CHAPTER II

LITERATURE REVIEW

2.1 Horticultural Characteristics of Cilibangi

Red chilies belong to the genus of *Capsicum.* This genus consists of approximately 25 species belong to the *Solanaceae* family. *Capsicum* was first introduced to Europe from New World in 1943 by Columbus. In the late sixteenth, it was first brought into Asia and in a short time it had spread through the tropical and subtropical region (Yamamoto, 2013). Through the long journey of dispersion, hybridization has occurred and resulted to evolvement of new species that include the domesticated species; *Capsicum annuum*, *Capsicum frutescens, Capsicum baccatum, Capsicum Chinese* and *C. pubescens* (Mo et al., 2015). In different geography, the pods developed different characteristics in shape, color, size and pungency. These made the exact classification of the genus difficult (Sven et al., 2015).

Cilibangi is classified under *C. frustecens* member. This variety has been produced by conventional breeding done by Dr. Ahmed Mahir and his team. A new genetically stable chili variety was successfully developed after 8 generations of selection breeding. The exclusive feature of this chili is not only synchronized fruit maturity but also upright fruit maturity (Anon, 2015). Upright matured chili fruits are easily visible without being hidden by leaves. The fruits are medium size and extremely hot. This variety resembled other chilies' varieties such as tabasco pepper, piri-piri, tjabe rawit and kambuzi pepper (Daniele et al., 2013; Mathur et al., 2000). Formulations of hot fermented sauce recipe require type of chilies that are hot, spicy and pungent in taste

(Koh, 2005). Therefore, this has made Cilibangi suitable to be used as the raw material for the hot fermented sauce production.

FIGURE 1: Cilibangi Plant and Fruits

2.2 Fermentation

Fermentation is one of the oldest methods of food preservation. Fermented products are generally appreciated for attributes such as pleasant flavor, aroma, texture and improved cooking and processing properties. Fermentation technology dates back at least 6000 years and probably originated from microbial interactions of an acceptable nature (Steinkraus, 1997). In general, it can be prepared from raw or heated raw materials either by spontaneous or controlled fermentation. Spontaneous fermentations are initiated without the use of starter inoculums whereas controlled fermentation is conducted with the use of starter under defined fermentation condition (Holzapfel, 2002). Spontaneous fermentation process takes a relatively long time with high risk of failure. The controlled fermentation is highly desirable for industry as it is cost and time effective (Sakai et al., 2008).

2.2.1 Chili Fermentation

Red chilies are grown for the fresh market and for processing. However, fresh chili are very perishable product since they have high moisture content (300 to 400% db) and only last long for two to three days based on cumulative loss (Kaleemullah & Kailappan, 2004). Chili fermentation is one kind of food preservation method that can be applied to prevent undesirable changes in food and food products. The idea of chili fermentation was adopted from wine aging process. Red chilies are normally ground into mash together with high salt concentration (10 to 15%) and fermented traditionally in closed tanks oak barrel with a vent to allow gas formed during the process to dissipate during the fermentation. Nowadays, the fermentation can be done in plastic barrel since there is no significant different between the qualities of fermented mash in either plastic or oak barrel (Koh, 2005). Time of chili mash to ripening will start at 4 to 6 weeks during spontaneous fermentation. Nowadays, chili mash that is traditionally fermented through spontaneous fermentation has been applied for industrial production in order to prevent spoilage during storage (Hakan & Mehmet, 2012). Preserving the chilies by using fermentation is beneficial as it can lower the production cost especially to small and medium manufacturers.

2.2.2 Spontaneous Fermentation

Spontaneous fermentation is a fermentation that is initiated without the use of starter inoculums. It is depending on natural microflora that present of food substrate. It is neither predictable nor controllable. This technique is a common traditional practice in vegetable fermentation. This technique was elucidated through trials and errors that perhaps over thousands of years to get desirable fermentation result (Holzapfel, 2002). Spontaneous fermentations typically result from the competitive activities of a variety of contaminating microorganisms. The production of metabolites (e.g. organic acids) inhibitory to other contaminating microbes (e.g. Enterobacteriaceae) may provide an additional advantage during fermentation (Fan & Truelstrup 2012). Bacteria typically dominate the early stages of fermentation processes, owing to their relatively high growth rate, followed by yeasts, in substrates that are rich in fermentable sugars (Holzapfel, 2002). Only best adaptive microorganisms can survive on food substrate and to technical control parameters dominate the fermentation process (Plengvidhya et al., 2007).

2.2.3 Controlled Fermentation

Controlled fermentation is a fermentation which is conducted with the use of starter culture. They are four types of starter culture that are commonly used which are single strain, multiple-strain culture, mixed-strain culture and black slopping (Table 1) (Holzapfel, 2002).

TABLE 1: Type of Vegetables Starter Culture

Foodstuff	Single strain-culture	Multiple Strain Culture	Mixed Strain culture	Black-slopping
Sauerkraut	+	-	-	+[a]
Various vegetables	+	-	-	-
Vegetable juices	+	-	-	-
Soy products	+	-	-	-
Sour dough	+	+	+	+
Wine	+	+	-	
Dry sausage	+	+	-	+
Dairy products	+	+	+	-

*- not used, + applied, [a] brine from previous fermentation

Black slopping is a traditional controlled fermentation technique which is still being applied until now. It is defined as inoculation of residue from previous batch contained numbered of adapted microorganisms that already tolerable in the food substrates facilitate and improve fermentation process. It is developed by batch to batch fermentation in order to select the adapted strains that capable to produce best fermentation result (Holzapfel, 1997).

The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. There are three types of starter culture; single starter, multiple-strain starter and mixed strain culture. Single starter means the addition of single strain of bacteria into fermentable substrate. In addition, de Castro et al. (1988) performed controlled fermentation of peeled, blanched garlic, using a starter culture of *L. plantarum*.

Multiple strain culture is inoculation of microorganisms from same species but containing different strains lead to significant fermentation process. Novel *L. pentosus* paired strains consist of *L. pentosus* LP RJL2 and *L. pentosus* LP RJL3 significantly accelerate the fermentation process of Spanish-style green olive fermentation as compared to the natural fermentation (Ruiz-Barba & Jiménez-Díaz, 2012). Mixed culture fermentation is combining microorganism from different species that can work synergistically which improve the fermentation process. Montaño et al. (1997) applied combination of bacteria and yeast which is a mixed culture of *L. plantarum* and *Saccharomyces cerevisiae* to produce a better harmonic taste of lye-treated carrot juice under rapid fermentation process.

In terms of solid vegetable substrate, it is really hard to achieve single culture results except if it in juice system since inoculation with single culture can eventually resulted to multiple-strain or mixed culture fermentation if the raw substrate is not sterilized and maintained axenic (free from foreign microorganisms) prior to inoculation (Panda & Ray, 2007; McFeeters, 2004).

2.3 Autochthonous and Allochthonous Starter

Two main options may be pursued for the controlled fermentation of vegetables and fruits either with the use of autochthonous or allochthonous starter. Autochthonous starter is defined as microorganisms that are selected from natural flora which is isolated and re-inoculate to ferment the same food substrate (Di Cagno et al., 2008a). Allochthonous starter means isolated from certain raw matrices but used to ferment various products. Most of commercial starter culture that are available in the market specifically for vegetable fermentation can be considered as allochthonous since their origin are from various matrices. Most researchers claimed that autochthonous starter is more promising since they are well adapted to the food environment and to specific manufacturing process instead of capable of dominating the microbiota of the product due to specific metabolic activity (Kargozari et al., 2014). Allochthonous starter are claimed to have several limitation and have low performance (i) do not lead to rapid acidification, (ii), poor adaptation to sensory and functional properties (iii) low metabolic flexibility; and (iv) diversity did not reflect ecosystem where they have to be used (Oberman & Libudzsiz, 1998). Therefore, allochthonous starter is not recommended to be used to ferment specific vegetable or fruit matrix. Only one report described the selection of allochthonous *L. plantarum* NK-312, *Pediococcus* *pentosaceus* AFERM 722 and *Leuconostoc, mesentroides* BLAC to ferment mixture of cabbages, carrots, beets and onion (Gardner et al., 2001).

2.3.1 Lactic Acid Bacteria

The most important bacteria in desirable food fermentations are the *lactobacillaceae* or lactic acid bacteria (LAB) which have the ability to produce lactic acid from carbohydrates. They are a group of Gram-positive bacteria, facultative anaerobic, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. Lactic acid bacteria are a dissimilar group of organisms with diverse metabolic capacity. This diversity makes them very adaptable to a range of conditions and is largely responsible for their success in acid food fermentations (Axelsson, 1998). Lactic acid bacteria carry out their reactions by the conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids without the need for oxygen. The lactobacillus can present two behaviors which then be divided into three groups which are Group I: Strict homofermenters; Group II: Facultative heterofermenters; Group III: Strict heterofermenters. Homofermenter produce mainly lactic acid while heterofermenters produce lactic acid plus appreciable amount of ethanol, acetate and carbon dioxide. Normal condition required are excess sugar but with limited oxygen. Table 2 summarizes the general description of three genera of lactic acid bacteria (Denis, 2006):

Homolactic Fermentation

The fermentation of 1 mole of glucose yields two moles of lactic acid;

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$

(Glucose) (Lactic Acid)

Heterolactic Fermentation

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH + C_2H_5OH + CO_2$

(Glucose) (Lactic Acid) + (ethanol) + (carbon dioxide)

Table 2: General Description of Bacterial Species

Lactobacilli	Facultative heterofermenters (Group I)	*Lactobacillus casei* *Lactobacillus plantarum* *Lactobacillus rhamnosus*
	Strict heterofermenters (Group III)	*Lactobacillus brevis* *Lactobacillus hilgardii*
Cocci	Homofermenters	*Pediococcus damnosus* *Pediococcus pentosaceus*
	Heterofermenters	*Leuconostoc oenos (Oenococcus oeni)* *Leuconostoc mesentroids Subsp. mesentroides*

2.3.2 Lactobacillus as Culture for Vegetable Fermentation

Starter cultures of the genus *Lactobacillus* are added into vegetables to achieve their desirable properties. Inoculation of lactic acid bacteria can fasten the fermentation reaction as it accelerates the initial phase of fermentation and results in the promotion of desirable changes during the fermentation process So far, various types of commercial starter cultures have been developed for specific kind of vegetables fermentation (Breidt et al., 1995; Fre´de´ric & Luc, 2004; Herrero-Fresno et al., 2012). Desirable properties of fermented vegetable juices can be achieved by choosing *Lactobacillus* strains suitable for the lactic acid fermentation of individual raw materials. The criteria used for finding out suitability of a strain are as follows the rate and total production of acids, change in pH, grow and perform well under harsh, high salt tolerance, wide pH and temperature range to carry out successful vegetable fermentation metabolism and ability of culture to create desirable sensory properties of fermented products (Kohajdová & Kročičová, 2004; McFeters. 2004; Wiander et al., 2011). However, specific criteria of selection are depending on the types of raw material involved. Table 3 summarizes the criteria of selection for various types of vegetable material (Herbert, 1993).

Table 3: Criteria Selection of Starter Culture for Vegetable Fermentation

Criteria	Saurkraut	Cucumber	Olives	Vegetable Juices*
Technologically relevant criteria				
Rapid and predominant growth	++	++	++	+
Homofermentative metabolism	-	++	++	+
Salt tolerance	+	++	++	0
Acid production and tolerance	++	++	++	+
Inability to metabolize organic acid	++	++	++	+
Growth at low temperature	++	++	++	0
Few growth factors required	0	0	+	0
Tolerance of phenolic glycosides	0	0	++	0
Formation of dextrans	-	-	-	-
Pectinolytic activities	-	-	-	-
Formation of bacteriocins	+	+	+	0
Bacteriophage Resistance	0	+	+	++
Sensorially relevant criteria				
Heterofermentative	++	-	-	-
Formation of Flavor Precursors	++	++	+	0
Nutritionally advantageous criteria				
Reduction of nitrate and nitrite	+	0	0	++
Forrmation of L(+) lactate	+	+	0	++
Formation of biogenic amines	-	-	-	-

++, importance; +, advantageous; 0, not relevant; -,detrimental; *, except sauerkraut juice

For the achievement of a fast and controlled fermentation of vegetable products, a pure culture of lactic acid bacteria is used. The strains of *Lactobacillus* genera can improve the aroma of fermented vegetables, allow a rapid decrease of pH and controlled the growth of spoilage microorganisms (Di Cagno et al., 2008b; 2001a). All species of lactic acid bacteria have their own particular reactions and niches.

L. acidophilus, L. bulgaricus, L. plantarum, L. caret, L. pentoaceticus, L. brevis and *L. thermophilus* are examples of lactic acid-producing bacteria involved in vegetable fermentations (Kohajdová et al., 2007; Rhee et al., 2011; Galal et al., 2012). The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the food. This characteristic made them important bacteria and desirable in food fermentations industry (Axelsson, 1998). Now, there are various starter has been developed to carry out specific vegetable fermentation For example sauerkraut and pickles can be produced by *Leuconostoc mesenteroides, L. brevis, L. acidilactici,* pickles by *Leuconostoc mesenteroides, Pediococcus cerevisiae, L. brevis* and *L. plantarum* (Fre´de´ric & Luc, 2004; Sukon et al., 2008).

2.3.3 *Lactobacillus plantarum* and *Lactobacillus pentosus* as Vegetables Starter Culture

Lactobacillus plantarum is a versatile bacterium that is found in a variety of ecological niches, ranging from vegetable and plant fermentations to the human gastrointestinal tract (Rhee et al., 2011; Galal et al., 2012). Inoculations of these bacteria allow rapid acidification process in vegetable substrates thus completing the fermentation process in advance. *L. plantarum* has been inoculated in various vegetable products to carry out controlled fermentation process. Inoculation of this bacterium in cabbage-carrot juice resulted various taste description with different time interval. For example, sweet acid acid taste can be obtained within 102 h fermentation while harmonic taste within 96 h of fermentation. Optimal quality of cabbage juice can be obtained within 96 h fermentation with content of histamine and thyramine under the quantification limit (Kohajdová & Karovičová, 2004). This bacterium also posses strong antagonistic effects against food poisoning bacteria such *Listeria monocytogenes* and *Staphylococcus Aureus* (Herbert, 1993). *L. pentosus* is a facultative heterofermentative bacteria that frequently associated with lactic acid fermentation on vegetable such as cucumbers, cabbage and olives (Servili et al., 2006).

2.4 Pre-treatment Prior to Fermentation Process by Pasteurization

Pretreatments can promote growth of lactic flora which will be inoculated into fermentation substrate. Washing fruits and vegetables prior to fermentation can reduce the initial microbial count of natural microflora that exists on vegetable surfaces favor the development of lactic flora (Ana et al., 2002). Vegetable that is macerated rich with pectinolytic enzymes allow for homogenization prior to lactic fermentation (Panda et al., 2007). In addition, many vegetables contain glycosides that hamper efficient fermentation (Drewnowski et al., 2000). Choosing a fully ripened fruit is highly recommended since the high solanin content of unripe fruits inhibits the growth of LAB.

Pasteurization also one popular pre-treatment for fruits and vegetable is a normal practice as method of sterilization technique prior to fermentation. It is important to achieve control fermentation by eliminating the natural microflora that exist on vegetable and fruits surface. Pasteurization for 80°C for 15 min significantly decreased the natural flora of total mold and yeast, lactic acid and mesophilic bacteria which later improve the fermentation result (Yaghi et al., 2007). de Castro et al. (1998) performed the controlled fermentation of peeled, blanched garlic, using a starter culture of *L. plantarum* and compared it with that of un-blanched garlic. The garlic which was first blanched in hot water (90°C) for 15 min resulted to the inoculated starter to grow abundantly producing mainly lactic acid and reaching pH 3.8 after 7 days. The fermented blanched garlic was microbiologically stable during storage at 30°C in acidified brine. However, sterilized and axenic condition of fruits and vegetable can only be obtained they are in form of juices and it is hard for solid vegetables and fruits (Saw et al., 2011; Tassou et al., 2002).

2.5 Role of Ingredients in Vegetable Fermentation

Addition of salt can be done by direct addition or by brining method. The optimum salt concentration depends on the type of vegetables or fruits. According to traditional recipe, the salt concentration used is around 6 to 18% salt concentration (Koh, 2005). Common salts used are NaCI and KCI. According to Choi et al. (1994) substituting the NaCl by KCI up to 50% in the preparation of *kimchi* from cabbage did not affect the sensory qualities (saltiness, bitterness, sourness, hotness and texture). The main role of salt is to promote the growth of LAB over spoilage bacteria and to inhibit the pectinolytic and proteolytic enzymes that can cause vegetable softening and further putrefaction. Salt induce plasmolysis in the plant cells and the appearance of a liquid phase, create anaerobic condition around product submerged product. Surface mold and yeast growth is common and should be removed eventhough not ruining the fermented vegetable (Ross et al., 2002). The aerobic yeast that develops on brines surface are distinct from mold. It is known as Kahm yeast. This is happened when sugar is used up and the pH dropped because of lactic acid production by LAB. Secondary oxidative yeast develops on the surface of brines form a thick folded layer of mold and yeast. Layering the vegetable surface with table oil will create anaerobic condition which is more effective to encourage the growth of lactic acid bacteria. This is the most effective way to avoid surface growth of undesirable mold and yeast (Sandor, 2013).

2.6 Physiochemical Analysis of Fermentation Process

Physiochemical analysis is especially relevant during the development and implementation of new processing and conversation procedure or when developing new formula and products. Chemical analysis of foodstuffs equally relevant steps with a profound effect on the validity of data generated. Various techniques considering chemical or instrumental technique can be applied for specific chemical analysis (Røssland et al., 2003). pH and total acidity are among of most important criteria need to be considered while developing a new lacto-fermented food (Kohajdová et al., 2007; Kohajdová & Karovičová, 2004).

2.6.1 pH and Total Acidity

pH information tells the whole basis of lactic acid fermentation on the ability of lactic acid bacteria to produce acid. Monitoring pH is crucial in order to monitor the fermentation process for determining a starter culture suitability to carry out vegetable fermentation. Rapid decrease in pH at the beginning of fermentation is of great importance for the quality of the end product as it minimizes the influence of spoilage bacteria (Viander et al., 2003). The information about pH and total acidity together with sensory test can determine the optimal ripening time of fermentation process. A study made by Mheen and Kwon (1984) use pH as dependent variable in order to monitor the ripening period of fermented kimchi under salt concentration and temperature as the independent variable. The end pH of fermented vegetable is depending on vegetables types. For example, pH for best taste of kimchi is 4.5 whereas sauerkraut is among 3.5 to 3.8. (Lee, 1997; Karovičová et al., 1999).

2.6.2 Microbial Profile of Inoculated Lactic Acid Bacteria Fermented Vegetable

Under controlled fermentation, number of inoculated LAB in vegetable to initiate the fermentation is normally within ranged of log 6 to 10. The 24 h culture of LAB can initiate the fermentation under controlled fermentation (Demir et al., 2006). Autochthonous LAB starter give comparable result compared to allochthonous starter in juice system (Di Cagno et al., 2008b). Allochthonous starter (isolated from green olives) showed longer latency phases of growth by achieving only $\log_{10}$ 8.5 cfu/mL as compared to autochthonous starter *L. plantarum* reached $\log_{10}$ 9.6 cfu/mL after 17 h fermentation at 25° C started with initial log $\log_{10}$ 7.0 cfu/mL. Prolonged fermentation after 40 days showed the existence of yeast in unstarted tomato juice and tomato juice with allochthonous starter. Fermented vegetable normally contains similar number of aerobic mesophilic bacteria and LAB count. As example, Gundruk, sinki and khalpi are lactic-fermented vegetable products of Sikkim in India, and inziangsang is a fermented leafy vegetable product of Nagaland and Manipur in India (Buddhiman & Jyoti, 2010). The LAB as well as aerobic mesophilic count were in the range of 10^7 to 10^8 cfu/g. In some samples, the LAB numbers exceed the mesophilic bacterial count. Yeast and mold detected present in the samples with ranging between $\log_{10}$ 4 to 6 cfu/mL (Tamang et al., 2005). Inoculation of LAB in vegetable fermentation can cause synergistic effect towards the growth of naturally present mold and yeast since LAB has affinity for the mannoproteins yeast (Peng et al., 2001). In some cases, LAB cause inhibitory result to the growth of mold and yeast. This inhibition might be due properties of LAB that has wide spectrum of anti-yeast and antifungal activity (Muhialdin et al., 2011; Pakorn et al., 2007).

2.7 Sensory Analysis

Sensory analysis is a great tool to understand the product characteristic. It helps to understand ingredient effects, processing variables, quality control and competitor's product through development of product itself (Cherdchu, 2008). Therefore, food companies pay great attention to train sensory expert to study the product attributes in understanding the consumers' reactions (Kulvadee & Chowladda, 2002). Sensory analysis can be divided into two parts; laboratory sensory and consumer sensory analysis. Laboratory analysis involved small panelists in the lab whereas consumer sensory requires population consumers as the instrument. Both analyses used the same methodology; consist of discriminative, descriptive and hedonic test. Within laboratory analysis, sensory analysis has been used widely to develop a new novel food product and improving the current products in fermentation industry (Jian, 2006).

Sensory analysis can be used to determine the optimum fermentation time with the most desirable taste. In the early study, applying this analysis showed that most harmonic taste lactic acid fermented cabbage juice was produced with *Lactobaciluus plantarum* 92H inoculation after 72 to 96 h fermentation (Karovičová et al., 2002). Then, it followed by development of cabbage-carrot juice lacto-acid fermentation within 96 h fermentation (Krovičová & Kohajdová, 2002). Novel lactic acid fermented cucumber juice with addition of onion juice has been successfully invented within 48 h applying this analytical and organoleptic profiles analysis (Kohajdová et al., 2007). Recently, new safe free hydrocolloid carrots purees inoculated which have thick texture accompanied by pleasant odour and flavor was obtained by the selection of exopolysacaccharide LAB through sensory evaluation (Rikka et al., 2015).

2.8 Detection of Volatile Organic Compound in Food

Foods contains fraction of small aroma compounds approximately 50 to 200 constituent made this analytical task rather complicated (Rijkens & Boelens, 1975). During the early stage of research, extraction and distillation methods in combination with gas chromatography were employed to measure the total volatile composition (Xie et al., 2008). Later finding found out that it appeared that concentration of volatile organic compound (VOC) in a food does not necessarily reflect their concentration in air not only depends on the concentration in the foods product but also on the interaction between the food matrix (Naknean & Meenune, 2010). The VOC in the air phase determined the sensory perception of aroma (Edgar & Kadri, 2013). This has made the headspace concentration relate better to the sensory properties of food product (Procida et al., 2015). Headspace offer versatile, rapid, efficient, green technique for volatile extraction and free of interference (Soria et al., 2015).

2.8.1 Static Headspace GC-MS for Detection of Flavor Compound

Nowadays many modern headspace-GC instruments employ static headspace sampling, where the gastight syringe is replaced by a heated transfer line that allows for rapid sample transfer. Food matrix such as wine, water, fruit, and juices contain high molecular weight and non-volatile matrixes remain in the GC system and result in poor analytical performance (Xie et al., 2013; Virginia, et al., 2011) Many laboratory analysts use extensive sample preparation technique to extract and concentrate the compounds of interest from this unwanted non-volatile material. Employing static headspace analysis, it avoids this times and cost by directly sampling the volatile headspace from the container in which the sample is place (Frankel, 1983). The analytical method employing static headspace been applied extensively for volatile fraction analysis of various types of samples (Xie et al., 2013). Braga et al. (2015) proposed that static-headspace GC technique is better than solid-phase microextraction headspace technique. Static headspace technique provides a simpler method and offers a better recovery of major volatile compound that present in passion fruit juice.

2.8.2 Split and Splitless Injection Mode in GC

Introduction of liquid samples in GC has been a problem ever since the introduction of this technique in laboratory analysis (Danilo et al., 2015). Injection of analyte from headspace to GC into the capillary column influences the detection of final volatile organic compound in GC-MS (Soria et al., 2015). Splitt and and splittless injection are among of the injection technique available for headspace GC-MS (Giorgia et al., 2015). In split injection, only small part of the vapor enters the column while the rest is being vented. On the other hand, splittless injection made all the vapor is transferred into the column (Grob, 2003). Splitt injection is a better source for concentrated samples while splittless injection is designed for trace analysis of volatile organic compound. Nawaporn et al. (2015) determined the highly volatile odorant present in Thai Fish sauce applying splittless injection mode in static-headpsace GC compared with different types of injection method. Twelve different types of aroma compounds were detected present in fermented whey inoculated with *Wickerhamomyces pijperi* applying 1:10 split injection mode of headspace GC-MS (Izawa et al., 2015).

2.9 Type of Organic Compound Present in Chilies

The fruits of Capsicum species have a relatively VOC which has been reported to range from about 0.1 to 2.6% in paprika and similar large forms of *Capsicum annum*. The initial volatile oil content of the freshly picked is dependent largely upon the species and cultivar grown on the stage of maturity at harvest (van Ruth et al., 2003). Different chilies variety will contain different VOC. In bell pepper 63 compounds were identified and include alcohols, aldehydes, ketones, acids, ester and sulphur-and nitrogen containing compounds. The five most abundant compounds were 3-methylbutanal, 2-methylbutanal, 3-methylbutyric acid, acetone and hexanal (van Ruth et al., 2003). The volatile fractions of the peppers species have previously been isolated and more than 200 compounds were identified after hydro distillation and dynamic headspace sampling (purges and traps procedure (Garruti et al., 2013). Hot chilies of *Capsicum frustaceans* contain 85 different types VOC that constantly changed during its ripening process. The aromatic compound, alcohol and ester decreased during maturity stage (Liu et al., 2009).

The aroma compound of raw materials also changed once they are converted into fermented food product (Apichartsrangkoon et al., 2013). The chemical composition of fermented food is highly complex and deviate compared to its raw material. It consists of both volatile and non-volatile compound. Some of these substances contribute to the flavor of foods have received most attention (Taylor et al., 2011). Thai green chili paste which is known as *Nam Prig Noom* has a unique aroma receive larger customer demand due to its unique flavor characteristic. The flavor aroma of this food derives from green chilies that changed during fermentation process. Srisajjalertwaja et al. (2012) found the most abundant VOC are aldehydes, ester hydrocarbon and alcohols.

2.10 Lactic Acid Bacteria Contribute into Aroma and Flavor

Lactic acid bacteria contributes to the aroma and flavor of fermented products. They acidify food, resulting in tangy lactic acid taste, frequently exert proteolytic and lypolytic activities and produce aromatic compound. For instance, amino acids form upon bio-conversion (van Kranenburg et al., 2002). Wild strain starter cultures play important role in flavor formation because they have high biosynthetic capacity and produce interesting aromatic compounds (Ayad et al., 1999). Homofermenative LAB convert available energy source (energy) completely into lactic acid via pyruvate to produce energy and to equilibrate the redox balance. However, pyruvate can lead to the generation of many other metabolites such as acetate, ethanol, diacetyl and acetyldyde (Kleerebezem et al., 2000). In this way, LAB produce volatile substances contribute to the typical flavor of certain fermented products, such as Korean fermented red pepper paste (diallyl disulfide), fermented durian tempoyak (Sulphide compound) and Thai green chili paste (sulphide compound) (Kang & Baek, 2014; Apichartsrangkoon et al., 2013; Neti & Virgilio, 2009).