCO_3 + \text{heat} \rightarrow Na_2CO_3 + CO_2 + H_2O \qquad \text{---(2.3)}$$

### 2.1.3 Effect of ingredients on the qualities of Chinese steamed bun

Dough characteristic are the main factor influencing the quality of the finished product. Traditionally, it is made by combining wheat flour, water, yeast, sugar, and mixing the mixture to form viscoelastic dough (Hoseney, 1986). The basic properties of dough are depending on its ingredients. Each ingredient contributes remarkable to the properties of dough.

Wheat flour from soft wheat (wheat cake flour) is the most suitable to use in Chinese steamed bun. The functions of major components of wheat flour including protein, starch and pentosan, are different (Linda and Stanley, 1999). Gliadin and glutenin are the main proteins in wheat flour. When, they are mixed, they form a substance called gluten. Glutenin is an important structure that provides the strength for gluten while gliadin contributes to soft and sticky structure. Therefore, the proportion of gliadin and glutenin in gluten indicate physical characteristics of dough (Linda and Stanley, 1999). Starch is the major component of wheat flour. Both amylose and amylopectin influence the physical properties of dough (Charley, 1970). In proofing process, amylose from damaged starch is converted to sugar that can used in yeast fermentation process. During gelatinization process, starch can help to diffuse the water out of gluten to form stable and strong film which helps dough to swell and form body of bread (Stear, 1990). Pentosan is a polysaccharide that serves as a bridge-linkage between constitution of protein and starch in dough (Linda and Stanley, 1999).

Sugar is used mainly for adding sweetness into products. It can absorb moisture that makes the texture of the product soft; in addition, the shelf -life of bread is also extended because sugar causes water to be retained longer in the bread. Salt plays many roles in dough. It adds flavour and influences gas retention (Finney, 1984). It enhances the dough strength and reduces water absorption in dough structure (Salovaara, 1988). However, adding too much salt can cause the gluten structure in dough to collapse (Niman, 1981).

Water is another important ingredient for the production of steamed bun dough. It is an intermediate substance, which leads to the changes in physical, chemical, biochemical and biological reactions, so it directly influences the quality of product. The major function of water is to make the dough moist and dissolve other ingredients during kneading and mixing processes (Jammek and Naivikul, 1982).

## 2.2 CUSTARD CREAM

Custard cream is based on milk and eggs, thickened with heat. Most commonly, it refers to a dessert or dessert sauce, but custard cream bases are also used for quiches and other savory foods. Custard cream is made from a combination of milk or cream, egg yolks, sugar, and vanilla. Sometimes flour or corn starch are also added. Depending on how much egg or thickener is used, custard cream may vary in consistency (Matringe, Phan Tan Luu, and Lorient 1999).

Custard cream is usually cooked in a double boiler or heated very gently on the stove in a saucepan, though custard cream can also be steamed or baked in the oven with or without a hot water bath, or even cooked in a pressure cooker. The trick to get custard instead of sweetened eggs is to add heated milk to the eggs, not to add eggs directly into the pan on the stove. A water bath slows heat transfer and makes it easier to remove the custard from the oven before it curdles (McGee, 1984).

Egg and milk are the main ingredients of custard cream and usually mixed in many food formulas (Matringe *et al.,* 1999). Many studies have been published on functional properties of eggs (Mineki and Kobayashi, 1997; Vadehra and Nath, 1973; Baldwin, 1986), milk (Kinsella, 1984; Leman and Kinsella, 1989; Cayot and Lorient, 1998) and protein mixture (Knapp *et al.,* 1978; Kwasniewska *et al.,* 1979; Porteous and Quinn, 1979; Hargett *et al.,* 1982; Nichols and Cheryan, 1982; Linn and Cunningham, 1984; Burgarella, Lanier, and Hamann, 1985).

Eggs are used in many food products because of their functional properties, coagulation and emulsification. Coagulation is a term used to describe the entire process that result in loss of solubility or change from fluid to a more solid state. Coagulation of protein of egg is responsible for the thickening and the gel structure of custard cream. Success in making custard cream depends on avoiding the application of excessive heat. Excessive heat results in overcoagulation and syneresis of the protein. Custard cream is likely to curdle if it is cooked above boiling water in a double boiler (Penfield and Campbell, 1990). Egg yolk, which is itself an emulsion, is a good emulsifying agent for fats or oils and water. Lecithin in egg yolk interacts at the surface of oil droplets to form a layer (Chang, Powries, and Fennema, 1972).

Milk is a complex physicochemical system whose various constituents differ widely in molecular size and solubility (Walstra *et al.,* 1999). Milk provides volume and it can also strengthen tastes and aromas in custard cream. Similar to egg, milk also coagulates but at a lower extent (Matringe *et al.,* 1999).

## 2.3 DETERIORATION DURING STORAGE

All foods undergo varying degrees of deterioration during storage. The factors affecting food deterioration include the following: growth and activities of microorganisms, principally bacteria, yeasts, and moulds; activities of food enzymes and other chemical reactions within food itself; infestation by insects, parasites, and rodents; inappropriate temperatures for a given food; either the gain or loss of moisture; reaction with oxygen; light; and physical stress or abuse. These factors can be divided into 3 types including microbiological, chemical, and physical factors.

### 2.3.1 Microbiological deterioration

Food spoilage is a complex process and excessive amounts of foods are lost due to microbial spoilage even with modern preservation techniques. Despite the heterogeneity in raw materials and processing conditions, the microflora that develops during storage and in spoiling foods can be predicted based on knowledge of the origin of the food, the substrate base and a few central preservation parameters such as temperature, atmosphere, $a_w$ and pH. Based on such knowledge, more detailed sensory, chemical and microbiological analyses can be carried out on the individual products to determine the actual specific spoilage organism.

There are thousands of genera and species of microorganisms. Several hundreds are associated with food products. Growth of microorganism in food is different up to water activity (Blakistone, 1999). Both of Chinese steamed bun and custard cream filling are high moisture food, $a_w$ is above 0.95 (Smith *et al.*, 2004), so they risk from bacteria, yeast and mould. However, if $a_w$ is lower than 0.60, microorganism can be inhibited (Fennema, 1996). Some microorganism can produce toxin that can cause illness in human but toxic bacteria can be controlled by $a_w$. For example, *Clostridium botulinum* cannot grow and produce toxin at $a_w$ lower than 0.94 (Leistner, 1999).

Mould spoilage is a serious and costly problem for bakeries. Spoilage of bakery products is caused mainly by moulds and yeasts and occasionally by bacteria such as the rope-causing heat-resistant endospore-forming *Bacillus subtilis* (Earle and Putt, 1984). Mould spores are killed in the baking process (Knight and Menlove, 1961), leaving after contamination to be the source of spoilage problems. Contaminants of wheat bread are mainly *Penicillium* species (90–100%), but *Clodosporium* and *Aspergillus* species also occur (Legan and Voysey, 1991), the latter especially in warmer climates. The most important mould species on bread are *Penicillium commune, Penicillium crustosum, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium roqueforti, Aspergillus versicolor* and *Aspergillus sydowii* (Northolt, Frisvad, and Samson, 1995). On rye bread *P. roqueforti* is the major contaminant (Hartog and Kuik, 1984; Lund *et al.,* 1996). In a four-year investigation of rye bread in Denmark *P. roqueforti* (27%), *Penicillium corylophilum* (20%) and *Eurotium sp.* (15%) were identified as the most important species (Lund *et al.,* 1996). Varieties of *P. roqueforti* have later been elevated to species; *Penicillium paneum* and *Penicillium carneum* (Boysen *et al.,* 1996).

### 2.3.2 Physical and chemical deterioration

Physico-chemical alterations during bread storage lead to crumb firming, flavour changes, and loss of crust cracking. All of them form part of the global process called staling. Bread staling is responsible for important economical losses to both the baking industry and the consumer. Overall, bread loses its sensory qualities, resulting in a negative perception from the consumer even though the product is still healthy. Crumb firming is the preferred parameter used to evaluate staling development. Compression assays proved to be the best correlation factor to staling perception of bread (Axford *et al.*, 1968; Bashford and Hartung, 1976).

Firming of bread crumb during storage is a common phenomenon and leads to a crumbly texture, and lower consumer acceptance (Biliaderis, 1990; Stollman and Lundgren, 1987). Staling of products is generally defined as an increase in crumb firmness and a parallel loss in product freshness (Short and Roberts, 1971). Staling is a complex process and the mechanism still remains unknown, despite numerous attempts to explain the mechanism responsible for bread staling (Dappolonia and Morad, 1981; Krog *et al.*, 1989; Piazza and Masi, 1995). Because starch is the major component of the system, a predominant role has been assigned to starch retrogradation, which involves the progressive association of gelatinized starch segments into a more ordered structure (Seow and Teo, 1996; Zobel and Kulp, 1996).

Although it has been extensively studied, it remains unsolved. It is a complex phenomenon in which multiple mechanisms operate (Grey and Bermiller, 2003). The mechanism of bread staling has been studied for several years. Several works showed that bread staling was closely associated to starch retrogradation (Inagaki and Seib, 1992; Kim and D'Appolonia, 1977; Leon Duran, and Beneditode, 1997a). Others authors suggested a mechanism about bread staling dealing with the increase of interactions between starch molecules and gluten proteins (Martin, Zeleznak, and Hoseney, 1991). However, it was shown that the addition of gluten in model systems did not change firming rate (Duran *et al.*, 2001; Leon, Duran, and Beneditode, 1997b).

Another deterioration of multi-domain foods that have regions of differing water activity is moisture migration. Internal moisture transfer destroys the sensorial balance of the product and trigger deleterious reactions. Moisture transfers compromise the quality, stability and safety of the product and limit its shelf-life (Kester and Fennema, 1986). Control of initial moisture content and moisture migration is critical to the quality and safety of multi-domain foods.

Moisture loss or gain from one region or food with higher $a_w$ to another region will continuously occur in order to reach thermodynamic equilibrium. Two main factors influencing the amount and rate of moisture migration are water activity equilibrium (thermodynamics) and moisture diffusion rate (dynamics of mass transfer) (Labuza and Hyman, 1998). Moisture migration can be prevented either by matching the water activity of different domains of the food, which is not always practical or, more often, by using an edible moisture barrier between the domains (Biquet and Labuza, 1988).

Moreover, the physical and chemical deterioration also affect the sensory qualities as perceived by consumers (Vulicevic *et al.*, 2004). For this reason consumers are the appropriate tool for determining food product sensory shelf-life (Hough *et al.*, 2003).

## 2.4 HURDLE TECHNOLOGY

Hurdle technology is a combination of several preservative factors (called hurdles), which microorganisms present in the food are unable to overcome (Leistner and Gorris, 1995). This is illustrated by the so-called hurdle effect, first introduced by Leistner (Leistner, 1978). The hurdle effect is of fundamental importance for the preservation of foods since the hurdles inherent in a shelf-stable product control microbial spoilage and food poisoning (Leistner and Gorris, 1995). Several hurdles are used minimally in optimum combination which contribute to improvement in sensory qualities, microbial stability and energy saving and pose either no or minimal legal problems due to lower levels of additives in the products.

There are more than 60 potential hurdles for food (e.g., temperature, pH, $a_w$, and redox potential), which improve the stability and/or quality of the products. The hurdle could have a positive or a negative effect on foods, depending on its intensity. If the intensity of a particular hurdle in a food is too small it should be strengthened, if it is detrimental to the food quality it should be lowered. By this adjustment, hurdles in foods can be kept in the optimal range, considering safety as well as quality, and thus the total quality of a food (Leistner, 1994).

Three examples of hurdle effect are showed in Figure 2.2 (Leistner, 1992). Example No. 1 represents a food that contains six hurdles: high temperature during processing (F value), low temperature during storage (t value), water activity ($a_w$), acidity (pH), redox potential (Eh), and preservative (pres.). The food is microbiologically stable and safe, because the microorganisms present cannot overcome these hurdles. However, No. 1 is only theoretical case, because all hurdles are of the same height, and this rarely occurs. In No. 2, likely situation, the main hurdles are $a_w$ and preservatives, whereas other are less important hurdles. If there are only a few microorganisms present at start (No. 3), then a few or only low hurdles are sufficient for the microbial stability of the product (Leistner, 1992).

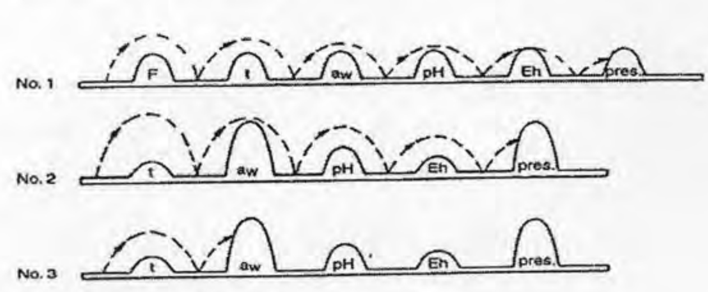


**Figure 2.2** Example of hurdle effect

### 2.4.1 Basic aspects

Food preservation implies putting microorganisms in order to inhibit their growth or shorten their survival or cause their death. The feasible responses of microorganisms to this hostile environment determine whether they may grow or die. Reactions of microorganisms in relation to hurdle technology are homeostasis, metabolic exhaustion and stress reactions.

#### 2.4.1.1 Homeostasis

Homeostasis is the tendency to uniformity and stability in the internal status of organisms. In food preservation the homeostasis of microorganisms is a key phenomenon which deserves much attention, because if the homeostasis of these microorganisms is disturbed by preservative factors (hurdles) in foods, they will not multiply and remain in the lag-phase or even die, before homeostasis is repaired. Therefore, food preservation is achieved by disturbing the homeostasis of microorganisms in a food temporarily or permanently (Leistner, 2000). Gould (1988, 1995) was the first to draw attention to the interference by the food with the homeostasis of the microorganisms present in this food, and more work in this direction is certainly warranted.

#### 2.4.1.2 Metabolic exhaustion

Another phenomenon of practical importance is metabolic exhaustion of microorganisms, which could cause 'autosterilization' of a food. Microorganisms in stable hurdle technology foods strain every possible repair mechanisms for their homeostasis to overcome the hostile environment, by doing this they completely use up their energy and die, if they become metabolically exhausted. Latin American researchers (Alzamora *et al.,* 1995; Tapia de Daza, Alzamora, and Chanes, 1996) observed the phenomenon in studies with high-moisture fruit products, because the counts of a variety of bacteria, yeasts, and moulds which survived the mild heat treatment, decreased fast in the products during unrefrigerated storage, since the hurdles applied (pH, $a_w$, sorbate, sulfite) did not allow growth.

2.4.1.3 Stress reaction

Some bacteria become more resistant or even more virulent under stress, since they generate stress shock proteins. The synthesis of protective stress shock proteins is induced by heat, pH, $a_w$, oxidative compounds, etc. Simultaneous exposure to different stresses will require energy-consuming synthesis of several or at least much more protective stress shock proteins, which in turn may cause the microorganisms to become metabolically exhausted (Leistner, 2000).

### 2.4.2 Hurdles used in food preservation

For the advanced application of hurdle technology a continually increasing number of preservative factors (hurdles) has become available (Table 2.1). The most important hurdles in common use for the preservation of foods, applied as either process or additive hurdles, are temperature, $a_w$, pH, Eh, and preservatives. At present, physical, nonthermal process (high hydrostatic pressure, mano-thermo-sonication, oscillating magnetic field, pulsed electric fields, light pulse, etc.) are receiving considerable attention. Hurdle used in this work are as follows.

**Table 2.1** Potential Hurdles for foods.

| |
|---|
| Temperature (low or high) |
| pH (low or high) |
| $a_w$ (low or high) |
| Eh (low or high) |
| Modified atmosphere (nitrogen, carbon dioxide, oxygen, etc.) |
| Packaging (aseptic packaging, vacuum or active packaging, edible coating, etc.) |
| Pressure (high) |
| Radiation (microwave, UV, irradiation, etc.) |
| Microstructure (emulsions, fermented sausage, ripened cheese, etc.) |
| Competitive flora (lactic acid bacteria, etc.) |
| Preservatives (lactate, acetate, sorbate, ascorbate, phosphates, etc.) |

Source: Leistner (1999)

2.4.2.1 Water activity

Reduction of $a_w$ is an effective preservation method of perishable material as growth of many of the spoilage bacteria is retarded due to low $a_w$. Each microbial species has an optimum, maximum, and minimum of $a_w$. When $a_w$ is reduced rapidly below the minimum level for growth of microorganism (Table 2.2), microbial cells will lose viability. Therefore, this information is used to control spoilage and pathogenic microorganism in food. Reduction of $a_w$ is performed by adding of humectant, such as sugar, honey, salt and glycerol. Each humectant has a different ability to reduce $a_w$ depending on molecular weight, amount of ionization and solubility (Cauvain and Young, 2000). However, addition of humectant is limited by physical, chemical and sensory qualities of product (Labuza and Hyman, 1998). In the present study, we use glycerol and fructose as humectants.

**Table 2.2** Minimum $a_w$ values for growth of microorganisms important in foods

| **Microorganism** | **Minimum $a_w$** |
|---|---|
| *Clostridium botulinum* type E | 0.96 |
| *Clostridium perfringens* | 0.96 |
| *Salmonellae* | 0.95 |
| *Escherichia coli* | 0.95 |
| *Vibrio parahaemolyticus* | 0.95 |
| *Clostridium botulinum* type A | 0.94 |
| *Bacillus cereus* | 0.93 |
| *Listeria monocytogenes* | 0.92 |
| *Staphylococcus aureus* (anaerobic) | 0.91 |
| *Bacillus spp.* (aerobic) | 0.89 |
| *Staphylococcus aureus* (aerobic) | 0.86 |

Source: Leistner (1999)

Glycerol is a colorless, odorless, viscous chemical compound which is widely used in pharmaceutical formulations. Also commonly called glycerin or glycerine, it is a sugar alcohol. The taste of glycerol is slightly bitter and sweet (about 0.6 times of sucrose). Glycerol has been given the status as a multipurpose "generally recognized as safe" (GRAS) food substance by the United States Food and Drug Administration (21 CFR 182.1320). There are no restrictions on functionalities or food category uses. Glycerol has three hydrophilic alcoholic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Moreover, it can extend shelf-life of product by reducing moisture migration (Labuza and Hyman, 1998) and increasing softness of cake (Cauvain and Young, 2000).

Fructose is a monosaccharide that found in many foods such as fruit, vegetable, cereal, and honey. It is 1.1-1.5 times sweeter than sucrose (Birch and Parker, 1982). Fructose is often recommended for, and consumed by, people with diabetes mellitus or hyperglycemia, because it has a very low glycemic index (GI) relative to sucrose (Deman, 1990). Fructose is normally used in bakery product such as cake and biscuit, because it increases softness of product and has pleasant taste (Birch and Parker, 1982).

2.4.2.2 pH

pH is one of the main factors affecting the growth and survival of microorganism in foods. Yeast and moulds are less sensitive than bacteria and capable of growing over wide range. The internal pH of cells is maintained near 7.0 which cell metabolism works best (Granum and Baird-Parker, 2000). When the microbial cell is subjected to extreme pH, cell membranes become damaged. Hydrogen ions can then leak into the cell, leading to cell death. When the pH is adjusted below the minimum for growth of microorganism (Table 2.3), it will be death. This method is used in mayonnaise to protect *Samonlla* hazard (Garbutt, 1997). The pH of processed foods is often modified using organic acids such as lactic acid and citric acid. Lactic acid was used as a hurdle for reducing pH because it did not affect the sensory quality of the product (Karthikeyan *et al.*, 2000).

**Table 2.3** Minimum pH values for growth of microorganisms important in foods

| **Microorganism** | **Minimum pH** |
|---|---|
| *Bacillus cereus* | 5.0 |
| *Clostridium perfringens* | 5.0 |
| *Vibrio parahaemolyticus* | 4.8 |
| *Clostridium botulinum* | 4.6 |
| *Escherichia coli* | 4.4 |
| *Listeria monocytogenes* | 4.3 |
| *Staphylococcus aureus* | 4.0 |
| *Salmonellae* | 3.8 |

Source: Leistner (1999)

2.4.2.3 Preservative

Preservative extends the shelf-life of foods by inhibiting the growth of bacteria and moulds. Bacteria can be carried through the baking process and can cause an unpleasant texture and odor. Moulds can contaminate products after baking and then grow on the outside surface. Careful sanitation is the key to avoiding both. Preservatives are of various types that are suited to certain products and are effective against specific chemical changes (Table 2.4).

**Table 2.4** Preservative used in foods

| **Preservatives** | **Foods** |
|---|---|
| Sorbate | Cheese, Cake, Meat |
| Banzoate | Pickles, Beverage |
| Propionate | Bread, Cake, Cheese, Grains |
| Phosphoric | Salad Dressing, Juice |
| Sulfite | Dried Fruit, Wine |
| Nitrite | Cured Meat |

Source: Leistner (1999)

According to Marin *et al.* (2002), the hurdle technology approach was used to prevent fungal growth of common contaminants of bakery products including isolates belonging to the genera *Eurotium, Aspergillus* and *Penicillium.* Several levels (0.003%, 0.03%

and 0.3%) of calcium propionate, potassium sorbate and sodium benzoate were assayed on a model agar system in a full-factorial experimental design in which the other factors assayed were pH (4.5, 6 and 7.5) and $a_w$ (0.80, 085, 0.90 and 0.95). Potassium sorbate and calcium propionate were found to be the more suitable preservative to be used in combination with the common levels of pH and $a_w$ in Spanish bakery products. Sub-optimal concentrations (0.003% and sometimes 0.03%) led to an enhancement of fungal growth. None of the preservatives had a significant inhibitory effect at neutral pH.

Marin *et al.* (2005) studied the inhibition of benzoate, propionate, and sorbate salts on intermediate moisture bakery products of low pH (4.5–5.5). The experiment was done as well as previous study at different levels of weak acid preservatives (0-0.3%), pH (4.5-5.5) and $a_w$ (0.80-0.90). Potassium sorbate was found to be the most effective in preventing fungal spoilage at the maximum concentration tested (0.3%) regardless of $a_w$ at pH 4.5 and its activity was slightly reduced at pH 5.5. The same concentration of calcium propionate and sodium benzoate was effective at low $a_w$ levels.

However, compared to other preservative options, propionates minimally impact yeast, making them the ingredient of choice for yeast-raised products. Calcium propionate is used as a preservative in a wide variety of products, including bread, other bakery goods, processed meat, whey, and other dairy products. When calcium propionate is dissolved in water, it dissociates in the same way as other chemical salts to form calcium ions and propionate ions (Equation 2.4). Once the propionate ions are in solution, a reaction with water occurs to reform propionic acid (Equation 2.5). The inhibitory agent is propionic acid, while the ionized or propionate form is not inhibitory (Davidson and Juneja, 1990). Therefore, calcium propionate was used as a preservative in this study.

$$\text{Ca(Propionate)}_2 \leftrightarrows Ca^{2+} + 2\ \text{propionate}^- \qquad \text{---(2.4)}$$

$$2\ \text{propionate}^- + 2\,H_2O \leftrightarrows 2\ \text{propionic acid} + 2\ OH^- \qquad \text{---(2.5)}$$

2.4.2.4 Modified atmosphere packaging

Modified atmosphere storage is one of the food preservation methods that maintains the natural quality of food products in addition to extending the shelf-life and improving product presentation in a convenient container (Jayas and Jeyamkondom, 2002). The changing of gaseous composition helps to reduce microbial growth, rate of internal chemical reactions. Gases generally used in gas packing are $N_2$ and $CO_2$ (Oberlender *et al.,* 1983). Each gas plays a distinct and specific role in modified atmosphere packaging (MAP) of food systems. $N_2$ is an inert gas that has no effect on food system and has no antimicrobial properties. It is used mainly as a filler to prevent package collapse. $CO_2$ is the most important gas in the mixture which is normally used for bakery product. It can inhibit the growth of spoilage microorganism by forming carbonic acid when soluble in water or fat.

Oxygen causes substantial deteriorative effects in many foods. Besides the destructive effects due to chemical oxidation of nutrients (especially vitamins A and C), food colors, flavours, and other food constituents, oxygen is also essential for mould growth. All moulds are aerobic and this is why they are found growing on the surface of foods. Therefore, $O_2$ is generally avoided in gas packaging. Suitable atmosphere modifier may be used to provide desired headspace atmosphere inside the package. Oxygen absorbers perform their action through a chemical reaction. They contain iron powder which reacts with the oxygen in the air causing the iron powder to rust. When all the iron powder has oxidized, the oxygen absorbers are "loaded" and the absorbing action stops.

According to Nielsen and Rios (2000), the effect of MAP on the most important spoilage fungi of bread was investigated. *Penicillium commune, P. roqueforti, Aspergillus flavus* and *Endomyces fibuliger* were able to grow at oxygen levels down to 0.03%, while the chalk mould *E. fibuliger* was capable of growing even in the presence of an oxygen absorber. High levels of carbon dioxide retarded growth but not completely.

Variations in permeability of packaging films like polyethylene (PE), polypropylene (PP), polyvinylidene chloride (PVDC), shrink film etc. used for MAP also affects the produce quality. Use of selective permeable packaging films on MAP could also be beneficial in extending the shelf-life of the product (Tiehua, Zhang, and Wang, 2007; Naik and Kailappan, 2007; Stewart *et al.,* 2005). Packaging serves a variety of purposes for food products from simply protecting food from outside contamination during distribution and storage to provide barriers that will maintain the correct moisture content, desired atmosphere in the headspace around a product (Risch, 2000). Plastic is a suitable food packaging material because of its properties including, light weight, good clarity, heat sealing and strength. Polymer plastic films generally exhibit a high resistance to the diffusion of water vapor and create an optimum modified atmosphere to keep the product under optimum condition. In this study, the suitability of PVDC as a packaging material was investigated.

PVDC enhances and upgrades packaging materials by giving them barrier properties which prevent the transmission of water vapor or oxygen, for example, the ingress of odors. PVDC provides strong seals to make hermetic packages, and it improves the appearance of packaging through its transparency, gloss, print adhesion and scratch resistance. It is mainly used for package of meal, tea, and medicines etc. because of its excellent moisture blocking and air blocking performance even in high temperature (Xaio, Zeng, and Xaio, 2008).

## 2.5 HIGH-MOISTURE FOODS

High-moisture foods (HMF) which have $a_w$ above 0.90 are common in industrialized countries. As they are often only minimally processed, their fresh-like properties and convenience appeal greatly to the consumer.

Lombard *et al.* (2000) studied the preservation of South African steamed bread using hurdle technology. The hurdles used are fat, salt, acid, preservative, glycerol, packaging material, heat and the processing technique. It was found that, low pH resulted in poor bread volume, fat had no effect on $a_w$ but increasing the salt content from 8 to 24 g/kg flour slightly reduced $a_w$ from 0.960 to 0.949. Glycerol was the most effective hurdle. Glycerol levels of 150 and 180 g/kg flour suppressed $a_w$ of the steamed bun to 0.908 and 0.880, respectively, which were sufficient to inhibit *Clostridium botulinum*. Yeast and moulds were effectively inhibited during the shelf-life study. Staling was not sufficiently retarded despite addition of high levels of fat.

Besides, Sangsawang (2004) studied the effect of glycerol and fructose in taro filling and found that addition of 7.5 %wt fructose and 7.5 %wt. glycerol could reduced $a_w$ of taro filling from 0.91 to 0.78, while maintained the acceptable sensory properties. The shelf-life of taro pie could extend from 7 days to 14 days at 30$^{O}$C when packed in polypropylene (PP) bag.

From another example, Karthikeyan *et al.* (2000) studied the effect of acidulant including citric acid, vinegar, acetic acid, sodium lactate, lactic acid and glucono-$\delta$-lactone (GDL), on the pH and sensory qualities of goat meat. It was found that, citric acid beyond 0.3%(w/v) gave an undesirable sour taste to the product and the pH reduction achieved at this level was not below 0.4 units. pH reduction by addition of 1% vinegar was very weak as evidenced by the decrease in pH to 6.22 from 6.34. The effect of GDL was moderate and pH decrease was low. Though sodium lactate improved the flavour of the product, the decrease in the pH was not significant. Lactic acid (90% purity) at less than 0.75% was acceptable to the panelist and at this level, the observed pH reduction was 1 unit. Therefore, lactic acid was preferred for the pH adjustment of the goat meat and the product with pH 5.8 to secure high sensory scores. Humectants was used for $a_w$ reduction to 0.90. Physico-chemical and microbiological characteristics as well as sensory attributes indicated that hurdle treated goat meat, that reducing pH with lactic acid and reducing $a_w$ with humectants, was safe and well acceptable up to the $3^{rd}$ day and fairly acceptable up to the $5^{th}$ day during storage at $36.2 \pm 1.2^{0}$C.