3. REVIEW

3.1. Roasted Coffee Composition Related to Antibacterial Activity

Composition of roasted coffee is determined by various factors, such as species, agricultural practices, ripeness, process (roasting, grinding, brewing) and storage conditions. Based on species, *Coffea arabica* and *Coffea canephora* as the most consumed species have quite distinctive composition. *C. canephora* have higher amounts of bioactive compounds such as phenolic acids and caffeine that leads to higher biological properties, such as antioxidant and antibacterial properties in comparison to Arabica (Ludwig *et al*., 2014).

Within numerous bioactive compounds, phenolic acid and melanoidin stand as the most reported antibacterial compounds in roasted coffee. During the studies, the separation and identification of these compounds are easily conducted as both of them have quite a large difference in their molecular weight. Phenolic acid have been reported as a low molecular weight (LMW) compounds on roasted coffee (1-3 kDa) while melanoidin (Maillard reaction product) have high molecular weight (10000 kDa) (Rurian and Morales, 2008).

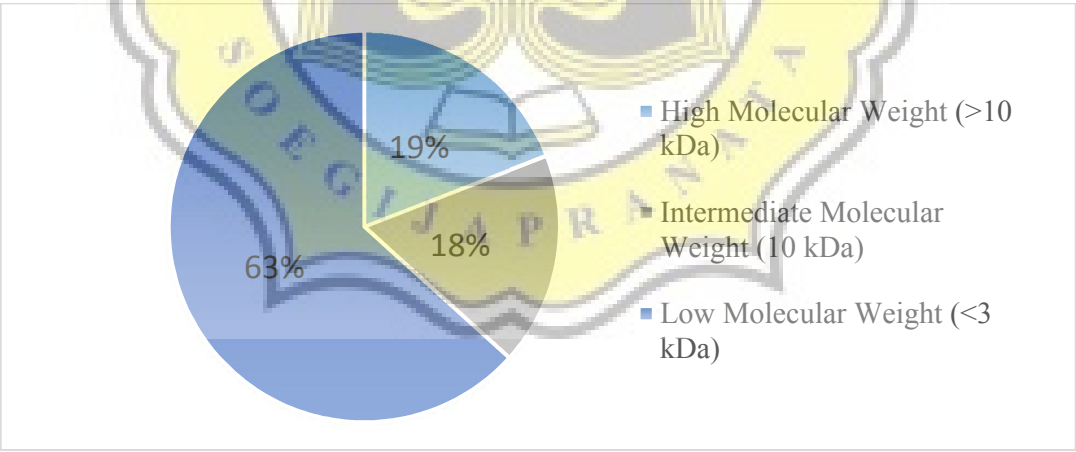


Figure 2. Roasted Coffee Composition Based on Molecular Weight

By gel filtration chromatography, roasted coffee are divided into three fractions as can be seen on **Figure 2**. Results showed that phenolic acid (indicated as low molecular

weight fraction on roasted coffee) correspondent to 63% of roasted coffee total composition. Despite of their sensitivity to high temperature, the expected low level of phenolic acid after roasting process are contradictive to the chromatography results. The expected low level of phenolic acid are based on the study of Daglia *et al.* (1994) that measure a decrease in coffee natural phenolic acid during roasting process (i.e., chlorogenic acid and its isomer, trigonelline). However, further research by Borrelli *et al.* (2002) has reported an increase in phenolic acid amount during the roasting process. Dark roasted coffee are observed to contain higher amount of phenolic acid in comparison to light roasted coffee or the raw coffee bean. The higher amount of phenolic acid are contributed from nicotinic acid that are form by the degradation of trigonelline during roasting process (Daglia *et al.*, 1994).

Other than that, several non-phenolic compound with low molecular weight are also contributed to the high percentage of low molecular weight fraction. Daglia *et al.* (1994) reported the formation of 5-hydroxymethylfurfural (5-HMF) in small amount. In the next research on 2007, this author also reported the formation of alfa dicarbonyl compounds. Both of this compound are formed by the Maillard reaction, thus are categorized into Maillard reaction product, other than melanoidin. In addition to that, melanoidin can be degraded to several low molecular weight compounds, including phenolic acid within the application of severe temperature (Rurian and Morales, 2008).

Despite of the huge proportion, the antibacterial activity of roasted coffee LMW compounds is reported to be 5 times lower than high molecular weight (HMW) compounds (Rurian-Henares and Morales, 2008). This result showed that melanoidin, as the main component of the high molecular weight fraction has better antibacterial properties than the phenolic acid of roasted coffee. The antibacterial properties of melanoidin has been recorded in several research, either on coffee or another food product (i.e., beer, chocolate, wine, cookies) (Moreira *et al.*, 2012; Rurian and Morales, 2008). The early studies of Daglia *et al.* (1994) had suspected the Maillard reaction product as compound with strong antibacterial properties as the increase of roasted coffee antibacterial activity is in line with the roasting degree (Daglia *et al.*, 1994)

When comparing the effect of roasted coffee melanoidin with a pure natural low molecular weight antibacterial compound (i.e. cinnamaldehyde, carvacrol), the antibacterial activity of melanoidin is weaker than natural LMW compounds. This opposing result may be related to the presence of other compounds in the extract as the separation are not conducted toward specific compound. Consequently, the interaction (synergism or antagonism) between each antibacterial compounds appear as an important factor to consider (Rurian and Morales, 2008).

Related to the hypothesis above, the different result of antibacterial activites are observed between the use of roasted coffee aqueous extract and roasted coffee specific compounds. The better antibacterial activity of roasted coffee aqueous extract are expected as the extract consist of several antibacterial compounds in comparison to a single antibacterial compound. However, it is important to consider the presence of other non-bacteriostatic compounds such as polysaccharides, amino acids, sucrose or milk. These compounds are already known as a nutrition source for bacteria. The effect of milk and sugar addition on coffee (1% on daily basis) has been reported by (Yew *et al*., 2013) from which the tested bacteria (*Vibrio chorelae*) are able to survive longer with the addition of sugar for 1 hour and 3 hours for addition of condensed milk (to be noted that condensed milk contain high level of sugar as well). In the other hand, the addition of condensed milk elevate coffee pH level to 6.0 whereas *Vibrio chorelae* can grow very fast under pH ranging from 5 to 9.6 (Yew *et al*., 2013).

For the same reason, the effect of sugar/sucrose may explain the absence of green coffee antibacterial activity. Natural sucrose concentration on green coffee beans are 4 mg/mL or 8346.9 mg/100 g coffee for Arabica and 7 mg/mL or 6401.6 mg/g coffee for Canephora that being a microbe-nutrient concern (McNeill and Hamilton, 2004). This conclusion was further supported by the fact that the addition of sucrose in the media can raise the bacteria growth by 4.4 times (Arora *et al.*, 2009). As the content of sucrose in green coffee is naturally high, therefore the contradiction between antibacterial activity of phenolic acid and sucrose contribution in bacteria survival are inevitable (Antonio *et al*., 2010).

In contrary, sucrose concentration on roasted coffee is quite low as it is an important precursor of Maillard reaction. Maillard reaction itself are triggered by the exposure of high temperature in roasting process. The higher the temperature / the roasting degree, leads to lower amount of sucrose in roasted coffee. In this sense, the roasting degree serve as an important factor for the amount of sucrose remained in roasted coffee. Therefore a high temperature/roasting degree is necessary to ensure the low level (or none) of sucrose in roasted coffee for the antibacterial purpose. Conversely, a certain level of sucrose is needed for roasted coffee in the purpose of consumption as it contribute in the pleasant taste and aroma (Perrone *et al*., 2008; Lanciotti *et al*., 1999).

3.2. General Antibacterial Activity of Roasted Coffee

Roasted coffee inhibitory activity on numerous bacteria is indicated in **Table 1**. Alongside the other natural source of antibacterial compounds (i.e, tea; ginger), roasted coffee showed stronger activity toward gram positive bacteria as well (showed by lower value of minimum inhibitory concentration [MIC] or higher distance of diameter disk). Those comparison can be observed on **Table 1**.

Table 1. Roasted Coffee Antibacterial Activity toward Numerous Strains of Bacteria

Bacteria	Gram (+/-)	MIC (mg/ml)	Inhibition Zone (mm)	Species	Roasted	References
Staphylococcus aureus	+	3	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		3.2	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		5.4	-	Arabica	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		24.9	-	Canephora	Medium roasted, 12% weight loss	(Daglia *et al*., 2007)
		0.84	-		Instant coffee (dark), acidic extract	(Daglia *et al*., 1998)
		15.5	-	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)

Table 1. (continued)

		12.5	26	-	18 minutes, 217°C	(Deressa *et al*., 2015)
		2	-	Arabica	Medium roasted	(Runti *et al*., 2015)
		-	35.44	Arabica	Roasted at 217°C, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al*., 2011)
Staphylococcus aureus	+	-	16.8		Instant coffee, dark	(Arora *et al*., 2009)
Bacillus subtilis	+	<3	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		13.23	-	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)man
Bacillus cereus	+	13.2	-	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)
Streptococcus faecalis	+	12	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
Streptococcus pyogenes	+	<3	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
Streptococcus mutans	+	12.4	-	Canephora	Medium roasted, 12% weight loss	(Daglia *et al*., 2007)
		0.76	-	-	Instant coffee (dark), acidic extract	(Daglia *et al*., 1998)
Streptococcus parasanguinis	+	5	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)
Lactobacillus rhamnosus	+	10	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)
Porphyromonas gingivalis	+	ND	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)
Prevotella intermedia	+	ND	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)
Prevotella nigrescens	+	ND	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)
Fusobacterium nucleatum	+	ND	-	Canephora	220°C for 6 minutes, light roasted	(Silva *et al*., 2014)

Table 1. (continued)

Staphylococcus epidermidis	+	-	19.6	-	Instant coffee (dark)	(Arora *et al.*, 2009)
Staphylococcus epidermidis	+	25	14	-	18 minutes, 217ºC	(Deressa *et al.*, 2015)
		-	1	Arabica	Medium roasted	(Runti *et al.*, 2015)
Enterococcus faecalis	+	-	22.5	-	Instant coffee (dark)	(Arora *et al.*, 2009)
		-	9.44	Arabica	Roasted at 217ºC, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al.*, 2011)
		-	15	Arabica	Medium roasted	(Runti *et al.*, 2015)
Listeria monocytogenes	+	-	17.22	Arabica	Roasted at 217ºC, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al.*, 2011)
		-	18	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)
Geobacillus stearothermophilus	+	0.001 ± 0.006	-	-	Bean colour : 60	(Rufián and Morales, 2006)
Shigella flexneri	-	-	21.3	-	Instant coffee (dark)	(Arora *et al.*, 2009)
Salmonella choleraesius	-	-	13.78	Arabica	Roasted at 217ºC, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al.*, 2011)
Citrobacter freundii	-	-	7.1	Arabica	Dark roasted	(Almeida et al., 2006)
		12.5	17	-	18 minutes, 217ºC	(Deressa *et al.*, 2015)
Escherichia aerogenes	-	-	8.4	Arabica	Dark roasted	(Almeida *et al.*, 2006)

Table 1. (continued)

Escherichia cloacae	-	-	9	Arabica	Dark roasted	(Almeida *et al*., 2006)
Klebsiella oxytoca	-	-	7.5	Arabica	Dark roasted	(Almeida *et al*., 2006)
Proteus hauseri	-	-	9.2	Arabica	Dark roasted	(Almeida *et al*., 2006)
Proteus mirabilis	-	-	8.1	Arabica	Dark roasted	(Almeida *et al*., 2006)
Salmonella enterica	-	-	8.1	Arabica	Dark roasted	(Almeida *et al*., 2006)
		-	15	Arabica	Medium roasted	(Runti *et al*., 2015)
Serratia marcescens	-	-	9.2	Arabica	Dark roasted	(Almeida *et al*., 2006)
Salmonella typhimurium	-	12	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		-	ND	-	Instant coffee, dark	(Arora et al., 2009)
Escherichia coli	-	23	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
		-	12.67	Arabica	Roasted at 217°C, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al*., 2011)
		-	8.1	Arabica	Dark roasted	(Almeida *et al*., 2006)
		24	-	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)
		12.5	23	-	18 minutes, 217°C	(Deressa et al., 2015)

Table 1. (continued)

Escherichia coli	-	15	-	Arabica	Medium roasted	(Runti et al., 2015)
Escherichia cloacae	-	6	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
Pseudomonas vulgaris	-	<3	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al*., 1994)
Pseudomonas aeruginosa	-	6	-	Canephora	8 minutes, 20% weight loss, dark roasted	(Daglia *et al.*, 1994)
		-	20.4	-	Instant coffee (dark)	(Arora *et al.,* 2009)
		-	14.22	Arabica	Roasted at 217ºC, 18-20 minutes, Colombia coffee, 300 µL	(Martínez-Tomé *et al*., 2011)
		16	-	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)
		12.5	14	-	18 minutes, 217ºC	(Deressa et al., 2015)
Yersinia enterocolitica	-	-	18	Canephora	Light roasted (high moisture), monsooned coffee	(Manonmani, 2009)
Citrobacter spp.	-	-	17	-	18 minutes, 217ºC	(Deressa *et al*., 2015)

In **Table 1.**, majority of the studies had reported the activity of roasted coffee toward pathogenic bacteria, especially toward *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* within different roasting condition. On the other hand, when comparing the sensitivity between gram positive and negative bacteria, a significant difference was observed. Lower value of minimum inhibition concentration (MIC) or wider inhibition zone are observed on gram positive bacteria, indicating the higher sensitivity of roasted coffee for this type of bacteria. In addition, the sensitivity of gram positive bacteria has been reported on another natural food products, such as on tea

(Arora *et al.*, 2009; Mohammed and Bayati, 2009), cinnamon and honey (Deressa *et al*., 2015).

This is caused by the difference in their membrane substances. Gram positive outer membrane are formed by thick layer of peptidoglycan, while gram negative have three layer of membranes (1) thin peptidoglycan layer (2) lipopolysaccharides (3) inner membrane formed by phospholipid bilayer. The additional layer of gram negative membrane cause a longer time for antibacterial compound to completely permeate those membranes (Rufián and Cueva, 2009; Bekedam *et al*., 2007; Borrelli *et al*., 2002;).

Results on **Table 1.**, also indicate the stronger antibacterial activity of dark roasted coffee bean. By comparison, the tested bacteria have higher sensitivity to dark roasted coffee than to light or medium roasted coffee. This is confirmed by the lower value of MIC and wider inhibition zone. The superior activity of dark roasted coffee is a result from two reactions that take place during roasting process (1) the formation of maillard reaction product (i.e., melanoidin, alfa dicarbonyl compounds) (2) breakdown of sucrose, as a precursor of maillard reaction. The higher the roasting temperature / degree, then the higher amount of maillard reaction product will generate, and the lower amount of sucrose will remain. Furthermore, Daglia *et al.* (1994) also reported the resistancy of *Salmonella thypimurium* (gram negative bacteria) toward roasted coffee extract as it exhibit no inhibition to light roasted coffee. However, a slight activity are observed for dark roasted coffee. Therefore, it is suspected that each bacteria has a certain sensitivity toward antibacterial compounds, other than the gram staining (Arora *et al.*, 2009; Daglia *et al*., 2007).

In the contrary, there are some number on **Table 1**., that in opposition to the previous observation. In example, the MIC value of light roasted coffee for *Staphylococcus aureus* is significantly lower than the medium roasted coffee; 15.5 and 24.9 mg/mL respectively. In the previous observation, coffee that are exposed with higher roasting degree is supposed to exert stronger antibacterial activity (Daglia *et al*., 2007). This type of data can not be avoided in this review as each of the author use different processing

condition, media, testing environment (i.e., pH, temperature) and different condition of raw material (Arora *et al*., 2009; Daglia *et al*., 1994).

Another important things to be observed is the categorizing of coffee beans based on their roasting degree. The category itself is divided into three categories; light, medium, and dark roasted. Additionally, there are some authors that add one further category, the severe roasted. However, the severe category are not available in commercial coffee as the dark roasted is set as the highest degree of roasting process. To evaluate the roasting degree of each authors, it is observed that each author have set a different roasting temperature for each category. Moreover, Belsc and Horz (2011) also reported the use of different roasting temperature for each of coffee sub variety, regarding being labelled with the same roasting degree (Antonio *et al*., 2010).

Table 2. Difference of Roasting Degree in Different Coffee Variety

Roasted Degree	Coffee Species			
	Minas (Arabica)	Cioccolatato (Arabica)	Vietnam (Robusta)	Cherry (Robusta)
Light Roasted (°C)	162	145	168	185
Medium Roasted (°C)	181	167	185	195
Dark Roasted (°C)	195	195	198	205

Note : Not a specific standard for roasting; a difference is expected in other place
(Belšc and Horz, 2011)

Furthermore, the categorizing of roasting degree are done in several method. There are method that measured the degree of roasting by the weight lost (in wet basis) due to vapor formation and cell fragment loss. In example, the loss of 12% of weight correspondent to medium degree of roasting (Daglia *et al*., 2007). Other author evaluated the colour difference to determine the roasting degree (Belsc and Horz, 2011). In addition, Antonio *et al.* (2010) mentioned the use of The Roast Colour Classification system to determine the roasting degree (Antonio *et al*., 2010). In this terms, the uncertainty condition of roasting degree may cause a difference in their chemical composition, even being labelled as the same degree. Therefore, there is a big possibility from some antibacterial data to differ from the theory being proposed.

Additionally, roasted coffee antibacterial activity has been reported to be equivalent with commercial antibiotics like chloramphenicol, amipicillin, ocytetracyclin and other antibiotics. It is reported as well that some bacteria with resistancy toward antibiotics are reported sensitive to roasted coffee (Arora et al., 2009; Rufián-Henares and Morales, 2006). Furthermore, *in vivo* research had been conducted (with mice) to ensure the antibacterial activity inside the human's body. By the research, the antibacterial activity of roasted coffee had been confirmed to inhibit the growth of *Escherichia coli* and *Enterococcus faecium* on colon. Other than that, oligosaccharides contained in coffee are able to promote the growth of *Bifidobacterium spp.* and *Lactobacillus spp.* which converts oligosaccharides into lactic acid for acquisition of adenosine triphosphate (ATP) (Nakayama and Oishi, 2013).

3.3. Antibacterial Compounds

3.3.1. Phenolics

Phenolic usually described by a hydroxyl group attached to an aromatic ring with low molecular weight (1-3 kDa prior to dialysis) (Borrelli *et al*., 2002). This compounds had been found in large number on plant and grouped in different class and subclasses depending on the difference in structure. Mainly, phenolic acid is divided into two big class, flavonoid compounds and non-flavonoid compounds. Flavonoid compounds, cover for subclasses like flavanols, flavones, flavanones, anthocyanidins, condensed tannin, and the other subclasses; while non-flavonoid subclasses are phenolic acid, benzoic acid, hydroxycinammic acid and lignans (Farah and Donangelo, 2006).

On coffee, condensed tannin can be found in huge number on the pulp while the bean mainly contain of chlorogenic acid. Chlorogenic acid (CGA) is an esters from hydroxycinammic acid and quinic acid that cover for around 14% of coffee bean composition. The asmount of CGA varies from 4 – 8.4% for *C. arabica* and 7 -14.4% for *C. canephora*. Chlorogenic acid main groups such as caffeoylquinic acid (CQA) and dicaffeoylquinic acid (dCQA) all with their isomer have been found in a higher amount on the green coffee bean (Farah and Donangelo, 2006).

As generally known, phenolic compounds are available in large proportion in green coffee beans (Antonio *et al*., 2011; Daglia *et al*., 1998). The amount is significantly higher on *C. canephora* than *C. arabica* leading to the less bitter flavour of Arabica coffee (Daglia *et al.*, 1994). Borrelli *et al.* (2002) recorded roasted coffee phenolic compounds for 67 mg Gallic acid equivalents (GAE) and it will degraded respectively until completely degraded at severe roasting resulting in 2 mg GAE. The rate of degradation vary from 50-90%, depending on the roasting degree itself (Fuster *et al.*, 2000). With the expected damage during the roasting process, coffee still contribute as the richest dietary source of its major phenolic compounds, caffeoylquinic acid (CQA). Regular coffee drinkers easily consume about 1-2 g of CQAs per day. (A single serving of espresso contains 24-423 mg of CQAs) (Ludwig *et al.*, 2014)

There are three theories reported regarding the factors that enhance phenolic compounds antibacterial activity; (1) the longer the side chain and the higher the number of reactive double bonds, the higher the antibacterial activity; (2) the higher the amount of undissociated acid, the higher the antibacterial activity; (3) the lower the polarity, the better permeability of compounds to permeate bacterial membrane. High amount of undissociated acid is expected when the pH of the extract is in a close range with pK_a value. For compounds with pK_a 4.5; there would be a higher percentage of the undissociated acid at pH 4 rather than at pH 5.5 (Herald and Davidson, 1983). This is related to the better permeability of undissociated acid to enter the bacterial cell and acidify the intracellular medium (Reguant *et al.*, 2000).

Conversely, other author reported that lower polarity compounds have better antibacterial activity as it has better permeability to pass bacterial cell membrane. However all of these authors stated that those theory can't be applied to all phenolic compunds. The current suggestion is that an equilibrium between solubility in both lipid and aqueous phase is necessary to be researched to gain better understanding on this topic (Daglia *et al*., 1994; Campos *et al*., 2003).

The mechanism of phenolic antibacterial is related to their ability to disturb the nutrient transport on bacteria outer membrane. Cirigliano *et al.* (2000) reported that phenolic

compound can cause a pH disturbance in bacteria outer membrane. Generally, bacteria cell membrane is on uncharged state that allows nutrient transport throughout the membrane. As the pH on outer membrane is disturbed, the bacteria can not conduct the nutrient transport (i.e., passive transport, electron flow, active transport). Additionally, removal of divalent cations on the membrane has been reported where those cations act as a bridge between phospate groups of phospholipids. Without the presence of those cations, each of the phospholipids will repel each other that cause swelling and instability of cell membrane, leading to interference in nutrient transport as well. This activity then has been related toward substances with anionic charge in general, such as phenolic acid and melanoidin (Rurian and Morales, 2008; Donnell, 1999). Besides the above-mentioned mechanisms, other authors reported phenolic activity to (1) permeate cell membrane and accumulate constantly inside the cell (cytoplasmic poison) (Donnell, 1999) (2) interaction with cell enzymes by the reaction of sulfhydryl groups with oxidised compounds or non-specific interactions with proteins (McManus *et al*., 1985).

Phenolic compounds that remained in roasted coffee is formed by different phenolic, like chlorogenic acid and trigonelline. Further elaboration regarding their antibacterial activity will be found below.

a. Chlorogenic Acid (λ=320 nm)

Chlorogenic acid is the main phenolic compound in roasted coffee as it cover for 14% of roasted coffee composition. It is formed by esterification between a hydroxycinammic acid (cinnamic acid) and quinic acid (Rodríguez *et al*., 2007). On coffee brew, chlorogenic acid is found in the range of 15 – 325 mg per 200 mL, depends on the bean fermentation, bean roasting, freeze or spray drying (in the terms of instant coffee), decaffeination, blending, addition and roasting. On raw bean, *Coffea arabica* has 5 g/100 g while *Coffea canephora* has 9 g/100 g. On other hand, it is observed that instant coffee have a similar amount of chlorogenic acid with regular coffee (Mills *et al*., 2013; Antonio *et al*., 2011)

Chlorogenic acid is known by its isomer such as 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA); 5-caffeoylquinic acid (5-CQA) (Almeida *et al*., 2006)

with 5-CQA that is commonly found in majority of plants. In coffee itself, 5-CQA account for 25-30% of the total CQA while CQA account for 80% of total chlorogenic acid (Silva *et al*., 2014). Beside of that, 5-CQA also has the highest antibacterial activity in comparison with the other CQA-isomer. Therefore, 5-CQA is usually use to represent chlorogenic acid and is the only one for which a commercial standard is available. The other subclasses of chlorogenic acid are also found in small amount (i.e., dicaffeoylquinic acids, feruloylquinic acid, p-coumaroylquinic acids) (Mills *et al*., 2013)

Beside the general phenolic antibacterial mechanisms, chlorogenic acid also possess a individual mechanism. Chlorogenic acid has been found to cause leakage on the outer membrane that leads to the loss of cellular cytoplasmic contents. Furthermore, the membrane will undergo depolarization because the cell lost the capability to maintain membrane potential and metabolites that end in the nucleotide leakage. This leakage will then cause the cell rupture (cell death) that can observed by the presence of celluler contents in media such as K^+, DNA, RNA and proteins. To reach the cytoplasmic leakage phase, it been reported that the use of 2-fold MIC will reach the phase after 90 minutes. This activity is reported to aid antibiotics activity as well. Within the mechanism above, bacterial outer membrane permeabilization is increased that give an easy access for antibiotics into the cell. Combined together, the needs of specific antibiotics is lower to reach the same activity and the risk of developing antibiotics resistancy can be evaded as well (Lou *et al*., 2011).

Chlorogenic acid and other phenolic compounds has been found with the capability to inhibit pathogenic bacteria as can be seen on **Table 3.**

Table 3. Phenolic Antibacterial Activity toward Numerous Bacteria

Bacteria	Gram (+/-)	Phenolic Compound	Inhibition Zones (mm)	MIC (µg/ml)	References
Streptococcus pneumoniae	+	Chlorogenic acid	-	20	(Lou *et al*., 2011)
Streptococcus mutans	+	Phenolic compounds	-	0.8	(Antonio *et al*., 2010)
		Chlorogenic acid	7.6 ± 0,38	-	(Almeida *et al*., 2012)

Table 3. (continued)

Streptococcus mutans	+	Caffeic acid	7.3 ± 0,29	-	(Almeida *et al*., 2012)
		Trigonelline	7.3 ± 0,29	-	(Almeida *et al*., 2012)
		Protocatheuic acid	7.5 ± 0,5	-	(Almeida *et al*., 2012)
		5-CQA (chlorogenic acid)		2.7 ± 0.5	(Daglia *et al*., 2007)
Streptococcus parasanguinis	+	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
	+	Trigonelline	-	2.56 – 1.28	
Lactobacillus rhamnosus	+	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
		Trigonelline	-	2.56	
Staphylococcus aureus	+	Chlorogenic acid	-	40	(Lou *et al*., 2011)
		5-CQA (chlorogenic acid)	-	6.3 ± 0.4	(Daglia *et al*., 2007)
		Chlorogenic acid	11	-	(Martínez-Tomé *et al*., 2011)
		Ferulic acid	7.33	-	(Martínez-Tomé *et al*., 2011)
		P-coumaric acid	ND	-	(Martínez-Tomé *et al*., 2011)
		Caffeic acid	26.33	-	(Martínez-Tomé *et al*., 2011)
Bacillus subtilis	+	Chlorogenic acid	-	40	(Lou *et al*., 2011)
Listeria monocytogenes	+	Ferulic acid	ND	-	(Martínez-Tomé *et al*., 2011)
		Chlorogenic acid	12.67	-	(Martínez-Tomé *et al*., 2011)
		P-coumaric acid	8.33	-	(Martínez-Tomé *et al*., 2011)
		Caffeic acid	23	-	(Martínez-Tomé *et al*., 2011)
Enterococcus faecalis	+	Ferulic acid	7.33	-	(Martínez-Tomé *et al*., 2011)
		Chlorogenic acid	11	-	(Martínez-Tomé *et al*., 2011)
		P-coumaric acid	ND	-	(Martínez-Tomé *et al*., 2011)
		Caffeic acid	26.33	-	(Martínez-Tomé *et al*., 2011)
Pseudomonas aeruginosa	-	Ferulic acid	ND	-	(Martínez-Tomé *et al*., 2011)
		Chlorogenic acid	10	-	(Martínez-Tomé *et al*., 2011)

Table 3. (continued)

Pseudomonas aeruginosa		P-coumaric acid	ND	-	(Martínez-Tomé *et al*., 2011)
		Caffeic acid	16.67	-	(Martínez-Tomé *et al*., 2011)
Escherichia coli	-	Chlorogenic acid	8.0 ± 0.5	-	(Almeida *et al*., 2006)
		Chlorogenic acid	10	-	(Martínez-Tomé *et al*., 2011)
		Protocatehuic acid	8.0 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	8.0 ± 0.4	-	(Almeida *et al*., 2006)
		Caffeic acid	17.33	-	(Martínez-Tomé *et al*., 2011)
		Trigonelline	8.1 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	8.0 ± 0.4	80	(Lou *et al*., 2011)
		Ferulic acid	9	-	(Martínez-Tomé *et al*., 2011)
		P-coumaric acid	ND	-	(Martínez-Tomé *et al*., 2011)
Klebsiella oxytoca	-	Chlorogenic acid	7.8 ± 1.0	-	(Almeida *et al*., 2006)
		Protocatehuic acid	7.6 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	7.5 ± 0.5	-	(Almeida *et al*., 2006)
		Trigonelline	7.4 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	7.5 ± 0.5	-	(Almeida *et al*., 2006)
Proteus hauseri	-	Chlorogenic acid	9.1 ± 1.1	-	(Almeida *et al*., 2006)
		Protocatehuic acid	9.3 ± 0.8	-	(Almeida *et al*., 2006)
		Caffeic acid	8.7 ± 0.8	-	(Almeida *et al*., 2006)
		Trigonelline	8.6 ± 0.8	-	(Almeida *et al*., 2006)
		Caffeic acid	8.7 ± 0.8	-	(Almeida *et al*., 2006)
Proteus mirabilis	-	Chlorogenic acid	8.9 ± 0.4	-	(Almeida *et al*., 2006)
		Protocatehuic acid	8.0 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	8.3 ± 0.3	-	(Almeida *et al*., 2006)
		Trigonelline	8.9 ± 0.8	-	(Almeida *et al*., 2006)

Table 3. (continued)

Proteus mirabilis	-	Caffeic acid	8.3 ± 0.3	-	(Almeida *et al*., 2006)
Salmonella enterica	-	Chlorogenic acid	7.9 ± 1.2	IC_{90} : 7.1	(Almeida *et al*., 2006)
		Protocatehuic acid	8.4 ± 1.4	IC_{90} : 4.1	(Almeida *et al*., 2006)
		Caffeic acid	8.3 ± 1.8	IC_{90} : 9.3	(Almeida *et al*., 2006)
		Trigonelline	8.8 ± 1.2	IC_{90} : 3.8	(Almeida *et al*., 2006)
		Trigonelline	9.6 ± 1.9	-	(Almeida *et al*., 2006)
Citrobacter freundii	-	Chlorogenic acid	6.8 ± 0.4	-	(Almeida *et al*., 2006)
		Protocatehuic acid	7.0 ± 0.5	-	(Almeida *et al*., 2006)
		Caffeic acid	7.2 ± 0.6	-	(Almeida *et al*., 2006)
		Trigonelline	7.0 ± 0.5	-	(Almeida *et al*., 2006)
Escherichia aerogenes	-	Chlorogenic acid	8.6 ± 0.4	-	(Almeida *et al*., 2006)
		Protocatehuic acid	8.4 ± 0.1	-	(Almeida *et al*., 2006)
		Caffeic acid	8.4 ± 0.8	-	(Almeida *et al*., 2006)
		Trigonelline	8.6 ± 1.2	-	(Almeida *et al*., 2006)
Enterobacter cloacae	-	Chlorogenic acid	9.6 ± 1.2	-	(Almeida *et al*., 2006)
		Protocatehuic acid	10.0 ± 0.9	-	(Almeida *et al*., 2006)
		Caffeic acid	10.0 ± 1.3	-	(Almeida *et al*., 2006)
		Trigonelline	9.1 ± 0.8	-	(Almeida *et al*., 2006)
Salmonella typhimurium	-	Caffeic acid	8.3 ± 1.8	-	(Almeida *et al*., 2006)
		Chlorogenic acid	-	40	(Lou et al., 2011)
Porphyromonas gingivalis	-	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
		Trigonelline	-	2.56 – 1.28	
Fusobacterium nucleatum	-	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
		Trigonelline	-	5.12	

Table 3. (continued)

Prevotella intermedia	-	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
		Trigonelline	-	2.56-1.28	
Prevotella nigrescens	-	Chlorogenic acid	-	ND	(Silva *et al*., 2014)
Porphyromonas gingivalis	-	Trigonelline	-	2.56	(Silva *et al*., 2014)
		Chlorogenic acid	-	ND	
Shigella dysentriae		Chlorogenic acid	-	20	(Lou *et al*., 2011)
Legionella pneumophila	-	Caffeic acid	-	IC_{90} : 0.625 mg/ml	(Almeida *et al*., 2006)

b. Trigonelline (N-methylnicotinic acid) (λ=260 nm)

Trigonelline is one of the biactive compounds that gain quite attention as it acts as a precursor of nicotinic acid (Daglia *et al.*, 1994). By the exposure to high temperature (starting at 210ºC), degradation of trigonelline into nicotinic acid or other LMW compounds is reported (Taguchi *et al.*, 2014). The loss of trigonelline during roasting range from 50-80% depending on the severity of roasting degree. Normally, trigonelline remained in small amount on roasted coffee, ranging from 279.7 – 955.9 mg/100 g coffee. To be noted that trigonelline is one of a few from phenolic acid that contained in higher level on Arabica than Canephora (Antonio *et al*., 2010; Almeida *et al*., 2006; Farah *et al*., 2006).

Despite of the small amount, the studies on trigonelline antibacterial activity is recorded toward several *Enterobacteria* strain (Almeida *et al.*, 2006). Moreover, trigonelline superior activity toward oral bacteria are recorded. Between all of roasted coffee phenolic compounds, trigonelline is reported as the most effective compounds toward oral bacteria (even superior to 5-CQA). The oral bacteria such as, *Streptococcus parasanguinis, Lactobacillus rhamnosus,* and *Streptococcus mutans* as the major oral bacteria. Specifically for *S. mutans*, the MIC recorded is 0.8 mg/mL with intervention towards streptococcal sucrose-independent adsorption to hydroxyapatite beads (Silva *et al*., 2014; Antonio *et al*., 2010; Almeida *et al*., 2004; Daglia *et al*., 2002)

However, the antibacterial activity of trigonelline is observed to be weak toward *L. rhamnosus* in comparison to other oral bacteria. The survival rate of this bacteria is reported to be enhanced in the presence of vitamin, such as d-biotin, pyridoxine, niacin (nicotinic acid), etc. As trigonelline is known to break down to nicotinic acid (niacin) on high temperature, the presence of niacin as a vitamin could contribute to *L. rhamnosus* resistance toward trigonelline (Silva *et al.*, 2014). Further antibacterial activity of trigonelline can be observed in **Table 3.**

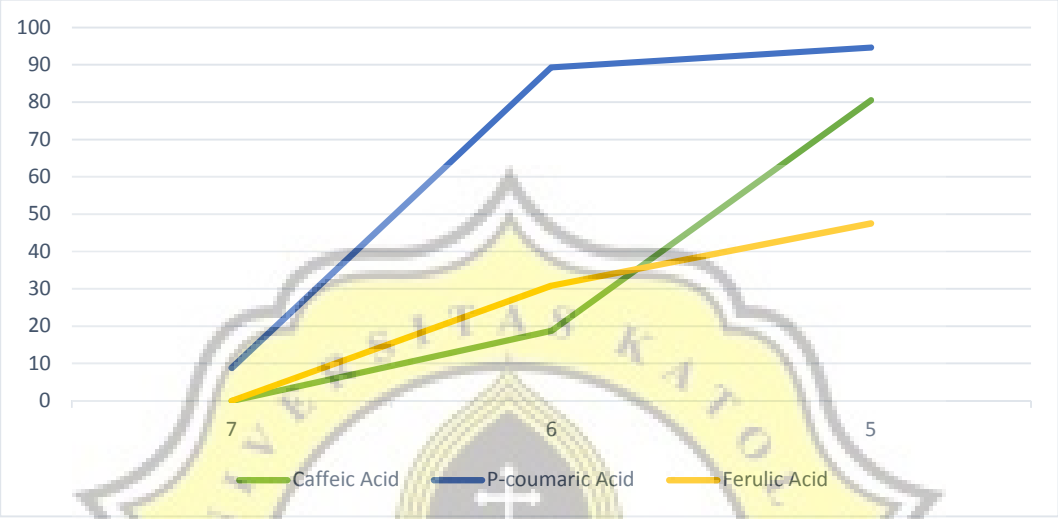
c. Caffeic Acid (3,4-dihydroxycinnamic acids) (λ=320 nm)

Antibacterial activity of caffeic acid are presented in **Table 3.** Other than bacteria, caffeic acid are able to inhibit the production of aflatoxin B_1 and G_1 at 1.8 mg/mL concentration. The concentration of caffeic acid on roasted coffee itself is very low (3 mg/g reported) or even none in the case of severe roasting that cause a fewer studies on caffeic acid. On the other hand, caffeic acid has been reported with synergistic effect toward caffeine and other bioactive compounds (Martínez-Tome *et al*., 2011; Farah *et al*., 2006; Franca *et al*., 2005; Nartowicz *et al*., 1979)

As hydroxycinammic acid, caffeic acid has propenoic acid chain that is less polar than other bioactive compounds. As previously mentioned, less polar compounds have better antibacterial activity as it can assist molecules transport easily across cell membranes. However, there are result showing intermediate polarity phenolic acid with higher activity (p-coumaric in comparison to caffeic and ferulic acid) thus the extensive research is needed to confirm this theory (Campos *et al*., 2003).

The other hydroxycinammic acid such as ferulic acid and p-coumaric acid are reported in numerous research as well, although not as much as caffeic acid. Towards *E. coli, S. aureus* and *B. cereus*, p-coumaric acid act as the most effective inhibitor rather than caffeic and ferulic acid. Generally, hydroxycinammic acid antibacterial activity has better activity at pH 5 rather than 7 that can be seen on the graphs. Hydroxycinammic acid has pKa near 4.5 and as been mentioned above, the nearer pH to the pKa, the higher level of the undissociated acid present. This makes

majority of hydroxycinammic acid and other phenolic compounds have better anibacterial activity at acid environment. Both ferulic acid and caffeic acid have no antibacterial activity at pH 7, in comparison p-coumaric acid exert a significant activity that imply p-coumaric as the most effective compound. The data on this activity can be seen on **Figure 3.** (Herald and Davidson, 1983).



Note : Data from the first 3 hour, the use of 500 µg/mL hydroxycinammic compounds

Figure 3. Hydroxycinammic Acid Inhibition Toward *E. coli*

While some of the hydroxycinammic acid emit low to none antibacterial activity, it been reported that the alkyl esters of those hydroxycinammic acid have better antibacterial activity. Alkyl esters of caffeic, cinamic and p-coumaric acid give the same effectiveness at 50 ppm in comparison to their free acids at 400 ppm. The increasing activity may relate to the ionization effects (the process of hydroxylation). In the opposite, methoxylation of alkyl cinnamate will decrease the antibacterial activity (Baranowski and Nagel, 1982).

d. Nicotinic Acid (λ=260 nm)

This compound is not present in green bean however it is present in very small amount on light and medium roasted coffee. The commercial coffee contain 9.6 – 30.4 mg/100 g while instant coffee contain 35.2 mg/100 g (Perrone *et al*., 2008). The formation of nicotinic acid as the explanation above depends on the trigonelline

degradation that occur on roasting process (Antonio *et al*., 2010). As trigonelline act as the precursor, nicotinic acid is contained in higher level on Arabica as well (Daglia *et al*., 1994). Nicotinic acid is reported to inhibit streptococcal sucrose-independent adsorption to hydroxyapatite beads causing bacteriostatic activity toward *S. mutans* (Daglia *et al*., 2002)

3.3.2. Melanoidin

Melanoidin as brown anionic polymeric is the final product of Maillard reaction occurring on thermally processed foods. Maillard reaction itself is a reaction between an amino group (free and/or protein bound) and carbonyl containing compounds such as reducing sugars and ascorbic acid that occurs in the presence of heat (Maletta and Were, 2012; Ames, 1992).

Melanoidin's structure can't be defined yet because the structure itself will vary according to the preliminary material, applied temperature, heating time, raw material water activity, pH, chemical composition and natural reagent present in the solution. Basically, melanoidin will contain nitrogen in their structure with carbohydrate or phenolic residues such as chlorogenic acid (Castillo *et al*., 2007; Friedman, 1996). The presence of chlorogenic acid or other phenolic in the structure result in melanoidin with better antibacterial activity. In this case, green coffee bean with high amount of phenolic has been used to generate melanoidin with better antibacterial activity (Borrelli *et al*., 2002)

Maillard reaction products have been well known for numerous functional properties such as antioxidant, antihypertensive and antimicrobial activity (Vignoli *et al*., 2011; Rufián and Cueva, 2009). At 1955, it been reported that oxidised orange juice (already turned to brown color) can't be fermented by *Saccharomyces ellipsoideus* (Einarsson, Snygg, & Eriksson, 1983). There also reported the use of them as a component for active packaging materials with a positive result on *E. coli* (Hauser *et al*., 2014; Stauder *et al*., 2010; Fuster *et al*., 2000; Hofmann, 1998; Daglia *et al*., 1994). *In vitro* experiments have reported the capability of hingut microflora to metabolize melanoidin therefore it can be safely use on food product (Rurian and Morales, 2008).

Melanoidin act as the representative for high molecular compounds of roasted coffee Maillard reaction product. Generally, Maillard reaction products are divided into two based on their molecular weight, low molecular weight (LMW) compounds with molecular weights less than 1000 Da and melanoidin assumed to weight up to 100000 Da. LMW compounds such as heterocycles amines, furans (hydroxymethylfurfural) and alfa dicarbonyl are the representative of LMW maillard reaction product. Those compounds contribute up to 40% of roasted coffee maillard reaction products and generally is a polar substances (Rurian and Morales, 2008).

High molecular weight of melanoidin contribute up to 25% of coffee dry matter and regular coffee brew reported to contained 2-4 mg/mL of melanoidin (Moreira *et al*., 2012; Nicoli *et al*., 1997). Numerous research has been conducted toward maillard reaction product (MRP), either with food produced MRP or made by model systems. Within different combination of amino acid and sugar available in the model system, some of the combination resulting in better antibacterial activity than the other. The comparison between arginine-xylose (AX) and histidine-glucose (HG) showed higher antibacterial activity of HG than AX combination (Stauder *et al*., 2010; Bekedam *et al*., 2007; Somoza, 2005; Morales, 2002; Ames, 1992; Einarsson *et al*., 1983).

As the same with the other antibacterial compounds, melanoidin is found to be more effective toward gram positive bacteria than gram negative as can be seen in **Table 4**.

Table 4. Minimum Inhibition Concentration of Melanoidin

Bacteria	Strain	Gram	Siderophore Production	MIC (mg/mL)	References
Escherichia coli	ATCC 35150	-	-	5.0	(Rufián and Cueva, 2009)
	ATCC 33475	-	+	8.0	(Rufián and Cueva, 2009)
Pseudomonas aeruginosa	ATCC 27856	-	-	4.0	(Rufián and Cueva, 2009)
	ATCC 15692	-	+	7.0	(Rufián and Cueva, 2009)
Proteus mirabilis	ATCC 7002	-	-	4.5	(Rufián and Cueva, 2009)

Table 4.(continued)

Salmonella typhimurium	ATCC 13311	-	-	4.5	(Rufián and Cueva, 2009)
Bacillus cereus	ATCC 11778	+	-	2.5	(Rufián and Cueva, 2009)
Stapyhlococcus aureus	ATCC 25923	+	-	2.0	(Rufián and Cueva, 2009)

Note: Isolated from Arabica, commercial roasted (medium to dark), freeze dried/lyophilized before analysis

At lower level of MIC concentration, melanoidin and other secondary product of Maillard reaction exert bacteriostatic activity. Bacteriostatic activity means that it's able to prolong the lag phase, but can't affect the maximum growth rate and final concentration of bacteria. Bacteriostatic activity of melanoidin has been reported with lower concentration (higher activity) compared to other coffee antibacterial compounds such as chlorogenic acid and caffeine. This comparation is done toward *Escherichia coli, Bacillus subtillis* and *Staphylococcus aureus* (Rurian and Morales, 2008; Rufián and Morales, 2006; Einarsson *et al*., 1983). At 2.5 mg/mL, melanoidin able to prolong *E. coli* lag phase and cause irreversible cell damage at 5 mg/mL (MIC). The inhibition toward *Streptococcus mutans* attachment to saliva-coated hydroxyapatite (90% of inhibition) and biofilm formation (100% inhibition) has been found in 5.92 mg/mL (twice the concentration of normal coffee) (Stauder *et al*., 2010; Rufián and Cueva, 2009; Rurian and Morales, 2008; Rufián and Morales, 2006).

A step from that, there are certain strain that generates siderophore (such as *E. coli* ATCC 33475 and *P. aeruginosa* ATCC 15692) during iron (Fe) deficiency (start at 23% of deficiency) that cause those strains to possess higher resistance. The starvation of this iron itself resulting in the decline of bacterial growth as Fe acts as the main protector of bacteria. Within those strains, siderophore will form tight stable complexes with ferric ion that aid those strain to colonize at adverse media such as the humand body. The presence of siderophore with ferric ions provide bacteria a capability to endure the direct attack from host defence mechanisms or to endure from nutrient starvation are resulting from this cation as the major factor (Ratledge and Dover, 2000). In the other hand, the presence of melanoidin will act as competitor toward siderophore to bind metals ion, including ferric ion (Rufián and Cueva, 2009).

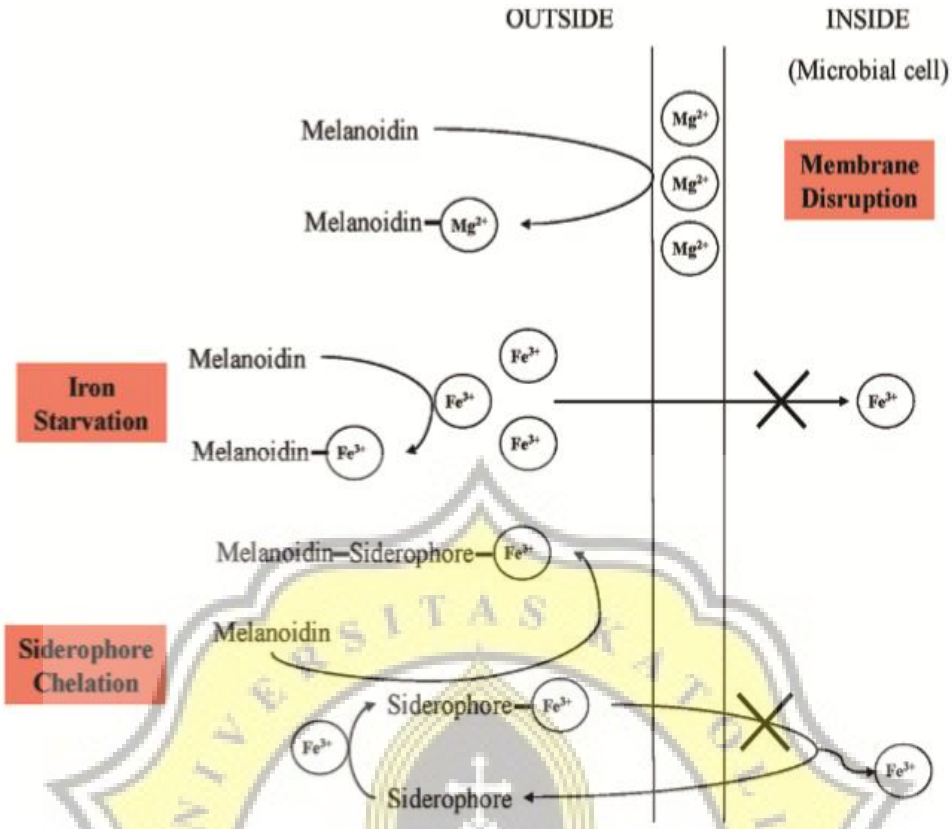


Figure 4. Melanoidin Antibacterial Activity Mechanisms (Siderophore Strain)

The use of low concentration of melanoidin (1 mg/mL used) resulting in the loss of melanoidin iron binding capability because of siderophore strong binding power. It is possible for siderophore to detach iron from molecules including the iron bound to melanoidin (Miethke and Marahiel, 2007; Winkelmann and Caranno, 1997). In medium concentration, melanoidin is able to chelate siderophores-Fe^{3+} complex and in high concentration melanoidin is capable to chelate siderophores-Fe^{3+} or siderophores-Mg^{2+} complex by non-covalent interactions with a recorded result of 5 mg/mL of melanoidin that able to chelate 97.2% of siderophores (bactericidal). The binding properties of melanoidin is reported in numerous studies as it showed high capability of binding properties toward low molecular weight compounds such as phenol and furfural derivatives (Rufián and Cueva, 2009) The comparison between the same bacteria with and without siderophore production can be seen at **Table 4** and the mechanisms can be seen on **Figure 4.**

Other than melanoidin antibacterial mechanisms toward siderophore-forming bacteria, other mechanisms of melanoidin has been reported. Metal chelating activity is the recently reported activity at which point melanoidin can bind numerous metal and form stable complexes with those metal that is essential for the growth of pathogenic bacteria, such as Fe, Zn, Cu, Mg. (Morales *et al.*, 2005; Nicoli *et al*., 1997; Homma and Murata, 1995; Einarsson *et al*., 1983; Gomyo and Horikoshi, 1976). This has been proven by the application of melanoidin toward magnesium (Rurian and Morales, 2008) and iron (Borrelli *et al*., 2002). In pH 5, melanoidin at 2.5 mg/mL concentration are able to chelate 50 µg of iron either from culture. The more acidic the culture, the higher capability of melanoidin to bind with metal. Another important factor is the presence of other ion in the culture that may compete with the specific metal toward coordination sites. Chelated metal can be identified as melanoidin-metal complex in the culture as well with the release of intracellular fluid (Rurian and Morales, 2008). The presence of ketone and hydroxyl groups of pyranone or pyridine in melanoidin's structure will aid the metal chelation as well (Fujimaki *et al*., 1986).

The other mechanisms are related to melanoidin negative charges. As melanoidin structure is determined by the parent reactants, it been reported that melanoidin generated from 5-HMF (5-hydroxymethylfurfural) with glycine possess a partially anionic character. 5-HMF is a product from sugar degradation whereas the most abundant sugar in coffee are cellulose, galactomannans and arabinogalactan type II. The arabinogalactan type II (Arabinogalactan proteins) are acidic in the cell wall of coffee beans. Other than that, melanoidin's negative charges may result from the covalent or ionic binding with chlorogenic acid as well (Stauder *et al*., 2010; Bekedam *et al*., 2007).

In the case of gram negative bacteria, lipopolysacharides (LPs) and protein constituted on the outer membrane were balanced by divalent cations that comprise as metal ions bridges between phosphate groups and phospholipids, therefore balanced the outer membrane. The negative charge of melanoidin will bind the cations from their binding sites resulting in the loss of membrane barrier function, cell rupture and blockage of nutrient flow as well (bactericide) (Moreira *et al*., 2012; Morales, 2002; Ibrahim *et al*.,

2000) The rupture itself will cause a disorder in cell substances as intracellular components such as DNA or RNA can be found on the culture. DNA or RNA on culture can be easily detected at 260 nm (used as indication of membrane damage) (Rurian and Morales, 2008; Chen and Cooper, 2002; Vaara, 1992).

After disrupting outer membrane, melanoidin will gain inner membrane (IM) access where melanoidin cause the discharge of cytoplasmic enzyme immediately. The concentration reported is 10 mg/mL that result in complete discharged of intracellular compounds in 60 minutes, resulting in cell dissolution. Both of activity toward outer and inner membrane indicated the mechanisms of melanoidin to disrupt bacterial membranes and interfere with biosynthetic processes at the membrane, such as transport of nutrient and macromolecules precursors that associated with bacterial viability. One of those activities is the interference of sugar catabolizing enzymes and intercepting serine, oxygen and glucose uptake (Rurian and Morales, 2008; Bekedam *et al*., 2007; Lanciotti *et al*., 1999; Tiina, 1989; Stecchini *et al*., 1991).

3.3.3. Caffeine

Caffeine is white alkaloid compounds that has been widely known with the worldwide consumption of 120.000 ton. Average US daily consumption of caffeine is about 200 mg, with adults taking about 2.4 mg/kg per day and children aged between 5 and 18 years old taking 1.1 mg/kg per day. The safety bar for caffeine consumption is 150-200 mg/kg (LD_{50}) in humans, depending on individual sensitivity and any medications that inhibit the metabolism of caffeine. It been reported that a daily consumption of 500-600 mg over some period of time can increase the risk of developing caffeinism that have adverse reaction to health such as muscle tremor, diuresis, cardiovascular symptoms, etc. (Rahman *et al*., 2014).

There has been numerous contradicting statements regarding the caffeine antibacterial activity. While (Corrao *et al*., 2001) reported that caffeine have no contribution toward roasted coffee antibacterial activity, (Antonio *et al*., 2010) reporting its inhibition toward *Streptococcus mutans* biofilm formation. Another author reported that caffeine are effective against *Streptococcus mutans*, but have no activity toward *Staphylococcus*

aureus (Daglia et al., 2007). This is regarding to the missing antibacterial activity with the elimination of caffeine in decaffeinate coffee. However, it is important to consider that the elimination of other antibacterial compounds is possible during decaffeination process especially with the use of non-specific solvent (Antonio *et al*., 2010).

Caffeine antibacterial activity toward numerous *Enterobacteria* (± 7-8 mm of inhibition zones) had been confirmed with weaker activity in comparison to other coffee antibacterial compounds, such as chlorogenic acid and trigonelline (Almeida *et al*., 2006). Despite the fact that caffeine possesses low antibacterial activity, it been reported caffeine are able to enhance the activity of other compounds such as alfa dicarbonyl compounds (glyoxal, methylglyoxal, diacetyl) (Daglia *et al*., 2007) and antibiotics (penicillin G and tetracycline (increased by the factor of 4) (Hosseinzadeh *et al*., 2006; Charles and Rawal, 1973) carbenicillin and ceftizoxime (Rahman *et al*., 2014)). Those synergistic activity is showed even at low concentration (5 mg/mL) (Antonio *et al*., 2010; Daglia *et al*., 2007).

Caffeine antibacterial mechanisms is to bind specifically with single stranded DNA that inhibit cell repair mechanisms. The other mechanism is to inhibit the synthesis of proteins and DNA by adenine and thymidine assimilation with enhanced genotoxicity after DNA at concentrations of 0.1%. The use of those concentrations are based on caffeine common concentration on food products that range from 0.01% - 1.00%. The other author reported bacteriostatic activity toward *E. coli* in the use of 0.5% caffeine on media. Afterward, each addition of 1% caused reduction of bacteria cells by an average of 2 log CFU/mL (Maletta and Were, 2012; Ibrahim *et al*., 2006; Kaufmann *et al*., 2003; Labbe and LL, 1987). Other antibacterial activity of caffeine can be seen in the **Table 5.**

Table 5. Caffeine Antibacterial Activity toward Numerous Bacteria

Bacteria	Gram (+/-)	Inhibition Zones (mm)	MIC (mg/ml)	Additional Information	References
Streptococcus mutans	+	7.5 ± 0,5	-	-	(Almeida *et al*., 2012)
		-	5.0 ± 0.2	-	(Daglia *et al*., 2007)
		-	0.8	-	(Antonio *et al*., 2010)
Staphylococcus aureus	+	-	>25	-	(Runti *et al*., 2015)
		12.5	125	Have 3-4 mm difference in inhibition zones to 10 µg gentamycin, unroasted coffee bean	(Al-Bayati and Mohammed, 2009)
Bacillus cereus	+	13.1	125	Have 3-4 mm difference in inhibition zones to 10 µg gentamycin, unroasted coffee bean	(Al-Bayati and Mohammed, 2009)
Lactobacillus pneumophila	+	-	IC_{90} : 0.625	-	(Almeida et al., 2006)
Streptococcus mutans	+	7.5 ± 0.6	-	-	(Almeida *et al*., 2012)
Staphylococcus epidermidis	+	-	25	-	(Runti *et al*., 2015)
Enterococcus faecalis	+	-	12	-	(Runti *et al*., 2015)
Serratia marcescens	-	7.2 ± 0.3	-	-	(Almeida *et al*., 2006)
Citrobacter freundii	-	7.0 ± 0.0	-	-	(Almeida *et al*., 2006)
Escherichia aerogenes	-	7.0 ± 0.0	-	-	(Almeida *et al*., 2006)
Escherichia cloacae	-	7.7 ± 1.2	-	-	(Almeida *et al*., 2006)
Escherichia coli	-	8.0 ± 1.0	-	-	(Almeida *et al*., 2006)
		-	3	-	(Runti *et al*., 2015)
		11.4	250	Have 3-4 mm difference in inhibition zones to 10 µg gentamycin, unroasted coffee bean	(Al-Bayati and Mohammed, 2009)

Table 5. (continued)

Klebsiella oxytoca	-	7.0 ± 0.0	-	-	(Almeida *et al*., 2006)
Proteus hauseri	-	9.7 ± 1.5	-	-	(Almeida *et al*., 2006)
Proteus mirabilis	-	7.3 ± 0.6	-	-	(Al-Bayati and Mohammed, 2009; Almeida *et al*., 2006)
		-	>1000	-	
Salmonella enterica	-	8.3 ± 0.6	IC_{90} : 2.6	-	(Almeida et al., 2006)
		-	>25	-	(Runti *et al*., 2015)
Pseudomonas aeruginosa	-	13.7	62.5	The use of caffeine (2 mg/ml) have similar result to gentamycin (antibiotic) (10 µg/ml) → 14.2 mm	(Al-Bayati and Mohammed, 2009)

Other than antibacterial activity, caffeine as antimicrobial compounds had been found to inhibit filamentous fungi and aflatoxin productions. It been reported that 2 mg/g of caffeine are able to inhibit the formation of aflatoxin. To be noted that caffeine content on regular coffee ranges from 10 – 20 mg/g with higher level on Canephora (17,01 mg/g) while Arabica contain 8-9 mg/g. Instant coffee is reported to have high levels of caffeine, as much as 21 mg/g. One cup of coffee contains different level of caffeine, ranging from 58-322 mg per cup, depending on the roasted coffee composition and barista techniques (Ludwig *et al*., 2014 Al-Bayati and Mohammed, 2009; Wang *et al.*, 2008; Perrone *et al*., 2008; Nartowicz *et al*., 1979; Daglia *et al*., 1994).

Despite of different result of antibacterial test, either toward caffeine or other coffee compounds or coffee extract, the outcome can be affected by many factors such as extracting method, volume of inoculum, culture medium used, pH of the media, volume of inoculum, and incubation time and temperature. Each of the research is different in many parts of test that may affect the result as well, for that reason the comparison of the data may be complicated. Other than that, bacterial resistance may be obtained through gene encoding enzymes that impair antibacterial agent, efflux pumps that force out antibacterial agent from the cell, certain metabolic pathway genes that yield substance that may alter antibacterial agent binding site on bacterial cell, and downregulation of porin genes causing agent limitation (mutation). One of them is the

possession of the *luxS* quorum sensing system by *Streptococcus* that trigger enzyme production which able to neutralize antibacterial compounds toxicity (Rahman *et al*., 2014; Al-Bayati and Mohammed, 2009; Tenover, 2006; Almeida *et al*., 2006).

3.3.4. Other Maillard reaction compounds

a. Hydrogen Peroxide (H_2O_2)

The assumption of hydrogen peroxide in roasted coffee arise by the addition of catalase which fully suspend coffee antibacterial activity. Catalase is known as an enzyme that decompose hydrogen peroxide into water and oxygen, thus eliminating the antibacterial activity. This studies then lead to the conclusion of H_2O_2 as major antibacterial compounds of roasted coffee, opposing to the other studies. Conversely, roasted coffee antibacterial compounds such as phenolic and melanoidin is stated to not contribute toward the antibacterial properties, but rather act as indicators of roasting degree (Mueller *et al*., 2011).

The formation of hydrogen peroxide is observed during the incubation time of roasted coffee in warehouse. It formed by the reduction of atmospheric oxygen by thermally degradated polyphenols in the presence of transition metals (Nicoli *et al*., 1997). Act as MRP, it been found that specific amino-reductone structure are the one responsible for H_2O_2 forming. Related to this, H_2O_2 formation will increase positively by the rising coffee concentration. The application of H_2O_2 on catalase positive bacteria leads to H_2O_2 detoxification as it's able to produce catalase on its own. Thus it is important to investigate those kind of bacteria and use high concentrations of roasted coffee (higher concentration of H_2O_2) to overcompensate bacterial catalase activity (Mueller *et al*., 2011)

Pure H_2O_2 showed a bacteriocidic effect on 1000 µM toward *Escherichia coli* and *Listeria spp.* while the initial H_2O_2 on coffee is reported for 290 µM. Upon incubation, H_2O_2 concentration will rise to 830 µM after 8 hours that exert bacteriocidic effect, which is higher than pure H_2O_2. This is found to be related

to synergistic effect toward other natural compounds, one which able to enhance H_2O_2 antibacterial activity (Mueller *et al*., 2011).

b. Alfa-dicarbonyl (glyoxal, methylglyoxal, diacetyl)

Alfa-dicarbonyl is one of the low molecular weight (less than 50 kDa) compounds of Maillard reaction product where the recent research found that these compounds responsible for roasted coffee antibacterial activity (been reported responsible for 50% of roasted coffee antibacterial activity). The other compound is 5-hydroxymethyl-furfuraldehyde that will explain at the latter section (Daglia *et al*., 2007).

Alfa-dicarbonyl is produced by multiple fragmentation of sugar moiety with weak acidic properties. The normal concentration of this compound on coffee brew is 4.73 µg/mL for glyoxal; 11.64 µg/mL for methylgyoxal, and 2.72 µg/mL for diacetyl. As these compound are formed by the heat, it been reported that alfa dicarbonyl, especially methylglyoxal are stable during the cooking process that give a huge advantage for the use of this compounds on pre-cook food product. On certain product, especially meat, the heating itself produces alfa dicarbonyl compounds as well (Maletta and Were, 2012; Daglia *et al*., 2007; Daglia *et al*., 1998; Antal *et al*., 1990).

The acidic properties of coffee, especially roasted coffee have been reported to assist in increasing the antibacterial activity. Other than the presence of phenolic acid, alfa-dicarbonyl compounds are contributing in these properties as well where methylglyoxal have pH 1.92 and glyoxal have pH 2.10 (Maletta and Were, 2012). This may explain the change in pH value (acidic) during the roasting process. There are three phase on roasting that are drying, roasting/pyrolysis and cooling. Initially, pH will increase to 4.3 and then slowly decrease until the beginning of pyrolysis phase. In the pyrolysis phase, sharp decrease of pH is reported to 3.3 (Franca *et al.*, 2005; Dutra *et al*., 2001).

All of α-dicarbonyl compounds were active strongly toward *S. mutans* in comparison with *S. aureus* within the finding of Daglia *et al*. (2007). *S. mutans* have higher sensitivity with MIC range from 11.8 – 34.3 μg/mL with comparison of 88.3-114.2 μg/mL for *S. aureus*. Another research reported its inhibition activity toward *Salmonella typhimurium* and *Salmonella enteridis* growth on ground chicken breast. The use of 2.28 mg methylglyoxal and 0.97 mg glyoxal per gram of chicken meat lower the growth of respective bacteria by 1.25-3.02 Log CFU/g (Maletta and Were, 2012). While the use of whole α-dicarbonyl compounds has been reported, the single use of diacetyl have been reported as well toward *S. typhimurium* and *E. coli* with the minimum concentration (MIC) 1.076 and 0.646 mg/ml correspondently (Olasupo *et al*., 2003).

Within those research data reported, majority reported an increase in concentration tends to give higher antibacterial activity. However, Maletta and Were (2012) reported different result regarding the increasing dose of concentsration. It been reported that at certain level, increasing concentration doesn't give a significant increase in antibacterial activity. This may related to the different use of media (chicken breast) and bacteria. Moreover, further research is needed to investigate the possible factor that affect alfa dicarbonyl concentration limit on antibacterial activity (Maletta and Were, 2012; Antonio *et al*., 2010).

Based on the single use, methylglyoxal held a superior activity compare to glyoxal. However, the safety concern toward this compound especially methylglyoxal arise with the reactivity and role in the formation of advanced glycation end products. The safety level for human has not been determined, but the data from the use in rats is an oral LD_{50} of 1165 mg/kg. As *in vivo*, alfa dicarbonyl compounds are detoxified by the glyoxalase to minimizing the toxic effect (carcinogenic effect) (Maletta and Were, 2012).

c. 5-hydroxymethyl-furfuraldehyde (5-HMF) (λ=272 nm)

Furans such as 5-HMF are sugar degradation product that formed specifically through hexoses-amines dehydration (Maillard reaction) or carbohydrate caramelization (Antal *et al*., 1990). Furans itself is the most abundant volatile chemicals in roasted coffee that responsible for coffee strong flavour. Arabica that known as the superior on flavour term have a high amount of this compound (Fuster *et al*., 2000; Daglia *et al*., 1994).

As this compound formed as a sugar degradation product during roasting process, it only found in roasted coffee bean, especially on light and medium roasted coffee. This compounds have been reported with weak activity toward *S. mutans* (2.8 mg/mL) and *S. aureus* (8.4 mg/mL). The normal concentration of this compound on coffee brew is 180.92 µg/mL where it exhibits no antibacterial activity of those concentration (Daglia *et al*., 2007).

3.4. Synergism Interaction on Antibacterial Compounds

Davidson and Parish (1989) mentioned that synergism effect are observed when the antibacterial activity of the combined substances is greater than the activity of individual substance. This particular effect on roasted coffee is observed by Daglia *et al.* (2007) that reported the enhancing effect of caffeine towards alfa dicarbonyl compound. Conversely, caffeine cause a reduction in alfa dicarbonyl compound mixture MIC values in comparison to the original values. This effect is observed even when caffeine is added in low concentration (5 mg/mL, correspondent to caffeine remained volume in roasted coffee). This finding is also supported by Malleta and Were (2012) that observed the increasing effectivity of several alfa dicarbonyl compounds by the addition of caffeine. Within the growth on chicken breast, the use of caffeine together with methylglyoxal deliver an increase in antibacterial activity toward *S. thypimurium* and the opposite effect toward *S. enteridis*. However, both enhancing effects of caffeine were observed in the combination with glyoxal with higher effect on *S. enteridis*. This showed that caffeine enhancing effect depends greatly on bacteria strain (Maletta and Were, 2012).

Other possible synergism interaction is related to the acidity of alfa dicarbonyl compounds. As mentioned before, alfa-dicarbonyl compounds have quite low value of pH, such as methylglyoxal with pH 1.92 and glyoxal with pH 2.10 (Olasupo *et al.*, 2003). On other hand, antibacterial properties of phenolic compounds is known to increase within the acid environment. In acid environment, phenolic compounds tend to exert more undisocciated acid as they have pKa value on acid range. Herald and Davidson (1983) explained that undissociated acid have better permeability on bacteria membrane as it is readily soluble in phospholipid membrane. Moreover, Baranowski and Nagel (1982) have also observed an increase in hydroxinammic acid antibacterial properties at pH 5 rather than pH 6 and 7 (Herald and Davidson, 1983).

The possible synergism interaction is observed on hydrogen peroxide as well. Mueller *et al.* (2011) reported that hydrogen peroxide extracted from roasted coffee have higher activity against bacteria than the pure compound. Therefore, further investigation is required to determine the compound responsible for the synergistic effect.