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Caffeine in foods and its antimicrobial activity

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Abstract

Caffeine is a widely known food constituent. Coffee and tea are major sources of caffeine. However, there is little data about caffeine's antimicrobial activity. Although caffeine is safe for the consumer and suitable as an antimicrobial agent in food, ingesting a lot of caffeine will affect the body (>400 mg day^{-1}). As a result, legislation has defined the amount of caffeine to be used in various food products. In term of antimicrobial activity, caffeine at concentration from 62.5 to >2,000 μg•ml^{-1} could inhibit bacteria. In addition, higher amount of caffeine (>5,000 μg•ml^{-1}) was found to inhibit mold growth.

Introduction

Natural coffee and tea contain some antimicrobial substances that could inhibit a variety of microorganism including pathogens (Pane *et al*., 2012; Chen *et al*., 2013). Caffeine was reported to inhibit mold (Suárez-Quiroz *et al*., 2004) and bacteria (Sandlie *et al*., 1980; Almeida *et al*., 2012). Therefore, the objective of this paper is to review the antimicrobial activity of caffeine extracted from these plants.

Definition of caffeine

Caffeine (1,3,7-trimethylxanthine, $C_8H_{10}N_4O_2$) (Figure 1) is a natural alkaloid or xanthine alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts and other plants It is one of the most widely used pharmacological substances in the world. Caffeine can be taken into the body by drinking coffee, tea, cocoa and cola and it is also used as component (Nawrot *et al*., 2003).

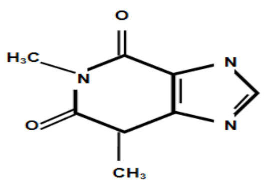

Figure 1. The chemical structure of caffeine

Natural sources of caffeine

Caffeine is a substance that can be found in various plants and beverages. Beverages such as coffee, tea, cocoa and soft drinks are the most produced from coffee and tea plants. Different caffeine concentrations in raw materials and food products are shown in Table 1.

Coffee

Coffee is the most importance source of caffeine (Minamisawa *et al*., 2004). Caffeine in coffee depends on the extraction method and types of coffee include fresh or roasted beans. For instant, fresh and roasted green beans of the Arabica genus from Los Altos de Chiapas, Mexico contain 4.00 and 9.33 mg•l^{-1} of caffeine, respectively (Salinas-Vargas *et al*., 2014). On the other hand, the amount of caffeine in Arabica fresh coffee was reported to be from 190 to 456 mg•l^{-1}. Higher concentrations of caffeine in fresh coffee from 314 to 646 mg•l^{-1} were found when using Robusta (Rodrigues *et al*., 2007).

Tea

Tea also provides a significant source of caffeine (Najafia *et al*., 2003). Dried tea contains a higher composition of caffeine when compared to the coffee bean (Chu and Juneja, 1997). Unfortunately, the tea brewing method causes reduced caffeine. The caffeine content in tea is also related to the tea type

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Table 1. Caffeine content

Products	Caffeine (mg·100g^{-1}food)	References
Coffee		
Instant	59	Burg, 1975
	66	Gilbert *et al.*,1976
	60	Lelo *et al.*,1986
	71	Stavric *et al.*,1988
	60	Barone and Roberts,1996
	71	Nawrot *et al.*, 2003
	75	Knight *et al.*, 2004
Roasted	83	Burg, 1975
	74	Gilbert *et al.*,1976
	80	Lelo *et al.*,1986
	82	Stavric *et al.*,1988
	83	Nawrot *et al.*, 2003
Tea		
Leaf	41	Burg,1975
	30	Barone and Roberts, 1996
	8	Stavric *et al.*,1988
	41	Nawrot *et al.*, 2003
Instant	28	Burg ,1975
	5	Barone and Roberts,1996
	28	Nawrot *et al.*, 2003
	28	Knight et al., 2004
Cocoa	5	Barone and Roberts,1996
	4	Nawrot et al., 2003
	6	Knight, et al., 2004
Chocolate	20	Gilbert et al.,1976
	20	Nawrot et al., 2003

and the extraction method. For dried tea leaves, the caffeine content varies from 1.0% to 3.5% (Fernandez *et al*., 2000). After brewing, caffeine in black tea (30.97 mg•g^{-1}) was found to be higher than green tea (18.70 mg•g^{-1}) and oolong tea (23.89 mg•g^{-1}) (Guo *et al*., 2011).

Cocoa

Cocoa is a source of caffeine (DeVries *et al*., 1981) and is the main ingredient to produce chocolate. Many reports (Zoumas *et al*., 1980; Blauch and Tarke, 1983; Craig and Nguyen, 1984) explained that chocolate products with caffeine such as hot chocolate, chocolate milk and chocolate cake can help to improve alertness and mood when consumed in low doses (<32 mg) (Lieberman *et al*., 1987). Depending on the plantation area, plant type and other factors, the caffeine content in cocoa powder was between 0.66 and 0.71 mg•g^{-1} (Li *et al*., 2012).

Chemical and physical properties

The chemical and physical properties of caffeine are shown in Table 2. Caffeine is an odorless white powder with a bitter taste. It has a density of 1.2 g•cm^{-3} and a neutral pH of 6.9. Solubility caffeine in water is about 21.7 mg•ml^{-1} at 25°C, 180 mg•ml^{-1} at 80°C and 670 mg•ml^{-1} at 100°C. Its boiling point is at 178°C and its melting point is at 238°C.

Caffeine biosynthesis pathway

Caffeine biosynthesis in plants has been reported by Suzuki *et al*. (1992); Ashihara and Crozier (1999); Ashihara *et al*. (2008). The purine alkaloid metabolism is used for the biosynthesis of caffeine in plants. The caffeine biosynthesis pathway in coffee beans, as shown in Figure 2 starts in the created substrate xanthosine which then changes to 7-methylxanthine by the methylxanthine synthase enzyme. Next, it changes into 7-methylxanthosine by the methylxanthosine nucleotidase enzyme and then changes into theobromine by the caffeine synthase enzyme. After that, theobromine changes into caffeine.

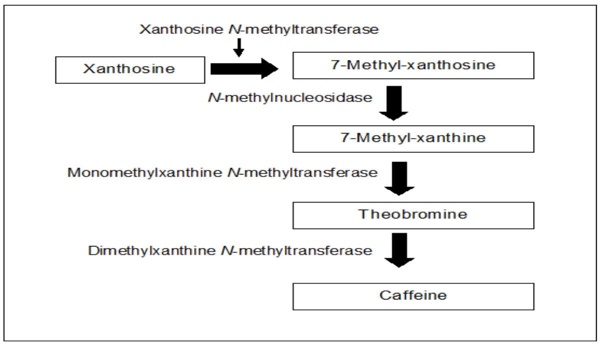

Figure 2. Caffeine biosynthetic pathway

Antimicrobial activity of caffeine in plant extract

In vitro *and* in vivo *test*

Caffeine has been reported to be an inhibitor of microorganism growth in various food crops and food products. The antimicrobial activity of caffeine in coffee and tea extracts is shown in Table 3. Extraction methods include microwave, ultrasonic, soxhlet, heat reflux extraction and solvent soaking (Jun *et al*., 2011). Although caffeine is the one of the main components in coffee and tea extracts, minor components or caffeine derivatives such as xanthine have also been found (Sun *et al*., 2006). Furthermore, various concentrations of theophylline and theobromine (a derivative of caffeine) as alkaloids caffeine were confirmed to be found in roasted coffee and the rough green beans in both Arabica and Robusta coffee (Huck *et al*., 2005). Therefore, Antimicrobial activity with caffeine as the main component and theirs minor components has been demonstrated by literature reviews. Caffeine from *Coffea arabica* (coffee beans) and *Camellia sinensis* (green tea leaves) showed antibacterial activity against gram-positive bacteria e.g. *Staphylococcus aureus* and *Bacillus cereus* and also gram-negative bacteria e.g. *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumonia*. The MIC values ranged from 62.5 to 250.0 µg•ml^{-1} for the coffee caffeine and from 62.5 to 500.0 µg•ml^{-1} for the green tea caffeine (Mohammed and Al-Bayati, 2009). Caffeine from tea *(Camellia sinensis)* can affect the mycelial growth, sporulation and spore germination of *Monacrosporium ambrosium* in media. In addition, concentrations of caffeine from 500 mg•l^{-1} showed a reduction colony size and inhibition of both mycelial

Table 2. Physical and chemical properties of caffeine

Properties of caffeine*[1,2]	Status
Molecular weight	194.191 $g \cdot mol^{-1}$
Physical characteristics	white powder , bitter taste and odorless
Density	1.2 $g \cdot cm^{-3}$ (solid)
Boiling point	178°C (Sublimation)
Melting point	238°C (Anhydrous)
Water solubility	21.7 $mg \cdot ml^{-1}$ (25°C) 180 $mg \cdot ml^{-1}$ (80°C) 670 $mg \cdot ml^{-1}$ (100°C)

*[1)] Florey, 1989; [2)]Agyemang-Yeboah and Oppong, 2013.

growth and spore germination of *Monacrosporium ambrosium* (Savitri Kumar *et al.*, 1995). In 1995, Fardiaz demonstrated that, at concentrations from 2.5 to 10 g•100 ml^{-1}, caffeine from coffee extracts inhibited the growth of *Rhizopus oryzae* on plate count agar. Wilmot (2006) used caffeine from tea extracts to inhibit *Fusarium solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* molds. Results showed that the mold growth percentage on cucumbers, tomatoes, and lettuce were reduced by 77%, 45%, and 50%, respectively. Chen *et al*. (2013) agreed with these results and found that the anti-fungal activity from green tea extracts was due to the caffeine.

Mode of action

Although caffeine from coffee and tea extracts has been recognized for its positive effect on human health, caffeine removal is being considered because of its negative effects. Some eukaryotic and prokaryotic microorganism cells can be degraded by caffeine (Gokulakrishnan *et al*., 2005). Therefore, only the minimum inhibitory concentration (MIC) value was reported from the literature review after using caffeine in coffee and tea extracts against microorganisms.

To explain the mode of action of caffeine against microorganisms, it has been found that caffeine can pass easily to the cell wall of the bacteria. Then, caffeine can begin to inhibit DNA synthesis. Lower DNA causes to lower activity in all bacteria cells. Therefore, enzyme synthesis and protein synthesis does not happen (Sundarraj and Dhala, 1965). However, this inhibition process of caffeine in bacteria can be stopped when caffeine is converted into theobromine and para-xanthine by bacteria enzymes (demethylases) before going to oxidation process (Blecher and Lingens, 1977). In mold, caffeine can inhibit its spore germination (Kumar *et al*., 1995). Therefore, the lag phase of mold spore contamination is extended and is observed. Caffeine degradation of molds (*Aspergillus tamari*, *A. niger*, *A. fumigatus* and *P. commune*) is started when the nitrogen source is insufficient. *Aspergillus* sp. showed the most efficient caffeine degradation (92%) (Hakil *et al*., 1998). Furthermore, caffeine can inhibit aflatoxins from molds by stopping the synthesis of glucose, fructose, and maltose. These sugars are

Table 3. Antimicrobial activity of caffeine in plant extract

Plant	Microorganism	Concentration	Reference
Tea	*Monacrosporium ambrosium*	500 to 2,500 ppm	Kumar *et al.*, 1995
	Fusarium solani, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*	100 to 200 g l^{-1}	Wilmot, 2006
	Mucor piriformis, *Aspergillus niger*, *Alternaria alternate* *Candida albicans*	-	Taley *et al.*, 2012
	Staphylococcus aureus, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*	62.5 to 500 µg ml^{-1}	Mohammed and Al-Bayati, 2009
	Salmonella typhimurium	2.0 mg ml^{-1}	Wu *et al.*, 2007
	Staphylococcus epidermidis *Micrococcus luteus* *Brevibacterium linens* *Pseudomonas fluorescens* *Bacillus subtilis*	0.156 to 0.313 mg ml^{-1}	Sharma *et al.*, 2012
Coffee	*Rhizopus oryzae*	10 g 100 ml^{-1}	Fardiaz, 1995
	Staphylococcus aureus, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*	62.5 to 250 µg ml^{-1}	Mohammed and Al-Bayati, 2009
	Aspergillus tamari *Aspergillus fumigatus* *Aspergillus niger* *Penicillium commune*	-	Hakil *et al.*, 1998
	Aspergillus flavus	-	Buchanan and Lewis,1984
	Streptococcus mutans	2.0 mg ml^{-1}	Antonio *et al.*, 2010; Almeida *et al.*, 2012
	Colletotrichum kahawae	20 $\%wv^{-1}$	Abera *et al.*, 2011

substrate for aflatoxin produce of mold (Buchanan *et al*., 1983; Hasan, 1999; Aneja and Gianfagna, 2001; Holmes *et al*., 2008).

Application of coffee and tea extracts in food

In the last decade, coffee and tea extracts have been researched to find ways to be applied in food products. Fresh coffee is very well known and popular - people around the world drink approximately 2.25 billion cups of coffee per day (Dicum and Luttinger, 1999). The tea market is also very big, especially in Asia. The popularity of tea in some countries has increased by more than 500% in the last ten years (New Products Trends, 2013). Of course, consumers know about caffeine in their coffee and tea. The debate regarding the impact of caffeine on human health in dietary items has been discussed and caffeine consumption levels have been set. For example, the amount of caffeine taken per day for pregnant women should be less than 300 mg. For children, intake should be below 2.5 mg•kg^{-1} (Nawrot *et al*., 2003). For normal adults though, levels are difficult to set.

The application of coffee and tea extracts against microorganisms continues to be looked at. For example, if there is a *Vibrio parahaemolyticus* contamination, a treatment of green tea extract could be applied to extend the shelf life of Pacific oysters *(Crassostrea gigas)* at the oyster per tea extract ratio of approximately 0.7 g•ml^{-1} during refrigeration storage (Xi *et al*., 2012). Also, the use of an edible film produced from tapioca starch with green tea extract can possibly reduce gram positive bacteria (e.g. *L. monocytogenes*, *B. cereus* and *S. aureus*),

Table 4. List of some foods with caffeine

Food	Caffeine (mg·250 ml^{-1}/g^{-1})	References
Espresso coffee (black coffee)	380-895	Desbrow *et al.*, 2012
Coffee-flavored milk	26-71	Desbrow *et al.*, 2012
Cappuccino or Latte	45-75	Health Canada, 2013
Iced tea	9-50	Knight *et al.*, 2004
Black tea	28-46	Bunker and McWilliams, 1979
Green tea	24.8	Mitchell *et al.*, 2014
White tea	15.2	Mitchell *et al.*, 2014
Lemon tea	31	Health Canada, 2013
Cocoa beverage	6	Knight *et al.*, 2004
Milk chocolate	54	Barone and Roberts, 1996
Baking chocolate	312	Barone and Roberts, 1996
Sweet chocolate	179	Zoumas *et al.*, 1980
Pepsi Cola	20	Chou, 1992

mold growth and yeast on fruit-based salads, romaine hearts, and pork slices by 1-2 log cycles (Chiu and Lai, 2010). And bioactive film incorporated with green tea extract and *Lactobacillus paracasei* L26, and *Bifidobacterium lactis* B94 were applied on hake fillets in order to evaluate the effect of the films during 15 days of storage. Results demonstrated that films with green tea and probiotics were able to extend the shelf life of hake for at least a week (Lacey *et al.*, 2014).

There are various food products based on coffee extract such as coffee cake, coffee-flavored milk. In Australia, espresso coffee and coffee-flavored milk were analyzed for their caffeine content. The concentration of caffeine from espresso coffee and coffee-flavored milk were 2,550 ± 1,030 mg•l^{-1} and 193 ± 90 mg•l^{-1}, respectively (Desbrow *et al.* 2012). For more about caffeine content in other food products is shown in Table 4. Table 3 and Table 4 indicate that consumption of food products containing caffeine may help to decrease a number of pathogenic microorganisms. However, more works on human laboratory test need to be performed.

Caffeine and food laws

According to some effects of caffeine on human behavior, food laws about caffeine are now being discussed for the safety of consumers. Nawrot *et al.* (2003) indicated that for the healthy adult, consumption of caffeine at a dose up to 400 mg a day does not affect general toxicity, cardiovascular effects, bone status and calcium balance. However, consumption of less than 300 mg per day is advised for woman and children. The caffeine limits for food products in various countries are found. In Thailand, the amount of caffeine in coffee is limited to less than 1000 mg•l^{-1}. However, there is no control of the caffeine limit in tea and tea products in Thailand (Thai Government Gazette, 2002a,b). In comparison with the EU food standards, Australia and New Zealand and Canada lower concentration (<150 mg•l^{-1}, 320 mg•l^{-1}, 400 mg•l^{-1} respectively) of caffeine is allowed in coffee and tea (Commission of the European Communities, 2002; Food Standards Australia New Zealand, 2013; Health Canada, 2013). On the other hand, no specific recommendations for caffeine intakes in the U.S., the FDA released a letter in August 2012 stating that for healthy adults, caffeine intake up to 400 mg•day^{-1} is not associated with adverse health effects (USFDA, 2012). Caffeine can be found naturally in more than 60 species of plants, especially in coffee and tea. It can be used to inhibit bacteria, yeast and mold growth. Food products around the world containing caffeine are accepted by consumers and business is increasing. However, various food law standards from various countries are used to control caffeine in food products.

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