Food microbiology

Science, Biology

CHAPTER I INTRODUCTION 1. 1 BackgroundFoodis one of human sources of calorie, protein, fats, and nutrition. Yet, because of the highly nutritious content, food is susceptible to growth of microorganisms. By the presence of microorganisms in food, the food is more likely to have shorter shelf life. Thus, mostly it is resolved by the addition of antimicrobial substances to food, such as condiments and preservatives. Condiments and preservatives could inhibit the growth of microorganisms or even destroyed them, as they have antimicrobial agents.

Some examples of condiments and preservatives are ginger, clove, sodium benzoate, garlic, and coriander. The factors contributing in the effectiveness of condiments and preservatives in inhibiting the growth of microorganisms are the concentration of the antimicrobial, temperature, characteristic of the microorganisms and food, storage time. As different types of microorganisms have different resistance toward the antimicrobial substance, it is important to understand the characteristic of the microorganisms towards the antimicrobial agent.

There are two types of resistance: intrinsic resistance and acquired resistance. There are some methods of observing the capability of the condiments in inhibiting the growth of microorganisms. In the experiment, the method used is well diffusion method, which used different type of condiments and added into holes of agar, where by the inhibition zone by the condiments could be observed. The larger the inhibition zone, the more effective the condiment was in inhibiting the growth of microorganisms. 1. 2 Objectives By conducting the experiment, students are expected to learn and observe he effectiveness of antimicrobial substance consisted in

condiments and potassium sorbate towards the growth of microorganisms. CHAPTER II LITERATURE REVIEW 2. 1 Antimicrobials Referring to Volk and Wheeler (1993), antimicrobials are the substances that are used to inhibit or kill pathogenic or non-pathogenic microbes. Antimicrobials are also often used as sanitizers and preservatives. Sanitizer is an agent to decrease the amount of microorganisms to the acceptable level. It is generally used in food processing equipments. Antimicrobials are grouped into natural antimicrobials and chemical antimicrobials.

According to Fardiaz (1992), antimicrobials may be microstatic, which is inhibitory to the growth of microbes, and microcidal, which means able to kill microbes. Fruit extract is one most susceptible food materials and thus, is often added with preservatives, especially chemical preservatives, stored at low temperature, or pasteurized. Pasteurization is a heating process at 63? C for thirty minutes. This process is aimed to preserve the stability of the food materials (Buckle et al. , 1987). The growth of microbes can be controlled by using various methods, namely physical method, chemical method, and immunological method.

The control of microorganism growth is performed to kill the microbes, to inhibit the microbes, and to destroy the microbes. Physical controls can be performed by sterilization with heating or radiation and filtering. Chemical controls can be carried out by using chemical antimicrobial compounds, such as disinfectants and preservatives. Whereas immunological controls can be performed by vaccination (Batzing, 2002). 2. 2 Active Antimicrobial Compounds in Spices 2. 2. 1 Garlic Garlic come from the onionfamilyand are an erect biennial herb, which grow annually. It has irregular roots,

condensed, flattened step and narrow and has lat leaves. Garlic’s bulb consists of 6 to 35 bulblets called cloves which enclosed in a thick whitish, glistening, and transparent covering (Anonym1, 2000). According to Ankri and Mirelman (1999), garlic or Allium sativum or lahsoon in Indian name is an edible plant, which has been generating a lot of interest as a medicinal panacea and a cure for a wide variety of different conditions since the human history has begun. It is reported to have anticancer effects and to reduce blood lipids in human body. Figure 2. 1 shows the approximate composition of fresh garlic. Figure 2. The approximate composition of fresh garlic Source: Ahmad (1996) The active compound found in garlic cloves which have an unusual concentration of sulfur-containing compounds (1-3%) is called allicin. It is a volatile molecule, which is poorly miscible in aqueous solution, and has a strong typical odor of crushed garlic. Chemically, allicin can be synthesized by mild oxidation of diallyl disulfide as presented in Figure 2. 1. It is to be noticed in Figure 2. 2 that there is a compound called alliin which is a stable precusor, that later will be converted to allicin by enzyme called alliinase present in cloves too.

Moreover, alliinase is surprisingly found in large amounts in cloves, which is about 10% of the total protein content. Practically, allicin is produced when garlic cloves are cut into or crushed (Ankri and Mirelman, 1999) Figure 2. 2 Generation of allicin in a garlic clove Source: Ankri and Mirelman (1999) According to Ankri and Mirelman (1999), there are several biological activities in allicin such as its activity as an antioxidant and its ability to attack the sulphur (SH) groups in enzymes and proteins while modifying their activities as well.

Furthermore, allicin can rapidly penetrate into cells through the cell membranes. In its pure form, allicin has been reputed to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, for instance Escherichia coli that is known to be a multidrug-resistant enterotoxicogenic strains, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, and Clostridium. It also has antifungal activity that prevents the formation of mycotoxins such as the aflatoxin of Aspergillus parasiticus.

Allicin has shown the anticandida activity towards and is effective against the group species of Candida, Cryptococcus, Trichophyton, Epidermophyton, and Microsporum at only low concentration since it inhibits both the germination of spores and the formation of hyphae. Referring to Dobre et al. (2011), allicin can also attack Aspergillus, Fusarium, and Penecillium species, which are responsible for food poisoning and food decay.

The main mechanism of the antimicrobial activity in allicin is the inhibition of certain thiol-containing enzymes in the microorganisms by the super fast reaction of thiosulfinates with thiol groups, such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase that later will affect the essential metabolism of cysteine proteinase activity. The reason why microbial cells are highly sensitive to allicin is probably because the lack of glutathione (thiol molecules such as trypanothione) which results of lack of the ability to reactivate the pivotal SH-enzymes that are thiolated by allicin (Ankri and Mirelman, 1999). . 2. 2 Coriander Coriander or Coriandrum sativum L. is originated from the Mediterranean region and has the appearance of flat shape in the one side while slightly pointed shape is found

in the other side. Coriander seed has various lengths between 3 to 5 mm with brown color in ripe state (Sarkar, 2012). Accodring to Rattanachaikunsopon and Phumkhachorn (2010), it has been traditionally used as an analgesic, aphrodisiac, antirheumatic, anti-inflammatory, diuretic, antispasmodic, circulatory stimulant, and antidiabetic. Beside that, coriander is known to have effect in lowering cholesterol. Table 2. shows the composition of coriander seed. Table 2. 3 The coriander seed composition in 1 tsp Total Fat 0. 9g 1% Saturated Fat 0. 00g 0% Monounsaturated Fat 0. 7g Polyunsaturated Fat 0. 1g Cholesterol 0mg 0% Sodium 2mg 0% Total Carbohydrates 2. 7g 1% Dietary Fiber 2. 1 g 8% Protein 0. 6g Vitamin A 0% Vitamin C 2% Calcium 4% Iron 5% Source: Sarkar (2012) The precise volatile compounds acting as antimicrobial compounds have not been examined clearly till now, although there are some volatile compounds suggested to be the antimicrobial compound inside coriander which are (2E) – hexenal and (3E) – hexenal (Kubo et al. , 2004).

They are reported to have antimicrobial activity against Gram-negative and Gram-positive bacteria such as Escherichia coli O157: H7, Listeria monocytogenes, and Staphylococcus aureus, which are foodborne pathogenic bacteria. It also exhibits bactericidal activity and is reported to have an effective antibacterial. The mechanism of the activity of coriander is membrane damage causing cell death to the bacteria (Silva et al. , 2011). However, according to Uma et al. (2009), coriander seems to not having an effective antifungal activity and its activity toward yeast have not yet been examined further. 2. 2. 3 Black Pepper

Black pepper (Pipper nigrum) is a condiment that has been used since ancient times and is native to India. Black pepper is useful for treatment of various sicknesses such as vertigo, asthma, fever and also cholera. The volatile oil of black pepper has been shown to have antimicrobial activity as well (Karsha and Lakshmi, 2009). The major antimicrobial compund found in black pepper are monoterpenes and sesquiterpenes (Davidson et al. , 2005). According to Karsha and Lakshmi (2009), black pepper shows strong antimicrobial activity against gram-positive bacteria such as Staphylococcus aureus, Bacillus cereus and Streptococcus faecalis.

Gram-negative bacteria such as Pseudomonas aeruginosa, Salmonella typhi and Escherichia coli are affected as well, although the effect on gram-positive bacteria is better. The mechanism of the antimicrobial activity appears to be loss of control over cell membrane permeability. According to Singh et al. (2004), black pepper has antifungal activity as well as it is effective in stopping the growth of molds such as Fusarium graminearum. Black pepper is also shown to be able to inhibit the growth of yeast such as Candida albicans (Joe et al. , 2009). 2. 2. 4 Potassium Sorbate

Potassium sorbate are currently one of the most widely used preservative and can be used to preserve foods, animal feeds, pharmaceuticals, and cosmetics. Potassium sorbate may be manufactured as a powder or granules and has an antimicrobial potency of 74% compared to sorbic acid. The molecular weight of potassium sorbate is 150. 22 and is the most soluble form of sorbate compared to the others, such as calcium sorbate and sodium sorbate. Besides good solubility, potassium sorbate is also has good stability

and easy to manufacture, making it the most used form of sorbate in food industry.

Sorbate is very effective when used against bacteria, molds, and yeasts. Yeasts inhibited by sorbate are Brettanomyces, Candida, Cryptococccus, Debaryomyces, Endomycopsis, Hansenula, Kloeckera, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Torulaspora, Torulopsis, and Zygosaccharomyces. Molds species inhibited by sorbate are Alternaria, Ascochyta, Ascosphaera, Aspergillus, Botrytis, Cephalosporium, Chaetomium, Cladosporium, Colletotrichum, Cunninghamella, Curvularia, Fusarium, Geotrichum, Gliocladium, Helminthosporium,

Heterosporium, Humicola, Monilia, Mucor, Penicillium, Phoma, Pepularia, Pestalotiopsis, Pullularia, Rhizoctonia, Rhizopus, Rosellinia, Sporotrichum, Trichoderma, Truncatella, Ulocladium, and others. While for bacteria, the species inhibited are Acetobacter, Achromobacter, Acinetobacter, Enterobacter, Aeromonas, Alcaligenes, Alteromonas, Arthrobacter, Bacillus, Campylobacter, Clostridium, Escherichia, Klebsiella, Lactobacillus, Micrococcus, Moraxella, Mycobacterium, Pediococcus, Proteus, Pseudomonas, Salmonella, Serratia, Staphylococcus, Vibrio, Yersinia, and others (Davidson et al. , 2005). 2. 3

Mechanism of Antimicrobials There exist some mechanisms of antimicrobial activity in inhibiting the growth of microbes. The antimicrobials are classified into: cell wall destructor, cell wall permeability intervention, destructor of proteins, nucleic acids, and enzymes, antimetabolites, inhibitors of nucleic acid synthesis, and cell plasmolysis (Fardiaz and Betty, 1989). Referring to Fardiaz and Betty (1989), the broken cell wall of microorganisms will cause

the cell content to depart from the cell and thus, inhibit the cell metabolisms. Severe destructions on the cell may cause the death of the cell.

Lysozyme is one of the enzymes that are able to destruct the cell wall of Gram-positive bacteria. An enzyme produced by a bacterium might be able to inhibit the growth of other microbes. There is also a type of antimicrobials that is able to inhibit the formation of cell wall materials. These cells that do not have cell wall are called protoplast. Protoplast is very easily broken, except when placed in isotonic medium. Penicillin and cycloserin are examples of compounds that retard the formation of peptidoglycan in developing cell. Gram-positive bacteria are susceptible to penicillin as they have lots of peptidoglycans.

The breaking of plasma membrane and leakage of cell content will inhibit the microorganism growth or even kill the microorganisms. The implication if the cell destruction is the enzymes will not be able to function properly in the cell metabolism. However, antimicrobials that intervene the cell wall permeability are rarely used in food industries (Fardiaz and Betty, 1989). Compounds destructing the protein and nucleic acid are able to destroy cells. This type of destruction is unfixable. For instance, certain amount of alcohol and sodium chloride are able to denaturate the proteins.

Those two compounds are often used in food industries (Fardiaz and Betty, 1989). Antimetabolites are the compounds that are similar to natural metabolites. These antimetabolites will interrupt the metabolisms in the cell. The interruption of the metabolisms might retard the cell growth or even kill the cell. Furthermore, the synthesis of DNA and RNA may be inhibited by some antimicrobial compounds, namely the compounds that are able to

retard the formation of nucleic acid arrangement and compounds that inhibit the nucleic acid polymerization (Fardiaz and Betty, 1989).

Plasmolysis or the breakage of a cell is caused by the high plasmolysis pressure. Materials that are often used in food to promote the cell plasmolysis are salt and sugar. Salt and sugar are considered to be able to increase the osmotic pressure in food materials therefore plasmolysis takes place (Fardiaz and Betty, 1989). 2. 4 Antimicrobial Sensitivity towards Microbial Defense Microorganisms have the ability to resist some types of antimicrobial substances. There are two types of resistance in microorganisms toward antimicrobial agents, such as intrinsic or natural resistance and acquired resistance.

The antimicrobial agents in intrinsic resistance could not affect the microorganisms, as they have no target sites, which are the microorganisms, to affect. In contrary, in microorganisms that don’t have the intrinsic resistance, the antimicrobial substance could gradually enter the microbial cell and affecting the activity of the microorganisms, as the microorganisms’ cell membrane have lower permeability to antimicrobial substance (Sosa et al. , 2010). Furthermore, in acquired resistance, the microorganisms are naturally vulnerable, as they need specific ways for preventing to be affected by the antimicrobial substances.

Some examples of the specific ways are the presence of enzyme that has the ability to inactivate the antimicrobial agent, or alternative enzyme that has the ability to inhibit the activity of antimicrobial agent. Then, it also happens when there is mutation and post-transcriptional and posttranslational in the microorganisms that are the target of the antimicrobial agent. Thus, these

will reduce the binding of the antimicrobial agent (Sosa et al. , 2010). Every microorganism has different sensitivity towards different type antimicrobial agents. Coriander (Coriandrum sativum L. is mostly used as seasoning condiment. According to Kubo et al. (2004), in the leaves of coriander, there are volatile oils that are suggested to have antimicrobial properties against food born pathogen, such as Salmonella species, which are gram-negative bacteria. Black pepper (Piper nigrum L.) is used mostly in food as seasoning condiments. The aqueous and ethanolic extract of black pepper is very effective for inhibiting antibacterial activity agains penicillin G resistant strain of Staphylococcus aureus, Bacillus cereus and Bacillus subtilis (Chaudhry and Tariq, 2006).

According to Karsha and Lakshmi (2010) experiment, gram-positive bacteria were more susceptible towards antimicrobial in black pepper than gramnegative bacteria. In gram-positive bacteria, the most susceptible towards antimicrobial in black pepper is Staphylococcus and followed by Bacillus and Streptococcus. Furthermore, among gram-negative bacteria, the most susceptible towards antimicrobial in black pepper is Pseudomonas, followed by E. coli, Klebsiella and Salmonella. Garlic (Allium sativum) is commonly used for antifungal, antiviral, antibacterial, antihelmantic, antiseptic and anti-infamatory.

Garlic extract is effevtive in inhibiting the microbial activity of both gram-positive and gramnegative bacteria. Several examples of the gram-negative bacteria are E. coli, Salmonella species and Citrobacter Enterobacter, Pseudomon, Kilabsella) and the gram-positive bacteria are Pseudomonas aeruginosa, Salmonella typhi, Proteus, spp. , Staphylococcus aureus, S.

pneumonia Group A streptococcus and Bacillus anthrax (Daka, 2011; Durairaj, 2009). Potassium sorbate is used as preservative in food as the sorbic acid is more effective than benzoic acid in preserving food. The preservation process occurs in higher pH.

It is effective to inhibit the microbial activity of Pseudomonas species, which are categorized as gram-positive bacteria (Beuchat, 1980). Mostly, gram-positive bacteria are more sensitive towards antimicrobial agent than gram-negative bacteria (Torrence and Isaacson, 2003). 2. 5 Factors Affecting Microorganism Strength towards Antimicrobial Compounds The resistance of microbes towards particular antimicrobial compound is dependent on several factors that contained in that particular microbe such as the cell wall, protein content, nucleic acid, and membrane cell (Lay, 2002).

There are several types of microbes that have cell walls, and an antimicrobial compound that can affect the mechanism of the cell wall that influence the microbial resistance. Anti-microbial compounds may interfere with the work of the cell wall as well as peptidoglycan biosynthesis, which is the process in prokaryotic cell wall structure construction. Disruption of peptidoglycan may affect the resistance and sensitivity of microbes to changes in osmotic perspective (Lay, 2002). Microbes also contain nucleic acids.

The nucleic acid can be affected by antimicrobial compunds where the enzymes that are going to be used for the synthesis of nucleic acid are inhibited. The example is rifampin where it binds the enzyme RNA polymerase, quinolone as well as binding enzyme DNA gyrase (Lay, 2002). The resistance of microbes can be affected by the metabolism of the

microbes. Enzyme that catalyzes the synthesis of essential molecules can be inhibited by antimetabolite. Antimetabolite is the antimicrobial that is used for inhibiting the growth of microbes. An example of antimetabolite is the sulfinylamide (Lay, 2002).

In general, most living cells including microorganisms has the ability to control what is going in and out of the membrane. If there is a rupture in the membrane, it will cause the spillage of the essential inorganic ions. Thus, rupture in the cell membrane can affect the growth and death of the microbes (Lay, 2002). 2. 6 Methods of Microbial Defense towards Antimicrobial Activity 2. 6. 1 Well Diffusion Method Well diffusion method is done by pouring the microbial suspension that is going to be tested into a sterile Petri dish continued by pouring the media agar into the dish. Then, the Petri dishes are mixed by the eight movement ethod to allow even mixing. The media is then allowed to be solidified and cynlidrical holes are made. The holes are then filled with antimicrobial agents to be tested. The cup was incubated at 37°C for two days and then observed on the antimicrobial activity of the tested microbial suspension. Antimicrobial ingredients poured into the well are able to spread evenly as it can diffuse in all directions around the microbial suspension. Once incubated, usually will form a clean circular zone of microbes. This method can be used to test several different types of antimicrobial agents. (Smith, 2005) 2. 6. Kirby Bauer Disc Method One of the easy methods to test the vulnerability of organisms towards the antimicrobial agents is by inoculating the agar withcultureand allowing the antimicrobes to diffuse to the media agar. The discs that contain antimicrobial agents are placed on the surface of the plate

that has the organisms that are going to be tested. At particular distance on respective discs, the antimicrobes will diffuse to a point where the antimicrobes are unable to inhibit the microbial growth. Antimicrobial effectiveness is shown by the inhibition zones. Inhibition zone appears as clean areas that surround the disc.

The diameter zone can be measured by using ruler and the result of the experiment considered to be one antibiogram. (Smith, 2005) 2. 6. 3 MIC and MBC The antimicrobial activity can be observed by knowing the concentration of the antimicrobial agents that are going to be used by reducing the total critical number of bacteria that caused their death. MIC (minimum inhibitor concentration) from an antimicrobes can be known by providing the antimicrobial agents into two serial dilutions in series tubes or can be done by well method in a media that has been inoculated with bacteria.

Series tubes that have been filled with antimicrobial agents are incubated to see the growth of the bacteria and the turbidity is observed. The increment in turbidity indicates the growth of the microbes. MIC is the lowest concentration of an antimicrobial agent where the grwoth can be inhibited completely. (Smith, 2005) MBC (minimum bactericidal concentration) is the total number of antimicrobial agents that is required to kill organisms.

MBC is done by taking a part from each serial tubes from the MIC that does not show any growth of the bacteria that has been incubated, The samples is taken from respective tubes that has been incubated in pour plate media. MBC is the lowest concentration of antimicrobial agents that are able to kill at least 99. 9% of the inoculums that has been incubated (Smith, 2005). 2. 7

Food Preservatives Preservatives are commonly used to prevent destruction of physical, chemical and microbiological food. Use of antimicrobial preservatives combined with other preservatives so that the preservation of the food will be maximized.

According Fellows (2000), the criteria for antimicrobial preservative in food are the usage and is more efficient which when dissolved in water, stable in storage, non-toxic, low antimicrobial concentrations but has a wide range, optimum efficiency at room temperature, non-corrosive, odorless, and its high penetrative ability to the food. Based on its mechanism, the antimicrobial agent acts as the rupture of the cell wall When there is a rupture on the cell wall, the cell contents will spill out thus inhibiting the metabolism of cells. The destruction of the cell wall can result in cell death.

Lysozyme can damage the cell walls of Gram-positive bacteria. Penicillin and sikloserin inhibit the growth of peptidoglycan in the cell develops. Gram-positive bacteria is sensitive towards penicillin as the high level of peptidoglycan content. Damage to the cell wall would also cause damage to the plasma membrane (Fellows, 2000). NaCl and alcohol is often used to preserve food because it can cause denaturation of proteins and nucleic acids that can destroy the cell and cannot be repaired. While H2O2 is an antimicrobial that can destroy the enzyme activity of microbes.

Compounds inhibiting the formation of nucleic acids and nucleic acid polymerization inhibitor compounds can inhibit the synthesis of both DNA and RNA. The use of salt and sugar can cause plasmolysis the microbial cell. Since the osmotic pressure on microbial too high, so that the cells undergo plasmolisis and can inhibit the growth of microbes (Fellows, 2000). Ascorbic

acid can inhibit the growth of bacteria and molds in a way to inactivate the enzyme fatty acid dehydrogenase. Ascorbic acid can work optimally at a pH above 6. 5.

Propionic acid is at its most effectiveness to inhibit molds and yeasts with maximal activity at pH above 5. Acetic acid as vinegar used to preserve bread in order to prevent mold contamination, but vinegar cannot inhibit the growth of yeasts. Acetic acid work optimally at low pH (acid). Ethylene oxide and propylene oxide can be used as a fumigant in spices and flour. Sodium or potassium nitrite and nitrate are commonly used for preservatives in meat products. Maximum working power of nitrite compounds is pH of about 57. Nitrite also serves to inhibit Clostridium botulinum, Acinetobacter, Moraxella, Flavobacterium,

Pseudomonas, Enterobacter, Escherichia, and some micrococcus. Nitrites are heated simultaneously with foods will provide the growth inhibitory effect against microbes ten times greater than that of nitrite without heating (Fellows, 2000). CHAPTER III MATERIALS AND METHOD 3. 1 Materials and Equipments Equipments that are used in the experiment are balance, blender, grater, dilution bottle, autoclave, waterbath, refrigerator, vernier caliper, tip with the cuttop, sterile toothpicks, sterile petri dishes, micropipette, tip (10 ml and 100µl), and Bunsen’s burner.

Materials that are used are spices (black pepper, garlic, and coriander), potassium sorbate, alcohol, aquadest, bacteria culture (Bacillus subtilis), yeast culture (Candida tropicalis), mold culture (Aspergillus oryzae), PCA media, and NA media. 3. 2 Procedures 3. 2. 1 Extract Preparation 1. Spices were weighed up to 3 grams and reduced in size. 2. The spices were mixed

with 10mL of ethanol into Erlenmeyer flasks. 3. 2. 2 Antimicrobial Assay 1. 1mL of culture is added into Petri plate 2. The matching medium is added into the Petri plates (NA for B. subtilis and PDA for A. ryzae and C. tropicalis) 3. The media were let to solidify. 4. Holes were made by using tips with cut-tops. 5. Mixtures of spices were added into the holes. 6. The plates were incubated were incubated for 48 hours in 37? C. CHAPTER IV RESULTS AND DISSCUSION 4. 1 Effect of Antimicrobial Compounds Towards Microbial Growth 4. 1. 1 Garlic Table 4. 1Observationresult of inhibitory activity by garlic From Table 4. 1, it can be seen that the microorganism that was the most susceptible to allicin contained in garlic, judging by the largest area inhibition, was Bacillus subtilis.

There were even no growths in some Petri dishes that meant that the effectiveness of allicin as the antimicrobial compound could be found most when it was applied for bacteria, which was Bacillus subtilis in particular. It matched with the previous literature discussed stating that allicin is an active compound in garlic that attacks the cell membrane in microorganism. However, there was an error found in the experiment from Group 4, which resulted in no inhibition found from Bacillus subtilis. This might happen due to the absence of the allicin itself or the ontamination happened which might result in the building of the resistance towards antimicrobial compound. Afterwards, it can be seen from the experiment that Candida tropicalis and Aspergillus oryzae shared almost the same result, which means that they were also susceptible to allicin. The uneven inhibition found in Candida tropicalis showing the largest inhibition (7. 23mm) beyond the inhibition done for Bacillus subtilis might happen because of the uneven diffusion of

the active antimicrobial compound to the dishes. It still matched the theory since allicin has antibacteria and antifungal activity towards the species mentioned above.

From the result, it can be concluded that garlic can be used as the antimicrobial compound in food to prevent foodborne disease and food poisoning. However, the concentration of garlic has to be concerned since it has strong odor and may be unpleasant to some people. 4. 1. 2 Coriander Table 4. 2 Observation result of inhibitory activity by coriander From Table 4. 2, it can be seen that coriander is found to be effective as an antimicrobial compound most in Bacillus subtilis, although some of the dishes showed no area of inhibitions.

The disappearance of Bacillus subtilis in most of Petri dishes shown by no growth sign indicated that coriander worked best to inhibit bacteria. This result matched with the previous literature stating that coriander inhibits food pathogenic bacteria, especially Gram-positive bacteria. However, coriander has the least inhibition activity towards Candida tropicalis and Aspergillus oryzae was found to be less susceptible to the volatile compound in coriander acting as antimicrobial compound. There were almost no area of inhibition found in every petri dish, proving that coriander did not affect mold and yeast.

Since the literature stated that coriander has almost no antifungal activity and its activity toward yeast has not been examined, the result matched them. 4. 1. 3 Black Pepper Table 4. 3 Observation result of inhibitory activity by black pepper From the result of the experiment, it can be seen that black pepper is most effective when used against Bacillus subtilis, as many

bacteria were inactivated, indicated by the area of clear zone, which in some plates, all of them doesn’t grow at all and so the area of clear zone can’t be measured.

Aspergillus oryzae are quite susceptible as well to black pepper’s antimicrobial compound as the area of inhibition overall is large. While the most resistant microorganism from the experiment shown is Candida tropicalis, as the area of inhibition is relatively small. From the theory, black pepper shows antimicrobial activity against bacteria, molds, and yeasts, but the strongest towards bacteria, especially Grampositive bacteria. The result of the experiment matches the theory, as black pepper is very effective in stopping the growth of Bacillus subtilis, which is a Grampositive bacterium.

It also inhibited the growth of Candida tropicalis and Aspergillus oryzae, so the result of the experiment matches the theory. 4. 1. 4 Potassium Sorbate Table 4. 4 Observation result of inhibitory activity by potassium sorbate From the result of the experiment, it can be seen that potassium sorbate is very effective in stopping the growth of Bacillus subtilis, as in some plates it inhibited the growth of all the bacteria and so the area of clear zone can’t be measured. Antimicrobial activity can be seen as well towards Candida tropicalis and Aspergillus oryzae.

According to the theory, potassium sorbate is very effective when used to inhibit the growth of bacteria, molds, and yeasts. The result of the experiment matches the theory, as potassium sorbate in this experiment is able to stop the growth of Bacillus subtilis, Candida tropicalis, and Aspergillus Oryzae. 4. 2 Microbial Defense Towards Antimicrobial Compounds 4. 2. 1 Bacteria (Bacillus subtilis) Table 4. 4 Table of diameter of inhibition zone of

Bacillus subtilis in some condiments From the table 4. 4, it is shown that the most effective condiment in affecting the microbial defense of the Bacillus subtilis for its growth is arlic. Also, the least effective condiment for inhibiting the growth of Bacillus subtilis is coriander. According to the theory, garlic is effective for both Gram-negative and Grampositive bacteria, thus the inhibition zone is larger. Also, coriander is effective in inhibiting Gram-negative bacteria, as in the experiment the bacteria used was Gram-positive bacteria, thus, coriander is not effective in inhibiting Bacillus subtilis. The experiment result has shown the same result as the theory given. 4. 2. 2 Yeast (Candida tropicalis) Table 4. 5Table of diameter of inhibition zone of Candida tropicalis in some condiments

In the table 4. 5, it shown that the most effective condiment in affecting the microbial defense and microbial growth of Candida tropicalis is garlic and the least effective is coriander. According to the theory, garlic is used as antifungal and is the most effective one in inhibiting the growth of bacteria, molds and yeast. Thus, the inhibition zone of garlic is the largest. Also, as coriander is mostly effective in inhibiting Gram-negative bacteria, the rate of inhibition of yeast is lower, so the inhibition zone is smaller. The experiment result has shown the exact result as the theory given. 4. 2. Molds (Aspergillus oryzae) Table 4. 6 Table of diameter of inhibition zone of Aspergillus oryzae in some condiments The experiment result has shown that the most effective condiment towards the inhibition zone in Aspergillus oryzae media is garlic and the least effective condiment is coriander. Referring to the theory, garlic is mostly used as antifungal and the most effective condiment used in the experiment to inhibit the growth of bacteria,

mold, and yeast. Also, coriander is least effective in inhibiting the growth of Aspergillus oryzae as coriander is most effective in inhibiting gramnegative bacteria.

In conclusion, the result of the experiment shows the same result as the theory given. CHAPTER V CONCLUSION Spices have different antimicrobial compounds able to inhibit the growth of various types of microorganisms. Based on the experiments, garlic, coriander, and black pepper have the most effective activity in inhibiting the growth of bacteria, or in this case Bacillus subtilis. However, coriander was proven to have no antifungal activity by the absence of inhibition zone on the Petri plates with yeast and molds, in this case Candida tropicalis and Aspergillus oryzae.

In addition, potassium sorbate is effective to inhibit the growth of all types of micoorganisms in the experiment: bacteria, yeast, and molds. The Bacillus subtilis bacteria, Candida tropicalis yeast, and Aspergillus oryzae molds are most susceptible by the presence of garlic. This means that the garlic has the highest effectiveness in affecting the defense of bacteria, yeast, and molds. In contrast, the growth of bacteria, yeast, and molds is least affected by the presence of coriander.