Identification of Lactic Acid Bacteriain Kimchi and Studied Their Sensory Profiles

By

Ms. Lalita Chuangchuan

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Special project submitted to the Faculty of Biotechnology, Assumption University

in part fulfillment of the requirements for the degree of Bachelor of Science

in Biotechnology

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Abstract

Four samples of kimchi were purchased for identification microbiological test and sensory evaluation by sensory profiles. Two samples were produced by Thai producer and two samples were produced by Korean producer in Thailand; (1.)Traditional Kim Chi (Thai producer) ®. Korea Wide Corporation Co., Ltd. Thailand, (2.) Miss Kimchi ®.(Thai producer) Uniriver Global Co., Ltd. Thailand, (3.) Hanpong E & C® (Korean producer) Co,. Ltd. Thailand, and (4.) Wuree Restaurant® (Korean producer). The first sample showed microbial population of 9:11 (*Lactobacillus spp.* : *Lactococcus spp.*), The second sample had 16:4 (*Lactobacillus spp.* : *Lactococcus spp.*), The third sample had 4: 16 : 1 (*Lactobacillus spp. : Lactococcus spp.: Streptococcus spp.*) and The fourth sample four had 12:7:1 (*Lactobacillus spp. : Lactococcus spp. : Leuconostoc spp.*). Sensory profile of each sample was studied, the attributes were sweetness, saltiness, sourness, spiciness, crispness, bitterness and cabbage flavor. The first sample was most sourness among samples. The second sample had lowest intensity of all attributes. The third sample was the most spiciness, bitterness and crispness among samples. The fourth sample had the highest intensity of cabbage flavor and sweetness. Kimchi made from Korean people had the same pattern of sensory profile in all attributes. The crispness and cabbage flavor were not found in Kimchi which was made by Thai people. The bitterness of Korean Kimchi were higher than Thai Kimchi. Preference and ranking test showed that the first sample was the most preferred. The third sample was ranked in second place. Followed by the fourth sample and second sample. The first sample had the highest rank because Thai consumer prefers sour, medium saltiness, varies spiciness and non-bitter kimchi. The first sample and the third sample were alike in the sense that the microbial population of *Lactobacillus spp.* is higher than *Lactococcus spp.*, this could have some effect on the taste of kimchi referring to the fact that both samples are preferred by Thai consumers. *Lactobacillus spp.* is homofermentative organism which mainly produces lactic acid, hence the sour taste.

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Introduction

The world is merging into one as the technology is improving all the time, better communication, faster travelling, culture is being exchanged, as well as food culture. There is also a trend for healthy food and healthy living and people are seeking new and healthy food. Kimchi has its unique taste and it is healthy with all the vitamins and minerals within it, it is also low in calories, hence an increase in demand. This also applies to Thai consumers.

There are some differences in Kimchi produced by Thai producers and Kimchi produced by Korean producers, but which one is preferred by Thai consumers is still unknown, what is Thai consumer's Kimchi taste preference is also unknown.

A sensory evaluation on Kimchi as well as study about the Lactic Acid Bacteria (LAB) present in the Kimchi can lead to better understanding about Kimchi, the difference between Thai made and Korean made Kimchi or discover possible relationship between the microbiological population and the taste of Kimchi.

Therefore more study on Thai consumer's preference on Kimchi and difference of Kimchi produced by Thai and Kimchi produced by Korean should be conducted.

Objectives

1. To identify the Lactic Acid Bacteria population in four samples of Kimchi.
2. To study the sensory profile of two Thai produced Kimchi and two Korean produced Kimchi.
3. To identify the differences between the two groups of Kimchi, on microbiological aspect and sensory aspect.
4. To identify the preference of Thai consumer regarding Kimchi.

Literature Review

1. Kimchi

Figure1: Kimchi. [8].

1.1 Definition

Kimchi (Figure 1) is a fermented food that mixes pickled Korean cabbage or radish with spicy vegetables and various condiments such as salted fish and red pepper powder. The term "Kimchi" was derived from "chimchae" which means soaking vegetables in salty water and storing them. To be prepared for the coldest season of Korea – 3 or 4 months in winter – when food was scarce, vegetables were pickled and stored beforehand, which was later developed into Kimchi. Kimchi is not a simple fermented vegetable but a complex and indigenous food of Korea involving diverse condiments and spices. [8]

1.2 Origin of Kimchi

In view that Koreans had enjoyed eating vegetables from the ancient times, and that salt had been made and used then, together with the old records on appearance of fermented foods like salted fish and soy in Korea, it is presumed that Kimchi had existed before the three-kingdom era. Red pepper was introduced into Korea via Japan around 1592-1598 when the Japanese army invaded Korea. The first detailed description on Kimchi is found in the book 'Gyeongdo Japji' (written in the late 1700s) which records that radish, cabbage, garlic, hot pepper powder, turban shell, ear shell, yellow corvina, etc. were mixed into boiled soup of salted shrimps, and then stored in jars during winter season for being fermented and transformed into a hot food. The book also records that Korean people in 1700s enjoyed the fermented food. Another record is found in 'Jeungbo Sanrim Gyeongje' (mountains and forest economy) written in 1766, which describes the use of Kimchi as daily side dish. The type of Kimchi seen today seems to have appeared after the 17th century when the 'cabbage with head' was introduced from

China. Around this time, condiments and spices were also in full use in Korea, which enabled Kimchi to develop into the current version. [8]

1.3 Types of Kimchi

Types of Kimchi found in one region may be different from another. Harvesting, weather condition, culture and other factors all have effect on which type of Kimchi is found in one region, religion also has an influence. Buddhism plays important role in Korea, Mahayana (Daseung Buddhism) has a tradition to serve food to the public in a way to accumulate their virtuous believes that making food and eating it is self-dicipline.

There is a vast variety of Kimchi throughout Korea, each type has different characteristic in each different region and is consumed in certain time of the year. Here are some examples of different types of Kimchi.

1.3.1 Baechu Kimchi

Baechu Kimchi is the most popular winter Kimchi. Baechu Kimchi uses whole uncut cabbage but the method of making varies by region. The taste also varies in different region, in the northern area where it is cold the Baechu Kimchi is not very tasty, quite insipid while in warm southern area it is rather salty, hot and juicy.

1.3.2 Kkakdugi

Kkakdugi is made from radish, preferably winter radish, because it has sweeter flavor and firmer texture. Green leaves of radish, leaf mustards, green onions or outer leaf of cabbage can be added to enhance the flavor. Fermented shrimp is used instead of anchovy paste if darker color and stronger sense is desired.

1.3.3 Nabak Kimchi

Nabak Kimchi uses cabbages and radishes with plenty of Kimchi stock, the less spicy the better the taste. Nabak Kimchi is a year-round Kimchi available at all seasons. Other seasoning should be shredded to avoid thick and sticky stock.

1.3.4 Oi Sobagi (Stuffed Cucumber Kimchi)

Oi Sobagi is the most popular Kimchi during spring and summer for its crunchy texture and refreshing juice. Because it contains a lot of juice it gets spoiled easily. Cucumbers are soaked in brine to maintain firm texture. Young radish can be put between layers for better taste and more yield quantity. A chopped Chinese chive is the most popular stuffing.

1.3.5 Yeolmu Kimchi (Young Summer Radish Kimchi)

Young radishes are one of the most common vegetables for Kimchi during spring and summer season despite its thin and small size. It is prepared without fermented fish. It can be served mixed with rice or cold noodles, which is a typical Korean delicacy on hot summer days.

1.3.6 Bo Kimchi (Bossam Kimchi: Stuffed Wrapped Kimchi)

Bo Kimchi is particularly famous in Kesong town but it is also famous nationwide. It is served in a bunble, with blended material inside, wrapped around with cabbage leaves. It is left to mature in a white container. It should be served and eaten in one time with no leftovers. It contains seafood and fruit with mild seasoning, it is fermented and softened quickly and easily.

1.3.7 Pa Kimchi (Green Onion Kimchi)

Pa Kimchi is most popular in Jeolla-do, it is made of medium-thick young green onions and tastes hot and spicy. Native young green onion with large white part is proper for this kind of Kimchi due to its sweet taste. It should be fermented for quite long to bring out its best flavor.

1.3.8 Got Kimchi (Indian Mustard Leaves Kimchi)

A Kimchi made of gat, a kind of mustard leaf. The stems and leaves are seasoned with pickled anchovy sauce. The strong flavor of the mustard leaf and the anchovy is what makes this Kimchi popular. Large amount of red pepper powder gives this Kimchi its unique bitter taste and aroma. Gat Kimchi usually takes around a month till it's ready to be consumed.

1.3.9 Dongchimi (Radish Watery Kimchi)

Dongchimi is rather vinegary than spicy. It is a water-based Kimchi and is famous among Koreans during winter months.

1.3.10 Chonggak Kimchi (Ponytail Radish Kimchi)

It is made from young radishes with stems still attached. The radish is usually cut vertically down the middle, leaving it in two halves and then a salty anchovy sauce and red pepper mixture is applied to the surface. The radishes are washed carefully before mixing with seasoning such as garlic, red pepper powder, ginger, green onions, salted shrimp and salt. The radishes are then placed in a jar and let sit for few days.

Temple Kimchi is famous for its mild flavor compared to others. Monks do not use strong flavored condiments and vegetables. Also, they do not use pickled fish paste since they believe the raw material causes anger. They use many kinds of herbs, pine nuts, wild sesame, peanuts, pumpkin, flour juices and potato-boiled water.

1.4 Nutritional Values of Kimchi

Kimchi is a fermented health food with unique flavor, which has been recognized as an excellent side dish in respect of nutrition of physiology as a result of its main ingredient (vegetable) and fermentation. [10] See Table1.

Table1: Nutritional compounds of Kimchi.

Nutrient (unit)	Contents in 100(g) Kimchi
Calorie (cal.)	32
Water (g)	88.4
Raw Protein (g)	2.0
Raw Fat (g)	0.6
Sugar (g)	1.3
Raw Fiber (g)	1.2
Raw Ash Content (g)	0.5
Calcium (mg)	45
Phosphorus (mg)	28
Vitamin A (IU)	492
Vitamin B1 (mg)	0.03
Vitamin B2 (mg)	0.06
Niacin (mg)	2.1
Vitamin C (mg)	21

Source: [10].

Apart from all the nutrition mentioned above, Kimchi is rich in live Lactic Acid Bacteria (LAB). From matured Kimchi 10~100 times more LAB than from milk fermentation products can be absorbed. These LAB also generates various organic acids which helps facilitate metabolism of inorganic contents such as calcium and iron inside human body. [10]

1.5 Ingredients of Kimchi

Kimchi contains many nutritious ingredients which combine together to give Kimchi its unique flavor and make Kimchi a wholesome dish. Although different types of Kimchi has different recipe, and the recipe also varies in different households and regions, the standard ingredients and seasonings are shown in Figure12 below.

Figure2: Ingredients of Kimchi. [10]

1.5.1 Korean Cabbage

The cabbage should have many green leaves and thin skin. Inner leaves should be tightly held to avoid cutting too many outer leaves. Bigger ones are preferred, in autumn medium sizes are preferred. Korean cabbage is full of vitamins and minerals. Methylmethionine, a biologically activated form of methionine found in the cabbage helps treat Atherosclerosis, it is also reported that methylsysteinsulfoxid in cabbage helps remove cholesterol. [13]

1.5.2 Radish

Radishes are rich in vitamin C, it also contains diastase, a digestive enzyme and is mostly composed of water, which makes it good for digestion.

Radishes' skin contains twice as much vitamin C as the inner part, it is better to clean the skin carefully than to peal off the skin. Good radish should be quite big, clean and fresh. Sweet radishes that are dense but soft are also good for making Kimchi

1.5.3 Red Pepper

Carotenoid, vitamin C and other healthy ingredients can be found in red pepper. The sterilizing effect of red pepper results from capsicin, which also promotes digestion by increasing the secretion of saliva and gastric juice and it also generally promotes metabolism.

1.5.4 Garlic

Allyl sulfide, a very pungent substance contained in garlic, is 15 times more germicidal than carbonic acid. This chemical has many other efficacies: helping metabolism, easing pain, preventing constipation, and detoxifying the body.

1.5.5 Green Onion

Green onion contains high sulfur which makes it acidic unlike usual vegetables. The green part is very rich in vitamin A and C. Green onion contains a sulfide of the radical allyl, a stimulant substance with sterilizing effects. When buying green onions, the freshness of their stem is important for thick green onions, while short thin ones should have short and fresh leaves. The root should look white in general with even thickness of the stem.

1.5.6 Ginger

Ginger contains a lot of inorganic contents, while generating peculiar scent and hot taste which comes from gingerol in the ginger. Gingerol facilitates blood circulation and has perspiring and antibiotic functions. Ginger gives very hot taste when used as mixture in various foods which often masks fishy smells or other unwanted aroma.

1.5.7 Salt

Korean salts are classified by their granular shape. In Kimchi making, whether using cabbage or radish, Hoyeom is mainly used. Hoyeom salt is not so black in color. Salt absorbed into human body does osmotic function within blood, digestive fluid and tissue fluid. It also controls acidity and regulates nerve and muscle excitability.

1.5.8 Cucumber

95% of cucumber is water, it is also rich in vitamin A, B1 and B2. Korean cucumbers are largely divided into "native cucumber", "Gasi cucumber", "young cucumber" and "old cucumber". Different types of cucumber are suitable for different case of cooking.

1.5.9 Leek

Allyl sulfide gives leek its unique aroma. When allyl sulfide is mixed with vitamin B1, it improves digestive power and generates sterilizing effect. Leek in Korea is divided into 'native leek' and 'reformed leek.' Native kind is suitable for making 'leek Kimchi' as it tastes better and dry. Leek with clear and green leaves is the good one, while bent or withered leek is to be avoided.

1.5.10 Godeulbbagi (Korean Lettuce)

Inulin is the main component of the vegetable which gives it sour and bitter taste.

1.5.11 Dalrae (Wild Garlic)

Dalrae, meaning "small garlic" is widely used in cooking and as a material of medicine. Dalrae Kimchi, a watery Kimchi with good aroma and taste is made with this vegetable.

1.5.12 Leaf Mustard

The stalk of leaf mustard has a unique scent and a little hot taste. Its leaves together with the stalk are put into making Kimchi. Leaf mustard contains protein, carotene and niacin and well as glucose and sugar. In addition, vitamin C, calcium and iron are also richly present in the plant. Red-colored leaf mustard will be suitable to the kinds of Kimchi that use red pepper, such as 'leaf mustard Kimchi,' 'cabbage Kimchi' and 'Ggakdugi' (cubed radish Kimchi). Green-colored leaf mustard will match with such kinds as 'white Kimchi' and 'watery radish Kimchi.'

1.5.13 Pickled Anchovy

Pickled anchovy is a kind of preserved food, made by spreading salt on anchovies and storing them for 2-3 months. During fermentation period, protein is hydrolyzed into amino acid, resulting in unique flavor. The bones of the fish turn into calcium which can be easily absorbed and the fat turns into volatile fatty acid which provides taste. It neutralizes body fluids, a very important function. Salted anchovy contains the highest percentage of fat, essential amino acids, and calories among salted fish.

1.5.14 Pickled Shrimp

The best pickled shrimp for making Kimchi should made with shrimps that are thick, plump, clean, pink colored and clearly shaped. Salted shrimps, the most commonly used type of salted fish, tastes particularly good because of its relatively small amount of fat.

1.5.15 Minari (Parsley)

It is an alkali food rich with vitamins and inorganic substances. It is mainly used as a vegetable but also utilized as a medicine since it has remedial effect such as alleviation of fever and dropping of blood pressure. Generally minari is washed, boiled and seasoned, to be taken as a cooked vegetable. Raw minari is mixed into preparing Kimchi, which then generates unique flavor and refreshing taste.

1.6 Kimchi Pickling

The steps and ingredients differ from different types of Kimchi being made, different households also have their cherished recipe which is handed down from generation to generation, and different regions have different ways of pickling Kimchi. The pickling method described here is general method for the most common Kimchi, Baechu Kimchi.

1.6.1 Trimming and Preparing Ingredients

a) Tear off tough outer leaves of cabbage, then wash and cut cabbage into half.

b) Put cabbages into brine and then pick them up. Sprinkle salt a little on the cabbages (towards their stalks) and store them, with cut section upwards, in big jars one by one. After preserving them for about five hours, turn each cabbage upside down so that salts may be fully and evenly soaked into the cabbages.

c) Cleanse the salted cabbages in water, and then drain them until they are dried. Cut large cabbages again into half, and then cut off their roots.

1.6.2 Preparing Kimchi Stuffing

a) After shredding radish, trim and wash the green onion, leaf mustard and parsley, cut all of them into 4cm pieces, and shred the white part of green onion.

b) Chop ginger and garlic. Then, take shrimps out of pickled fish and chop them (soup of pickled fish should remain).

c) After taking off shells, cleanse oysters in salty water and then pick them up.

d) Remove motes and impurities from raw shrimps, which will be then washed, drained and ground fully in a grinder.

e) Add red pepper powder on shredded radish until they become reddish. Mix parsley, leaf mustard, green onion and green onion with radish. And then, put garlic, ginger and salted fish into the mixture. Lastly, season them with salt and sugar.

1.6.3 Storing Kimchi

a) The salted cabbage leaves will be filled with Kimchi stuffing made from condiments and trimmed radishes. The filling shall begin from back side of the cabbages, and carried out evenly.

b) Wrap the whole package with large outer leaves of cabbage and put them in the jars.

c) Lay outer leaves of vegetable on top of them, and sprinkle rough salts.

2. Fermentation of Kimchi

As the Kimchi is fermented, osmotic pressure (water exhaustion) helps Kimchi to mature. As a result, the green smell of vegetables disappears and essential bacteria and enzymes are produced.

The vegetables become dehydrated after salt penetrates into them. The salt on the outside of the vegetable increases the osmotic pressure and enhances permeability. This effect of salt also happens on other organic materials which results in stopping or lower bacterial activity and disappearance or slowdown of enzymes.

The higher the temperature, the faster the Kimchi matures, although at high temperature and low salt content Kimchi fermentation was faster than at low temperature and high salt content. [15] Kimchi is most flavorful when it is matures at 5-10°C for two to three weeks. In the initial maturing stage, lactic acid fermentation occurs as a result of increased bacteria. The generated lactic acid and salt prevent the vegetables from spoiling. After the peak of maturity, some bacteria continue to create acid which softens the fiber of Kimchi and transforms the ingredients. This chemical phenomenon is called over-maturation, and it is often found in long-preserved summer Kimchi. [1]

The type of fermented vegetable product similar to Kimchi is sauerkraut, the difference between sauerkraut and Kimchi is the preferred end-point of fermentation. The best tasting Kimchi is attained before overgrowth of *Lactobacillus brevis* and *Lactobacillus plantarum* with an optimal product pH of 4.5. The overgrowth of *L. brevis* and *L. plantarum* diminishes product quality, but sauerkraut production depends on these organisms as has been shown in earlier study. [2]

As mentioned earlier, the fermentation is manipulated by salt concentration and temperature, the optimal range of salt concentration of Kimchi is 3-5%. [2]

3. Microorganism in Kimchi

Studies on Kimchi have been actively performed since the middle of 1990's.

In Kimchi made recently, general bacteria propagate rapidly at the maximum rate of ten times and again die out speedily. The reason is that the rapid propagation of bacteria makes carbon dioxide saturated where the bacteria cannot live anymore, so in that situation, only the LAB, the anaerobic microorganisms that can live without the air start their action in it. The LAB gradually ripens the cabbage or radish to be Kimchi as making lactic acid with sour taste. This situation allows only the LAB to be alive, and they make antibiotics called 'Bacteriocin' which stops the growth of other microorganisms. There exist one billion to ten billion lactic bacteria in 1ml of Kimchi at maximum. [18]

The classical identification of bacterial isolates from Kimchi revealed that *Leuconostoc mesenteroides* and *Lactobacillus plantarum* were the predominant species in Kimchi. However, other studies exploiting molecular identification methods have reported that a variety of *Leuconostoc* species, including *Leuconostoc citreum, Leuconostoc gasicomitatum, Leuconostoc gelidum* and *Lactobacillus sakei* were detected in several different Kimchi samples. Two recent studies, both of which used culture-independent identification methods, have confirmed these previous findings, and indicated that the majority of bacterial cells in Kimchi are culturable.

Other examples of important LAB found in kimchi are such as *Lactobacillus Brevis, Streptococcus faecalis,* and *Pediococcus pentosaceus*. Most kinds of bacteria belonging to the genus *Lactobacillus* have been found to be present in Kimchi. Another experiment shows that *Weissella confusa,* and *Lactobacillus curvatus* were the main microorganisms responsible for Kimchi fermentation. [12]

L. mesenteroides, hetero fermentative LAB, actively grows in the early stage to the middle stage of Kimchi fermentation, thereby producing lactic acid and carbon dioxide which could acidify Kimchi and create an anaerobic state to suppress the growth of aerobes this condition is favorable for the stabilization of ascorbic acid and the natural color of the vegetables. [2] *L. mesenteroides* has the ability to grow more rapidly and over a wider range of temperatures and salt concentrations than any other LAB and reaches its maximum value at the optimum-ripening stage of kimchi, and decreases sharply when the pH of kimchi is decreased to 4.0. [20] *L. citreum* as well as *L. gasicomitatum* are also abundant during the first growth phase. *L. citreum* IH22 dominates over and retards the growth of other LAB in Kimchi, suggesting it can be used as a bacterial starter culture to maintain the quality of Kimchi for prolonged periods. [5] The sugars in raw materials are converted to lactic acid, acetic acid, carbon dioxide and ethanol by hetero fermentative LAB during kimchi ripening, and these acids and carbon dioxide are

responsible for the fresh and carbonated taste of kimchi. It was also confirmed that low salt concentration and low temperature (e.g. 2% and 10° C) favor growth of hetero fermentative LAB.

It is presumed that acids or bacteriocins produced from fermentative bacteria may eliminate pathogenic bacteria during fermentation. However the bacteriocin-producing microbes lagged in the growth at the early fermentation stage and antibacterial activity of the bacteriocin may therefore be limited which makes the potential of pathogenic contamination fairly high in the early fermentation stage of Kimchi processing. [4]

After a certain period of time, excessive lactic acids are formed and off flavors are developed due to the growth of homo fermentative LAB and yeasts. [20] A homo fermentative type LAB, *L. plantarum*, which has a strong pH tolerance under high organic acid concentrations, has been continuously increased in their number during kimchi fermentation to the last stage and was dominant at the over-ripening and acid-deteriorated stage of kimchi. It has been reported that the acidification of kimchi is mainly caused by *L. plantarum*. [20]

The rate of Kimchi fermentation is significantly affected by salt concentration and fermentation temperature, and Kimchi is at an optimum for consumption when it contained 0.6-0.8% titratable acidity (pH 4.2), 3% salt, and high volatile organic acids. High salt concentration and high temperature (e.g. 3.5% and 30° C) favor growth of homo fermentative LAB. However, at 5° C to 15° C, the times for the increase and decrease of LAB are delayed. [20] *Pediococcus cerevisiae* was dominant at higher salt content (5-7%) and also appeared generally at over-ripening stage of kimchi.

Generally, kimchi fermentation has been carried out relatively at low temperature, and psychrotrophic LAB were isolated and characterized from kimchi fermented at 5° C). Therefore, psychrotrophic LAB isolated from kimchi fermented at low temperature were studied as starters for their effect on kimchi fermentation. The results indicated that the fermentation period could be shortened by using the LAB starters which isolated from low temperature kimchi. Among the LAB used as kimchi starters, it was confirmed that *Leuconostoc* species were more effective than any other *Lactobacillus* species tested for kimchi fermentation. [20]

The patterns of microbial changes in each LAB group, *Leuconostoc spp., Lactobacilli spp.,* and *Streptococci spp.,* were similar at different fermentation temperature, and the microbial changes accelerated by increasing the temperature. Kimchi fermentation is dominated by *Lactobacillus spp.* at 25° C and *Leuconostoc spp.* at 5° C.

Until recently, the identification of LAB isolated from kimchi has mostly depended on traditional phenotypic analyses. However, this type of identification method using biochemical and morphological characteristics is limited in its discrimination and accuracy. Therefore, studies on the systematic taxonomy have been reported. Accordingly, the effective study of kimchi fermentation requires the development of rapid and accurate LAB identification methods, such as genotypic approach using modern molecular typing and identification tools. Recently, LAB in kimchi were identified and differentiated rapidly by using the Biolog system. *Leuconostoc* and *Lactobacillus* are reported as the main genera associated with kimchi fermentation. [20]

One recently discovered change in Kimchi is the fact that *Weissella koreensis* is now the predominant species in the product, which was not determined before 2001. [6] Other novel strains such as *Leuconostoc kimchi* and *Lactobacillus kimchi* were also recently found.

The combination of various strains such as *L. mesenteroides, L. brevis, L. plantarum* and *P. cerevisiae* which were isolated from kimchi could be used as starters for kimchi fermentation. These starters increase the fermentation rate, and mixed strains are more effective than a single strain to produce better organoleptic quality of kimchi. [20]

4. Descriptive Analysis

4.1 Scope and Goal of Descriptive Analysis

The goal of descriptive analysis is to provide a quantitative specification of the important sensory aspects of a product.

- It deals with perceptions not ingredients, causes or implications.
- It does not ask questions about consumer acceptability.
- It uses panels consisting of trained or calibrated observers.
- It uses well-defined terminology.
- Data are quantified through ratings of perceived intensities on scales.
- It seeks to answer questions about how products differ on specific sensory bases.

Applications of descriptive analysis:

- Sensory diagnositics of ingredient, processing or packaging changes.
- Prediction of consumer acceptance.
- Correlation with instrumental measures.
- Matching of sensory profiles in quality assessments.

All descriptive analysis methods involve the detection (discrimination) and the description of both qualitative and quantitative sensory aspects of a product by trained panels of 5 to 100 judges (subjects). Smaller panels of five to ten subjects are used for the typical product on the grocery shelf while the larger panels are used for products of mass production such as beets and soft drinks, where small differences can be very important.

Panelists must be able to detect and describe the perceived sensory attributes of a sample. These qualitative aspects of a product combine to define the product, and include all of the appearance, aroma, flavor, texture or sound properties of a product which differentiate it from others. In addition, panelists must learn to differentiate and rate the quantitative or intensity aspects of a sample and to define to what degree each characteristic or qualitative note is present in that sample. Two products may contain the same qualitative descriptors, but they may differ markedly in the intensity of each, thus resulting in quite different and easily distinctive sensory profiles or pictures of each product. The numbers used represent intensity ratings on a 15 cm line scale on which a zero means no detectable amount of the attribute, and 15 means a very large amount.

4.2 Components of Descriptive Analysis

4.2.1 The Qualitative Aspect

Those perceived sensory parameters which define the products are referred to by various terms, descriptors or terminology.

These qualitative factors include terms which define the sensory profile or picture or thumbprint of the sample. An important aspect is that panelists, unless well trained, may have very different concepts of what a term means. The selection of sensory attributes and the corresponding definition of these attributes should be related to the real chemical and physical properties of a product which can be perceived. Adherence to an understanding of the actual chemistry of a product make the descriptive data easier to interpret and more useful for decision making. Statistical methods such as ANOVA and multivariate analysis can be used to select the more descriptive terms.

The components of a number of different descriptive profiles are given below. The repeat appearance of certain properties and examples is intentional.

a) Appearance characteristics
    a. Color (hue, chroma, uniformity, depth)
    b. Surface texture (shine, smoothness/roughness)
    c. Size and shape (dimensions and geometry)
    d. Interactions among pieces or particles (stickiness, agglomeration, loose particles)
b) Aroma characteristics
    a. Olfactory sensations (vanilla, fruity, floral, skunky)
    b. Nasal feeling factors (cool, pungent)
c) Flavor characteristics
    a. Olfactory sensations (vanilla, fruity, floral, chocolate, skunky, rancid)
    b. Taste sensations (salty, sweet, sour, bitter)
    c. Oral feeling factors (heat, cool, burn, astringent, metallic)
d) Oral texture characteristics
    a. Mechanical parameters, reaction of the product to stress (hardness, viscosity, deformation/fracturability)
    b. Geometrical parameters, i.e., size, shape and orientation of particles in the product (gritty, grainy, flaky, stringy)
    c. Fat/moisture patameters, i.e., presence, release and adsorption of fat, oil or water (oily, greasy, juicy, moist, wet)
e) Skinfeel characteristics
    a. Mechanical parameters, reaction of the product to stress (thickness, ease to spread, slipperiness, denseness)
    b. Geometrical parameters, i.e., size, shape an orientation of particles in product or on skin after use (gritty, foamy, flaky)

c. Fat/moisture parameters, i.e., presence, release and absorption of fat, oil or water (greasy, oily, dry, wet)
d. Appearance parameters, visual changes during product use (gloss, whitening, peaking)

Again, the keys to the validity and reliability of descriptive analysis testing are:

- Terms based on a thorough understanding of the technical and physiological principles of flavor or texture or appearance
- Thorough training of all panelists to fully understand the terms in the same way and to apply them in the same way
- Use of references for terminology to ensure consistent application of the carrier and descriptive terms to a perception

4.2.2 Intensity: The Quantitative Aspect

The intensity or quantitative aspect of a descriptive analysis express the degree to which each of the characteristics (terms, qualitative components) is present. This is expressed by the assignment of some *value* along a measurement scale.

As with the validity and reliability of terminology, the validity and reliability of intensity measurements are highly dependent upon:

- The selection of a scaling technique which is broad enough to encompass the full range of parameter intensities and which has enough discrete points to pick up all the small difference in intensity between samples
- The thorough training of the panelists to use the scale in a similar way across all samples and across time
- The use of reference scales for intensity of different properties to ensure consistent use of scales for different intensities of sensory properties across panelists and repeated evaluations.

4.3 Training for Descriptive Testing

The important aspect of any training sequence is to provide a structured framework for learning based on demonstrated facts and to allow the students, in this case panelists, to grow in both skills and confidence. Most descriptive panel training programs require between 40 and 120 h of training. The amount of time needed depends on the complexity of the product, on the number of attributes to be covered, and on the requirements for validity and reliability (a more experienced panel will provide greater detail with greater reproducibility)

4.3.1 Terminology Development

The panel leader or panel trainer in conjunction with the project team must identify key product variables to be demonstrated to the panel during the initial stages of training. The project team should prepare and prototype or collect from commercially available samples an array of products as a frame of reference, which represents as many as possible of the attribute differences likely to be encountered in the product category. The panel is first introduced to the chemical (olfaction, taste, chemical feeling factors) and physical principles (rheology, geometrical, etc.) which govern or influence the perception of each product attribute. With these concepts and terms as a foundation, the panel then develops procedures for evaluation and terminology with definitions and references for the product class.

Typically, the first stage of training may require 15 to 20 h as panelists begin to develop an understanding of the broad array of descriptors which fall into the category being studied (appearance, flavor, oral texture, etc.) This first phase is designed to provide them with a firm background in the underlying modality and for them to begin to perceive the different characteristics as they are manifest different product types.

4.3.2 Introduction to Descriptive Scaling

The scaling method of choice may be introduced during the first 10 to 20 h of training. By using a set of products or references which represent 3 to 5 different levels of each attribute, the panel leader reinforces both the sensory characteristic and the scaling method, by demonstrating different levels or intensities across several attributes.

The continued use of intensity reference scales during practice is meant to provide continued reinforcement of both attributes and intensities, so that the panel begins to see the descriptive process as a use of terms and numbers (characteristics and intensities) to define or document any product in the category learned.

4.3.3 Initial Practice

The development of precise lexicon for a given product category is often a three-step process. In the first step a full array of products, prototypes, or examples of product characteristics is presented to the panel as a frame of reference. From this frame of reference the panel generates an original long list of descriptors to which all panelists are invited to contribute. In the second stage, the original list, containing many overlapping terms, is rearranged and reduced into a working list in which the descriptors are comprehensive (they describe the product category completely) and yet discrete (overlapping is minimized). The third and last stage consists of choosing products, prototypes and external references which can serve to represent good examples of the selected terms. At this early stage of development, which lasts 15-40 h, the

panel gains skills and confidence. The disparate samples allow the panel to see that terms and scales are effective as descriptors and discriminators, and help the members to gain confidence both as individuals and as a group.

4.3.4 Small Product Differences

With the help of the project/product team, the panel leader collects samples which represent smaller differences within the product class, including variations in producing variables and/or bench modifications of the product. The panel is encouraged to refine the procedures for evaluation and terminology with definitions and references to meet the needs of detecting and describing product differences. Care must be taken to reduce variations between supposedly identical samples; panelists in training tend to see variability in results as a reflection of their own lack of skill. Sample consistency contributes to panel confidence. This stage represents 10 to 15 h of panel time.

4.3.5 Final Practice

The panel should continue to test and describe several products during the final practice stage of training (15 to 40 h). The earlier samples should be fairly different, and the final products tested should approach the real world testing situations for which the panel will be used.

During all five stages of the training program, panelists should meet after each session and discuss results, resolve problems or controversies, and ask for additional qualitative or quantitative references for review. This type of interaction is essential for developing the common terminology, procedures for evaluation, and scaling techniques which characterize a finely tuned sensory instrument. [21]

Materials

Raw Materials

- Cabbage Kimchi from four different producers
  1. Traditional Kim Chi ®. Korea Wide Corporation Co., Ltd. Thailand
  2. Miss Kimchi ®. Uniriver Global Co., Ltd. Thailand.
  3. Hanpong E & C® Co,. Ltd. Thailand
  4. Wuree Restaurant®.

Media and Equipments

- de Man, Rogosa and Sharpe (MRS)
- Tryptic Soy Broth (TSB)
- Tryptic Soy Agar (TSA)
- TSB with 0.5% Salt
- TSB with 5% Salt
- Electrical Balance, Mettler, PJ300

Method

1. Identification of Microorganism in Four Brands of Kimchi

1.1 Preparing the samples

Four samples of Kimchi were purchased, two samples were produced by Thai (Traditional Kimchi® and Miss Kimchi) and two were produced by Korean in Thailand (Hanpong® and Wuree®). The Kimchi samples were studied within 24 hours after purchasing. 10g of each sample were put in sterile plastic bags and mixed with 90ml of 0.1% peptone solution to make 1:10 ratio (Kimchi : peptone solution). This was undertaken under aseptic technique.

The samples were mixed, then make serial dilutions to 10^{-5} and perform spread plate on de Man, Rogosa and Sharpe (MRS) duplicate in each dilution. which means there were 10 plates MRS for each sample. The plates were then incubated at 37°C for 48-72 hours.

1.2 Selecting Single Colonies

Once the colonies are formed, 20 single colonies were selected from each sample, meaning 80 colonies were selected in total. Each of the selected colony, half was transferred to Tryptic Soy Broth (TSB) to enrich the colony while another half was transferred to fresh MRS plate by streaking technique. The MRS plates were kept in refrigerator as reference and for further usage. The TSB tubes were then incubated for 24 hours.

1.3 Identification

After 24 hours, the bacteria in TSB tubes were determined in a series of tests to identify genus of Lactic acid bacteria. The tests were gram stain, catalase activity, gas production and NaCl tolerance. Characteristics that were used to identify genuses can be seen in Table2 below.

Table2: Genuses likely to find and their characteristics.

Genus	Gram stain	Catalase	Cell shape	Gas production	0.5% NaCl in TSB	5% NaCl in TSB
Leuconostoc spp.	+	-	Spherical	+	-	-
Streptococcus spp	+	+ or - [a]	Spherical [b]	-	+	-
Pediococcuc spp.	+	-	Spherical [c]	-	-	-
Lactobacillus spp.	+	+	Rod	- [e]	-	-
Lactococcus spp.	+	-	Spherical [d]	-	-	-

Source: John G. Holt et. al. (1994) . William & Wilkins, Baltimore. *Bergey's manual of determinative bacteriology*, 9

Note: *a = Weakly + or -, hard to identify*
b = Usually cluster.
c = Not form chain
d = Pair or short chain
e = Hard to identify

1.3.1 Gram Staining

1. Prepare and heat-fix smears.
2. Flood the smear with crystal violet for one minute
3. Rinse completely with water
4. Flood the smear with Gram's iodine for one minute
5. Rinse completely with water
6. Wash with 95% alcohol drop by drop till the color stops coming out
7. Flood with safranin for 45 seconds
8. Wash with water and dry carefully with heat

Positive = Blue, violet

Negative = Red

1.3.2 Catalase Activity Test

1. Drop 3% H_2O_2 on a colony, make sure the drop touches the colony directly.

Positive: bubbles

1.3.3 Gas Production

1. Prepare TSB tubes, put Durham tube in each of them.
2. Inoculate the culture to the tubes
3. Incubate at 37°C for 24 to 48 hrs.

Positive: bubble(s) in Durham tube

Negative: no bubbles in Durham tube

1.3.4 Ability of Cell Growth in Salt Containing Media

1. Prepare TSB solution, divide into two containers
2. Add NaCl in one of the container to make up 0.5% NaCl and another to make 5% NaCl.
3. Use syringe to transfer the already mixed TSB and NaCl into tubes, careful not to mix the two concentrations.
4. Inoculate the culture to each tubes.
5. Incubate at 37°C for 24 to 48 hours.

Positive: The media solution turns cloudy

Negative: The media remains clear.

2. Sensory Evaluation of Kimchi Samples

2.1 Sensory profile of Kimchi samples

This part of the experiment was conducted to identify the profile of attributes that Thai people perceived on Kimchi. The descriptive analysis was conducted in the following steps.

- Six panelists was selected and trained on sensory evaluation.
- Panelists tasted Kimchi samples and described on appearance, taste, flavor and texture. (Appendix: Questionnaire 3.1). Water and plain cracker were provided and break for 10-15 minutes after each sample. Group discussion was conducts on terms to choose the attributes. This step was repeated again.
- Panelists were trained with standard solutions (table 3 and Appendix : Questionnaire 3.3). The data was analyzed. This step was repeated again till SD did not over than ± 2.
- Panelists were trained with standard solution together with Kimchi samples (Appendix: Questionnaire 3.3,). The data was analyzed. This step was repeated again till SD did not over than ± 2.
- The last step was conduct with four samples. (Appendix: Questionnaire 3.3) the data was analyzed and summarized in sensory profile.

Table3: Attributes and concentrations of the standard solutions.

Attribute	Concentration (%)
Sweetness (Glucose)	1
	2
Sourness (Citric acid)	0.025
	0.050
	0.080
Saltiness (NaCl)	0.2
	0.25
	0.35
	0.45
	0.50
	0.80
	1.0

2.2 Preference and Ranking Test of Kimchi samples

Preference and ranking test was conducted in order to evaluate the preference of each attribute and overall preference among the four samples. An example of the questionnaire is provided in the Appendix section (Questionnaire 3.2).

Results and Discussion

The results and discussion of this experiment are divided into two major parts: identification of LAB in four Kimchi samples and descriptive analysis. The results from both parts will be looked at and discussed together in the end, to identify if there were any relationship or not between microbiological aspect and sensory aspect.

1. Identification of Microorganism in Four Brands of Kimchi

From this point onward, the samples are referred to as sample one, two, three and four for convenience.

The results showed that there were two main genus of LAB in these four samples; *Lactococcus spp.* and *Lactobacillus spp.* The criteria used to identify the genus in this experiment were shown in the method section, and all the results are shown in Table 2, in Appendix.

The colonies of sample one were white, round and definite with clear cut which is the characteristic of Lactic Acid Bacteria (Figure3). Gram staining showed that all the selected colonies were gram-positive with 9 rod and 11 cocci. All selected colonies showed negative catalase activity result, and formed pairs with. The colonies showed negative results in both TSB containing 0.5% and 5% NaCl, all these results suggested that the suspected colony could be *Lactococcus spp.*

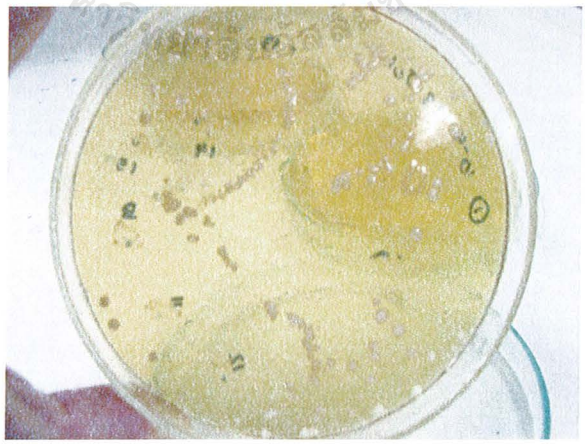

Figure3: Sample one, white, clear-cut colonies on MRS agar.

Among the selected colonies, all the cocci colonies were alike, the cells usually formed pairs, see Figure4. None of the selected colonies gave positive result in the gas production test.

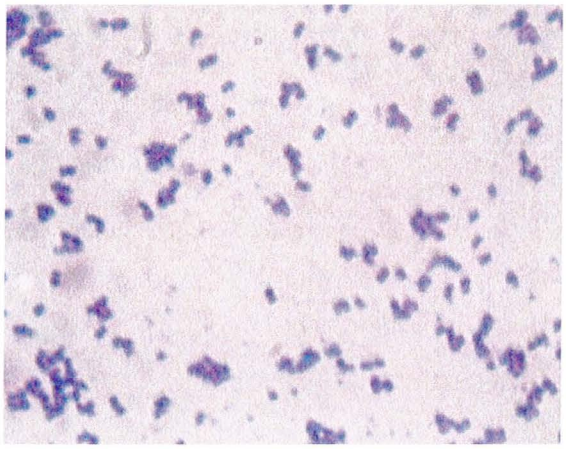

Figure4: Gram positive, cocci of sample one. Short chains and pairs can be seen.

Therefore, sample one consisted of *Lactobacillus spp.* and *Lactococcus spp.* in the ratio of 9:11.

Sample two colonies had the same characteristics as sample one; white, round and clear cut. The gram staining showed that they were all gram-positive with 4 cocci and 16 rod. The cocci cells also formed pairs like sample one. Catalase activity test showed weakly positive results in colony number 2 and 14, other colonies gave negative results. Gas production test and NaCl tolerance tests both showed negative results in all selected colonies. Therefore, sample two consisted of *Lactococcus spp.* and *Lactobacillus spp.* in the ratio of 4:16.

Sample three's gram staining result showed 4 rod and 16 cocci colonies, all gram-positive. Catalase activity test were positive in colony number 11 and 18. All colonies showed negative results for gas production. Colony number 1 showed positive result in 0.5% NaCl in TSB which was likely to be *Streptococcus spp.* while other colonies gave negative result. There were 1 *Streptococcus spp.*, 4 *Lactobacillus spp.* and 15 *Lactococcus spp.* among the selected colonies from sample three.

Sample four had 8 cocci and 12 rod colonies, all gram-positive. Colonies 7, 12 and 17 showed positive catalase activity test results, colonies 11, 14, 15 and 19 gave positive gas production results, none of the colonies gave positive result in the NaCl tolerance test. Colony number 11 was a cocci, gram-positive with positive gas production result, which categorizes into *Leuconostoc spp.* characteristics,

which made sample four consisted of 1 *Leuconostoc spp.*, 7 *Lactococcus spp.* and 12 *Lactobacillus spp.*.

For more accurate results, genetic engineering technique should be undertaken.

2. Descriptive analysis of Kimchi Samples

After three sessions of tasting and describing the samples, attributes were chosen for further study (table 4). The chosen attributes were; sweetness, sourness, spiciness, bitterness, saltiness, crispness and cabbage flavor.

Table4: Attributes and their frequencies of Kimchi samples

Attribute	Frequency
Crispness	3
Saltiness	4
Bitterness	9
Cabbage Flavor	10
Spiciness	23
Sourness	24

Standard solutions in standard solution were prepared for the training sessions. During the training session, a test was conducted to examine the panelists' accuracy and the result was in the acceptable range therefore another test was conducted to confirm the result, which was also in the acceptable range. The results were as shown in the Table 5.

Table5: The standard deviations of the tests during training.

Atrribute	SD 1st test	SD 2nd test
Sweetness	0.488	0.378
Sourness	0.013	0.012
Saltiness	0.082	0.049

The next stage was the sensory profile with sweetness, sourness, saltiness, spiciness, crispness, bitterness and cabbage flavor of the samples. Spider web diagram has been illustrated according to the results obtained for better understanding.

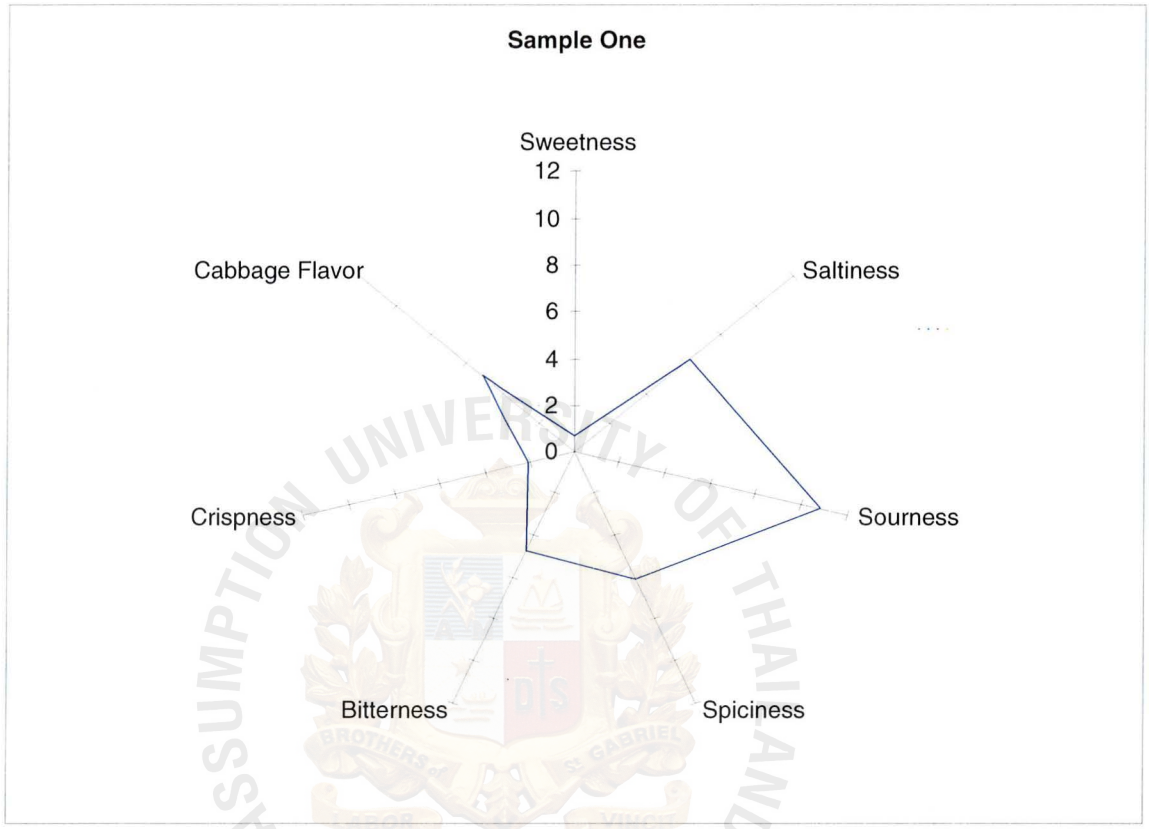

Figure5: Flavor profile of sample one.

From Figure5, it can be seen that sourness was the strong attribute of sample one with score of 10.860 from 15, sweetness and saltiness had the score of 0.648 and 6.431 respectively. Spiciness, bitterness, crispness and cabbage flavor showed score of; 6.107, 4.679, 2.086 and 5.150 in respective order. The panelists commented on sample one that it was attractive, bright red color, nice smell and juicy and that the flavor was just right. The panelists liked that it was quite sour and not too spicy.

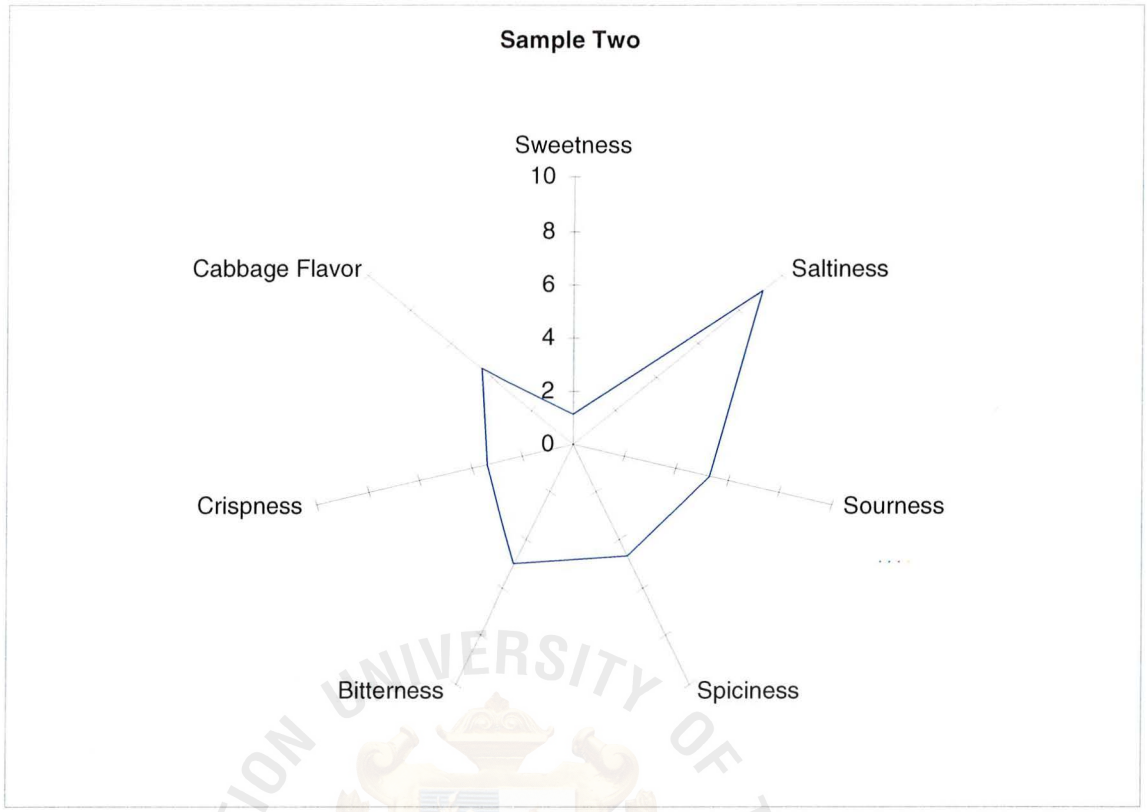

Figure6: Flavor profile of sample two.

From the Figure 6 above, it can be seen that saltiness had the highest intensity out of the seven attributes with the score of 9.127. Sweetness, sourness, spiciness, bitterness, crispness and cabbage flavor had the scores as follow; 1.164, 5.231, 4.700, 4.986, 3.329 and 4.479. Sample two was very pale in color, poor odor and the overall comment on the taste was that it was very bland. None of the panelists gave good comments on sample two at all. It was also noted that the cabbage of sample two was mainly the stalk part and not the leaf part which was harder to chew and the panelists preferred more leaves and stalk.

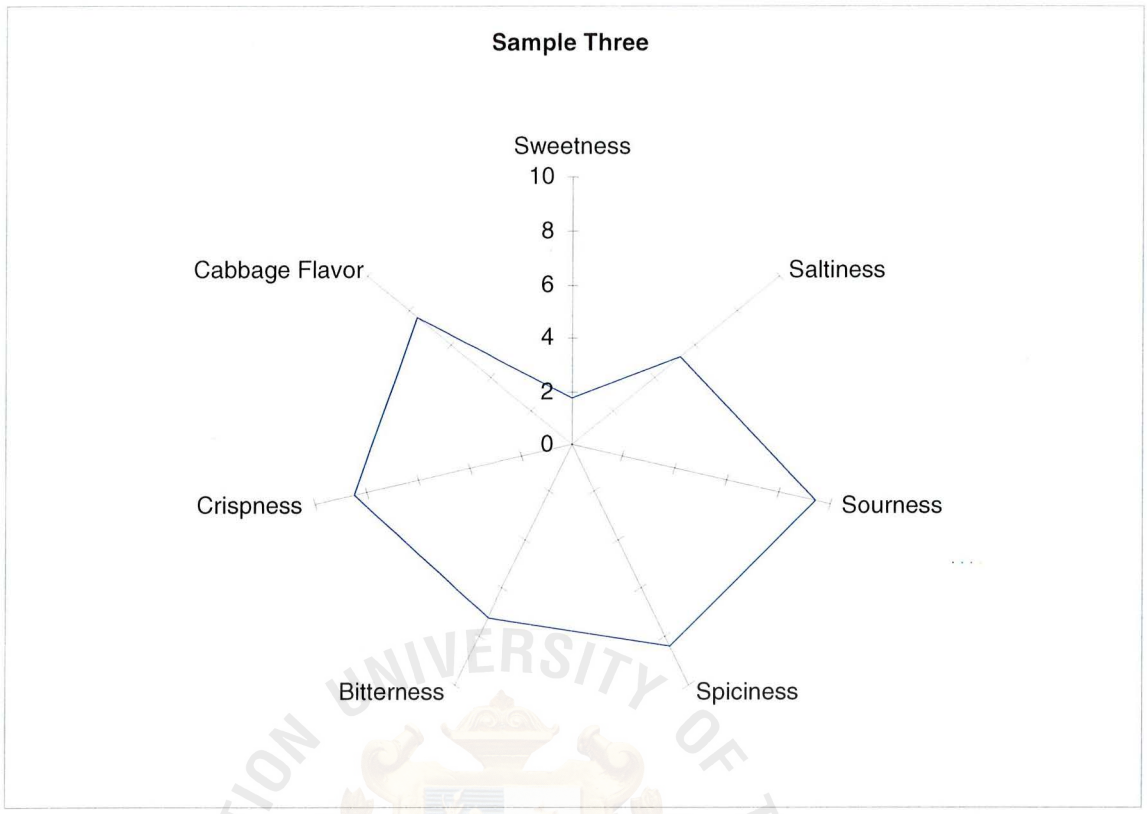

Figure 7: Flavor profile of sample three.

Sample three, from Figure7 above, has small difference between each attribute. Sourness had the highest intensity, spiciness and crispness had about the same intensity and came second while bitterness and cabbage flavor was quite similar then saltiness and sweetness. The scores are as follow; 1.714, 5.211, 9.356, 8.386, 7.207, 8.479 and 7.550. Sample three was quite red in color, although it wasn't too spicy like the color suggested it to be. Other comments were that it was crispy and a little bit bitter.

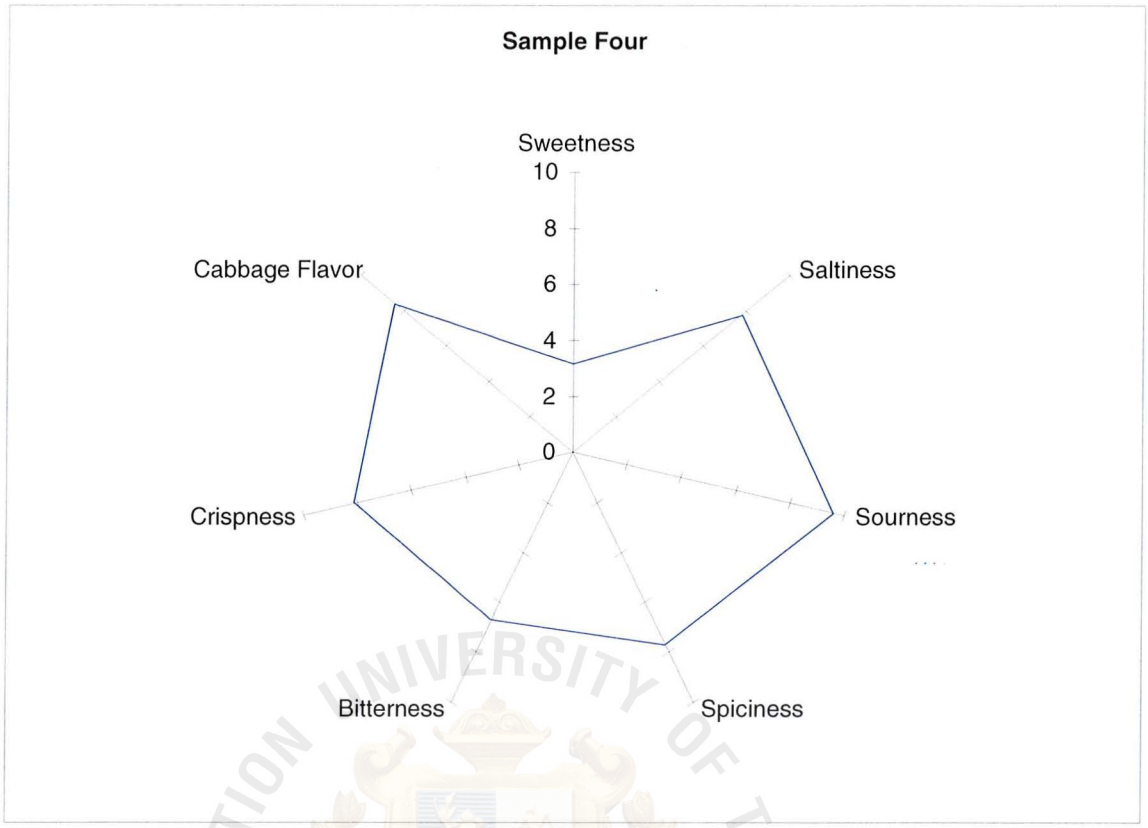

Figure8: Flavor profile of sample four.

Sample four, from Figure 8, was alike sample three in the sense that there was no major difference between each attribute. The scores are as follow; 3.156, 7.778, 9.589, 7.664, 6.714, 8.143 and 8.357 from sweetness to cabbage flavor. Sample four was the reddest out of the four samples, but it wasn't too spicy. It was also the bitterest one out of the four samples. Most of the panelists thought sample four was too bitter, but they liked that it was crispy and juicy. Some mentioned that it was too dark-red, and that they preferred the Kimchi to be in brighter shade of red.

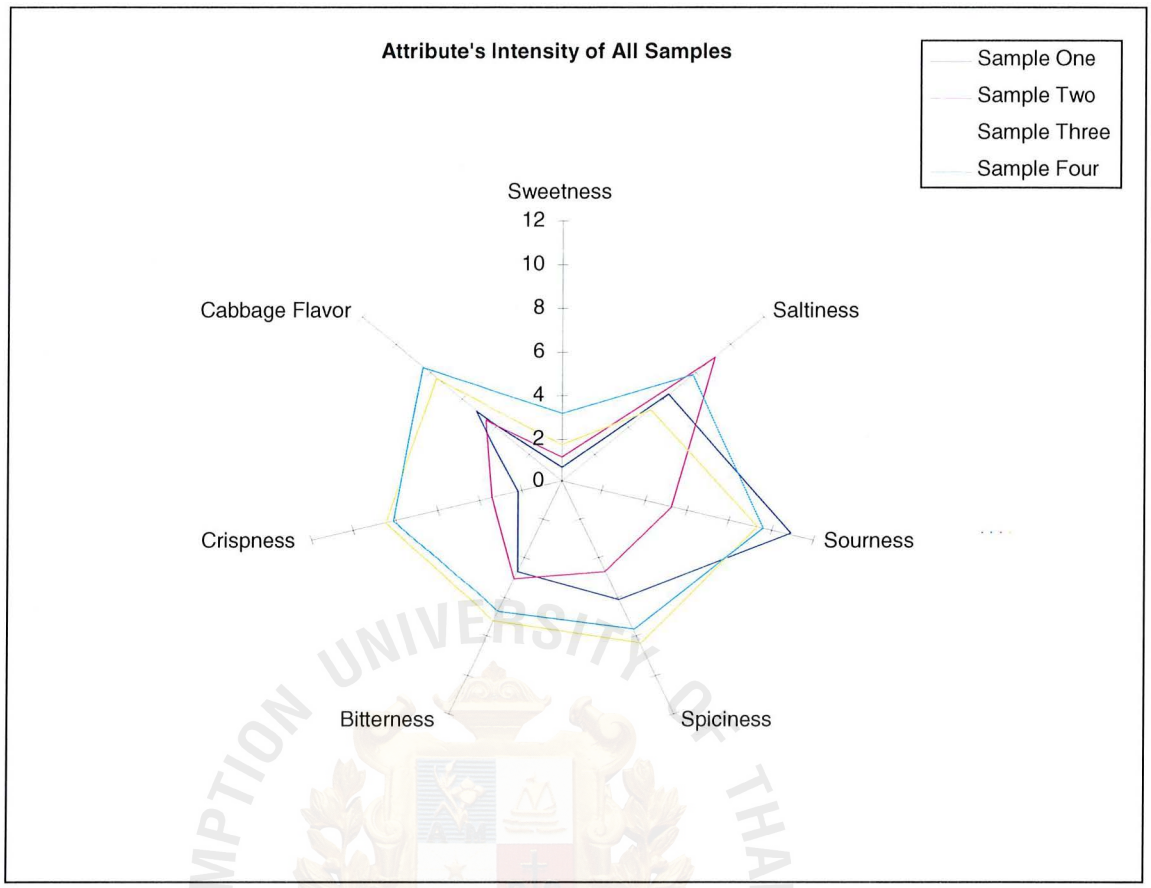

Figure9: Flavor profile of four samples.

The sensory profiles of Thai made Kimchi were shown in same pattern in low bitterness, crispness and cabbage flavor when compared with Korean made Kimchi, see Figure 9. Sourness and spiciness of sample one were higher than sample two, meanwhile, saltiness of sample two was higher than sample one. The higher salt content of sample two might be suppressed the growth of LAB and lead to lower intensity in other attributes. Sample one gained the highest score of sourness attributes. The higher acid production might lead to over-ripening of Kimchi and related to lower in crispness and cabbage flavor. The less bitterness might be the difference in pickling method or difference in ingredients and raw materials used.

The sensory profile of Korean made Kimchi were same pattern in many attributes except saltiness. Even saltiness of sample four was higher but other attributes still gained high score. The higher saltiness of Thai made Kimchi lead to poor fermentation but Korean made Kimchi showed better fermentation. This was the Korean skill on Kimchi production while Thai people had less. Spiciness and bitterness of Korean made Kimchi were higher than Thai made Kimchi. This might be difference in ingredients and raw materials used.

Table6: Ranking Test of four commercial Kimchi samples

Rank	Sample	Average score
1	1	3.43
2	3	1.14
3	4	3.14
4	2	2.29

Table7: Sensory Evaluation of four commercial Kimchi samples.

Sample	Sourness	Saltiness	Spiciness	Crispness	Bitterness	Overall
1	6.86 ± 1.07^{a}	7.00 ± 0.00^{a}	7.00 ± 0.82^{a}	5.00 ± 0.82^{b}	7.71 ± 0.49^{a}	7.43 ± 0.53^{a}
2	3.86 ± 0.90^{c}	3.57 ± 0.53^{d}	4.43 ± 0.53^{c}	4.71 ± 0.49^{b}	7.00 ± 0.00^{ab}	4.57 ± 0.53^{c}
3	5.86 ± 0.69^{b}	5.43 ± 0.53^{b}	7.29 ± 0.49^{a}	8.00 ± 0.00^{a}	6.43 ± 0.53^{b}	7.29 ± 0.49^{a}
4	4.71 ± 0.49^{c}	4.29 ± 0.76^{c}	6.14 ± 0.90^{b}	7.57 ± 0.53^{a}	5.43 ± 1.13^{c}	6.43 ± 0.53^{b}

The preference and ranking test was conducted to determine the most preferable sample. Ranking test (table 6) showed the most preferable sample in general while preference test (table 7) showed each sample's characteristic and their preference

There were significant differences in all attributes among samples ($p< 0.05$). Sample one had the highest preference for all attributes except crispness. Sample three was the second preferred, sample four was the third preferred and sample two was the least preferred. The moderate salt content was more preferred in sample one and three. Tested panelists moderately liked salt attributes in sample three. The highest salt intensity, the lowest preference which were found in sample two and followed by sample four. Tested panelists disliked high salt content which found in sample two and four. Sample three had lower salt intensity and gained neither like or dislike by tested panelists. But the higher score of sourness, the higher preference score. In sourness attributes, tested panelists liked slightly to moderately in sample one while neither liked or disliked in sample three. They disliked slightly to moderately in sample four and two, respectively. The intensity of spiciness of sample three and four were similar but tested panelists liked moderately in sample three and liked slightly in sample four. Even the spiciness ranking of sample one was the third position but tested panelists liked moderately as the same in sample three. This might be types of chili pepper in sample one was familiar with them. Sample two had low spiciness intensity and was disliked slightly by the tested panelists. The preference test of bitterness and crispness were opposite. The higher intensity of bitterness gained the lower preference. The lower intensity of crispness gained the higher preference. Crispness attribute replied to

freshness and moderate fermentation or acid production. The higher sourness of sample one affected to the lower crispness attribute. But crispness attribute was lowered by the effects of salt. The higher saltiness of sample two affected to the lower crispness attribute.

These results revealed the major preference attribute were high in sourness, spiciness and crispness, moderate in saltiness, low in bitterness attributes among Thai people.

Packaging of the kimchi also has an effect on the kimchi taste or the LAB found in each sample. Sample one was packaged in a glass bottle with tight seal, sample two was packed in a plastic bag, tighten with rubber band then insert into another plastic bag. Sample three was packed in a plastic bag, and then put in a plastic box. Sample four was packed in a plastic box, same type as sample three. The anaerobic condition created within the container promotes the growth of anaerobic LAB.

In microbiological aspect, sample one had ratio of 9:11 (*Lactobacillus spp. : Lactococcus spp.*). The difference between the two genuses is not very much, which is quite different from other three samples. Sample two had ratio of 16:4 *Lactobacillus spp. : Lactococococus spp.*), higher ratio of *Lactobacillus spp.* while sample one had higher ratio of *Lactococcus spp*. Sample three also had higher ratio of *Lactococcus spp.* but with larger difference of 4: 15: 1 (*Lactobacillus spp. : Lactococcus spp. : Streptococcus spp.*) . Sample four had the ratio of 12: 7: 1 (*Lactobacillus spp. : Lactococcus : Leuconostoc spp.*).

This suggests the possibility that sample four was fresh because *Leuconostoc spp.* is often found in the early fermentation process, hence its high crispiness. Streptococcus is also usually found in the early stage of fermentation which could explain the crispiness of sample three as well.

As the results suggest, the Korean made samples (sample three and four) were very much alike, high intensity in most of the attributes which reflects freshness of the sample (especially crispness). Together with the fact that sample three and sample four had suspected culture of *Streptococcus spp.* and *Leuconostoc spp.* which are usually found in the early stage of fermentation, the possible explanation for this situation would be that the Kimchi samples made by Korean producers were fresher than those of Thai made samples (sample one and two). Sample three and four were purchased from Korean restaurant and from Korean convenient store, in respective order. Which means that the Kimchi from these two sources were likely to be fresher than the Thai made Kimchi. Thai made Kimchi samples were purchased from supermarket which requires transportation from the producers to the supermarket, while sample three and four were packaged and stored in cool condition and put on sale immediately.

These results also suggests that Korean method of pickling Kimchi is different from Thai method since the sensory profile of the Korean made and Thai made Kimchi were different. The comments from the panelists on the appearance and taste of the two groups of Kimchi also agree with the sensory profile results. Thai made Kimchi were not as red in color, the juice was not as thick and the vegetable was not as crispy as Korean made Kimchi samples. If Thai producers would improve their pickling, storing and transportation method, the freshness, crispness and overall quality of the Kimchi could be improved. Thai consumers like tasty and sour Kimchi, but from the results obtained, they also like the Kimchi to be crispy which is what Thai producers still lack.

Suggestions

Further study could be done with more number of samples, study more number of attributes (tastes in Kimchi) and more thorough study in microbiological aspect to find possible relations between microbiological aspect and physical characteristics of Kimchi.

Conclusion

- Sample one was the most preferable sample, the LAB selected had the ratio of 9:11 (*Lactobacillus spp.: Lactococcus spp.*)
- Sample two was the least preferred, the LAB selected had the ratio of 16:4 (*Lactobacillus spp.: Lactococcus spp.*)
- Sample three was preferred second ranking position, the LAB selected had the ratio of 4: 15: 1 (Lactobacillus *spp.: Lactococcus spp.: Streptococcusspp.*)
- Sample four was preferred third ranking position, the LAB selected had the ratio of 12: 7: 1 (*Lactobacillus spp.: Lactococcus spp.: Leuconostoc spp.*)
- Kimchi produced by Korean producer was crispier, spicier, had more cabbage flavor and bitterer.
- Thai consumer preferred spicy, crispy, a little salty and quite sour Kimchi, but should low in bitterness.

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Appendix A

Microorganism Study in Four Brands of Kimchi

Sample One (Traditional Kim Chi ®)

Table8: LAB identification from sample one.

No.	Gram	Catalase	Cell Morphology	Gas	0.5% NaCl	5% NaCl	Suspected Culture
1.	+	-	Coccus,Pairs	-	-	-	*Lactococcus spp.*
2.	+	-	Rod	-	-	-	*Lactobacillus spp.*
3.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
4.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
5.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
6.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
7.	+	-	Rod	-	-	-	*Lactobacillus spp.*
8.	+	-	Rod	-	-	-	*Lactobacillus spp.*
9.	+	-	Rod	-	-	-	*Lactobacillus spp.*
10.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
11.	+	-	Rod	-	-	-	*Lactobacillus spp.*
12.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
13.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
14.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
15.	+	-	Rod	-	-	-	*Lactobacillus spp.*
16.	+	-	Rod	-	-	-	*Lactobacillus spp.*
17.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
18.	+	-	Rod	-	-	-	*Lactobacillus spp.*
19.	+	-	Rod	-	-	-	*Lactobacillus spp.*
20.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*

Note: * = Weakly + or -, hard to identify.

Ratio: 9:11 (*Lactobacillus spp.* : *Lactococcus spp.*)

Sample Two (Miss Kimchi ®. Uniriver Global Co., Ltd. Thailand.)

Table9: LAB identification from sample two.

No.	Gram	Catalase	Cell Morphology	Gas	0.5% NaCl	5% NaCl	Suspected Culture
1.	+	-	Rod	-	-	-	*Lactobacillus spp.*
2.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
3.	+	-	Coccus, Short Chains	-	-	-	*Lactococcus spp.*
4.	+	-	Rod	-	-	-	*Lactobacillus spp.*
5.	+	-	Rod	-	-	-	*Lactobacillus spp.*
6.	+	-	Rod	-	-	-	*Lactobacillus spp.*
7.	+	-	Rod	-	-	-	*Lactobacillus spp.*
8.	+	-	Rod	-	-	-	*Lactobacillus spp.*
9.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
10.	+	-	Rod	-	-	-	*Lactobacillus spp.*
11.	+	-	Rod	-	-	-	*Lactobacillus spp.*
12.	+	-	Rod	-	-	-	*Lactobacillus spp.*
13.	+	-	Rod	-	-	-	*Lactobacillus spp.*
14.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
15.	+	-	Rod	-	-	-	*Lactobacillus spp.*
16.	+	-	Rod	-	-	-	*Lactobacillus spp.*
17.	+	-	Rod	-	-	-	*Lactobacillus spp.*
18.	+	-	Rod	-	-	-	*Lactobacillus spp.*
19.	+	-	Rod	-	-	-	*Lactobacillus spp.*
20.	+	-	Rod	-	-	-	*Lactobacillus spp.*

Note: * = Weakly + or -, hard to identify.

Ratio = 16:4 (*Lactobacillus spp.* : *Lactococcus spp.*)

Sample Three (Hanpong E & C®)

Table10: LAB identification from sample three.

No.	Gram	Catalase	Cell Morphology	Gas	0.5% NaCl	5% NaCl	Suspected Culture
1.	+	-	Coccus, Pairs	-	+	-	*Streptococcus spp.*
2.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
3.	+	-	Rod	-	-	-	*Lactobacillus spp.*
4.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
5.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
6.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
7.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
8.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
9.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
10.	+	-	Rod	-	-	-	*Lactobacillus spp.*
11.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
12.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
13.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
14.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
15.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
16.	+	-	Rod	-	-	-	*Lactobacillus spp.*
17.	+	-	Rod	-	-	-	*Lactobacillus spp.*
18.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
19.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
20.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*

Note: * = Weakly + or -, hard to identify.

Ratio = 4: 16: 1 (*Lactobacillus spp.: Lactococcus spp.: Streptococcus spp.*)

Sample Four (Wuree Restaurant®)

Table11: LAB identification from sample four.

No.	Gram	Catalase	Cell Morphology	Gas	0.5% NaCl	5% NaCl	Suspected Culture
1.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
2.	+	-	Rod	-	-	-	*Lactobacillus spp.*
3.	+	-	Rod	-	-	-	*Lactobacillus spp.*
4.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
5.	+	-	Rod	-	-	-	*Lactobacillus spp.*
6.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
7.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
8.	+	-	Coccus, Pairs	-	-	-	*Lactococcus spp.*
9.	+	-	Rod	-	-	-	*Lactobacillus spp.*
10.	+	-	Rod	-	-	-	*Lactobacillus spp.*
11.	+	-	Coccus, Pairs	+	-	-	*Leuconostoc spp.*
12.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
13.	+	-	Rod	-	-	-	*Lactobacillus spp.*
14.	+	-	Rod	+	-	-	*Lactobacillus spp.*
15.	+	+	Rod	+	-	-	*Lactobacillus spp.*
16.	+	-	Rod	-	-	-	*Lactobacillus spp.*
17.	+	-*	Coccus, Pairs	-	-	-	*Lactococcus spp.*
18.	+	-	Rod	-	-	-	*Lactobacillus spp.*
19.	+	-	Rod	+	-	-	*Lactobacillus spp.*
20.	+	-	Rod	-	-	-	*Lactobacillus spp.*

Note: * = Weakly + or -, hard to identify.

Ratio= 12: 7: 1 (*Lactobacillus spp.: Lactococcus spp.: Leuconostoc spp.*)

Appendix B

Media Formula

de Man, Rogosa and Sharpe Media (MRS) (1L solution)

Peptone	10g
Beef extract	10g
Yeast extract	5g
Glucose	20g
Tween80	1ml
K_2HPO_4	2g
Sodium acetate	5g
Tri-ammonium citrate	2g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	0.2g
Distilled water	1L

Water 1000 ml with 55.5 g of MRS, autoclave at 121℃ for 15 mins.

Tryptic Soy Broth (TSB) (1L solution)

Tryptein	17g
Soy peptone	3g
Sodium Chloride	5g
Dipotassium phosphate	2.5
Dextrose	2.5

Water 1000 ml with TSB 30 g, autoclave at 121℃ for 15 min.

TSB with 0.5% Salt

Tryptein	17g
Soy peptone	3g
Sodium Chloride	5g
Dipotassium phosphate	2.5
Dextrose	2.5
NaCl	0.5g

Water 1000 ml with TSB 30 g, autoclave at 121℃ for 15 min.

TSB with 5% Salt

Tryptein	17g
Soy peptone	3g
Sodium Chloride	5g
Dipotassium phosphate	2.5
Dextrose	2.5
NaCl	5g

Water 1000 ml with TSB 30 g, autoclave at 121℃ for 15 min.

Appendix C

Sensory Questionnaires

1. Describing Tastes

Name:

I.D.:

Date:

Describe the taste of the given samples of Kimchi. Also consider the first impression, while chewing and the taste after swallowing. Rinse your mouth with water before tasting another sample, crackers are also provided. Try to use adjectives to describe your perceptions, e.g. spicy, sweet.

Sample no....

_____
_____
_____

Sample no.....

_____
_____
_____

Sample no.....

_____
_____
_____

Sample no.....

_____
_____
_____
_____

2. Preference and Ranking Test

Name _____ Date _____

Part I Instruction

1. Please rinse your mouth with water before testing. You may rinse again at anytime during the test you need to
2. Please test the sample in the order presented, **from left to right**
3. Evaluate hedonic scale in each attribute of sample by using the following number:

1 = Dislike Extremely
2 = Dislike Very Much
3 = Dislike Moderately
4 = Dislike Slightly
5 = Neither Like nor Dislike
6 = Like Slightly
7 = Like Moderately
8 = Like Very Much
9 = Like Extremely

Attributes	**547**	**274**	**355**	**172**
Sourness				
Saltiness				
Spiciness				
Crispness				
Bitterness				
Overall				

Part II Instruction

1. Please rank the samples from most preferred to least preferred using the following number: 1 = most preferred, 4 = least preferred

Sample Rank (1 to 4)

1. _____ 2. _____ 3. _____ 4. _____

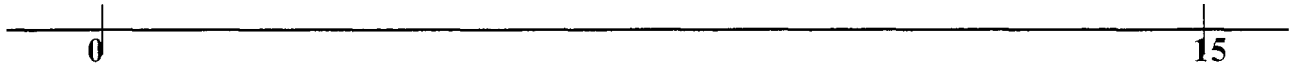
3. Intensity Test

Name: _____ **I.D.:** _____

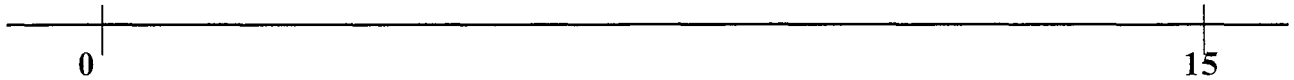
Product: Kimchi (intensity) **Date:** _____

Instructions: Taste each of the samples and rank on the line according to intensity of each attribute.

Spiciness



Cabbage Smell

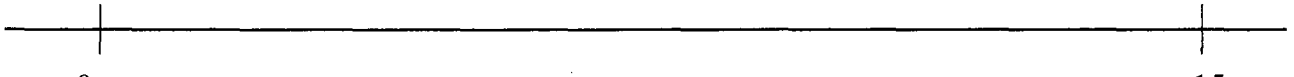


Crispness

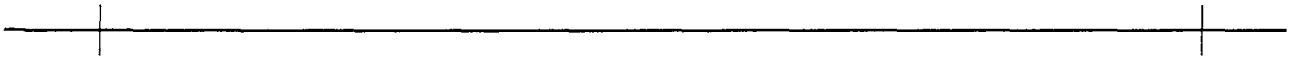
Bitterness

Sweetness

Saltiness



Sourness



4. Statistic analysis of Preference Test

Tests of Between-Subjects Effects

Dependent Variable: Bitterness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	19.571[a]	3	6.524	14.421	.000
Intercept	1235.571	1	1235.571	2731.263	.000
treatment	19.571	3	6.524	14.421	.000
Error	10.857	24	.452		
Total	1266.000	28			
Corrected Total	30.429	27			

a. R Squared = .643 (Adjusted R Squared = .599)

Bitterness

Duncan[a,,b]

treatment	N	Subset		
		1	2	3
4.00	7	5.4286		
3.00	7		6.4286	
2.00	7		7.0000	7.0000
1.00	7			7.7143
Sig.		1.000	.125	.058

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .452.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable: Crispness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	60.964[a]	3	20.321	68.280	.000
Intercept	1118.893	1	1118.893	3759.480	.000
treatment	60.964	3	20.321	68.280	.000
Error	7.143	24	.298		
Total	1187.000	28			
Corrected Total	68.107	27			

a. R Squared = .895 (Adjusted R Squared = .882)

Crispness

Duncan[a,,b]

treatment	N	Subset 1	Subset 2
2.00	7	4.7143	
1.00	7	5.0000	
4.00	7		7.5714
3.00	7		8.0000
Sig.		.337	.155

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .298.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable : Saltiness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47.000[a]	3	15.667	54.833	.000
Intercept	720.143	1	720.143	2520.500	.000
treatment	47.000	3	15.667	54.833	.000
Error	6.857	24	.286		
Total	774.000	28			
Corrected Total	53.857	27			

a. R Squared = .873 (Adjusted R Squared = .857)

Saltiness

Duncan[a,,b]

treatment	N	Subset			
		1	2	3	4
2.00	7	3.5714			
4.00	7		4.2857		
3.00	7			5.4286	
1.00	7				7.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .286.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable: sourness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.107[a]	3	12.036	18.054	.000
Intercept	792.893	1	792.893	1189.339	.000
treatment	36.107	3	12.036	18.054	.000
Error	16.000	24	.667		
Total	845.000	28			
Corrected Total	52.107	27			

a. R Squared = .693 (Adjusted R Squared = .655)

sourness

Duncan[a,,b]

treatment	N	Subset		
		1	2	3
2.00	7	3.8571		
4.00	7	4.7143		
3.00	7		5.8571	
1.00	7			6.8571
Sig.		.061	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .667.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable:Spiciness

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34.714[a]	3	11.571	23.143	.000
Intercept	1081.286	1	1081.286	2162.571	.000
treatment	34.714	3	11.571	23.143	.000
Error	12.000	24	.500		
Total	1128.000	28			
Corrected Total	46.714	27			

a. R Squared = .743 (Adjusted R Squared = .711)

Spiciness

Duncan[a,,b]

treatment	N	Subset		
		1	2	3
2.00	7	4.4286		
4.00	7		6.1429	
1.00	7			7.0000
3.00	7			7.2857
Sig.		1.000	1.000	.457

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .500.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable: Overall

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.286[a]	3	12.095	44.174	.000
Intercept	1157.143	1	1157.143	4226.087	.000
treatment	36.286	3	12.095	44.174	.000
Error	6.571	24	.274		
Total	1200.000	28			
Corrected Total	42.857	27			

a. R Squared = .847 (Adjusted R Squared = .828)

Overall

Duncan[a,,b]

treatment	N	Subset 1	Subset 2	Subset 3
2.00	7	4.5714		
4.00	7		6.4286	
3.00	7			7.2857
1.00	7			7.4286
Sig.		1.000	1.000	.614

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .274.
a. Uses Harmonic Mean Sample Size = 7.000.
b. Alpha = .05.