Food ScienTech Journal Vol. 1 (2) 2019 ISSN : 2685-4279 DOI:10.33512/fsj.v1i2.6427

A SHORT REVIEW OF BONGKREKIC ACID IN FOOD SAFETY PERSPECTIVE

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ABSTRACT

Food safety is one of the concern today for consumer and producer of food products. One of its aspect is the availability of dangerous toxin. Bongkrekic acid belongs to foodborne toxin commonly produced by bacteria *Burkholderia cocovenenans*. This toxin's name comes from Indonesian local food, tempe bongkrek, and made several outbreaks in Indonesia with casualties. Bongkrekic acid causes lethal food poisoning which is associated with hyperglycemia. Studies of the bacteria and toxin itself had developed the strategies to prevent the outbreaks. Supported by the hygiene and technologies in parts of the world, bongkrekic acid could be considered under control in the perspective of food safety today.

Keywords: Bongkrekic Acid, Food Safety, Toxin, Tempe

INTRODUCTION

The quality of the foodstuff is measured by many aspects, one of it is food safety. Grunert (2005) distinguished two schools of thought about quality. The first one, the holistic approach, equates quality with all the desirable properties a product is perceived to have. The second, the excellence approach, suggests that products can have desirable properties that consumers, in their own language, may not view as part of quality. Food safety is part of food quality based on the holistic approach.

Microbial indicators are oftenly used to assess food safety and sanitation (Jay, 2000). Presence of metabolites or toxins from specific microorganisms are parts of the microbial indicators for the food safety. Uniquely, bongkrekic acid is a microbial toxin that caused outbreaks but only occurred in very narrow range of foodstuff.

This short review will cover the history, biochemistry, detection, epidemiology, contamination prevention, and regulatory standard of bongkrekic acid.

HISTORY OF BONGKREKIC ACID

The name *bongkrek* comes from Indonesia's most famous (or infamous) types of solid fermented food called *tempe bongkrek.* This fermented food is mainly made of coconut presscake or the residue from homemade coconut milk inoculated with *Rhizopus oligosporum*, rather than common tempe which made of soybeans. This tempe could become toxic when contaminated and for as long as the local people can remember, food poisoning and death in Central Java, mainly Banyumas and surrounding areas, were periodically caused by contaminated tempe bongkrek (Shurtleff, 2007).

The first outbreak of bongkrek poisoning was recorded by Dutch authorities in 1895 and reported several types of tempe bongkrek in 1902 by Vorderman. Historically between 1931 and 1937 during Indonesia's economic depression, some villagers tried to make tempe bongkrek by themselves rather than buying it from experienced producers, the poisonings become very numerous, recorded up to 10 or 12 a year (Shurtleff, 2007).

A group of Dutch scientist named W.K Mertens and A.G. van Veen from Eijkman Institute Jakarta began to investigate the causes of bongkrek poisoning in the early 1930s. They found the cause of poisonings and discovered the producing bacterium is *Pseudomonas cocovenenans*. Furthermore they isolated and named the two poisonous substances as toxoflavin and bongkrekic acid (van Veeg, 1967). Recent genetic sequencing studies have confirmed the bacteria producing bongkrekic acid belongs to *Burkholderia cocovenenans* (Lynch, 2009).

BIOCHEMISTRY OF BONGKREKIC ACID

Bongkrekic acid is a highly unsaturated and heat-stable tricarboxylic fatty acid with a molecular weight of 485 kDa (Fig. 1) (Moebius, 2012). The IUPAC name of this acid is (2E,4Z,8Z,10E,14E,18E,20Z)-20-(carboxymethyl)-6-methoxy-2,5,17-trimethyl docosa-2,4,8,10,14,18,20-heptaenedioic acid with molecular formula of $C_{28}H_{38}O_7$ (NCBI, 2019). It was before considered as biologically active secondary metabolites to impart a survival advantage such as inhibiting the growth of other microorganism, known as polyketides.

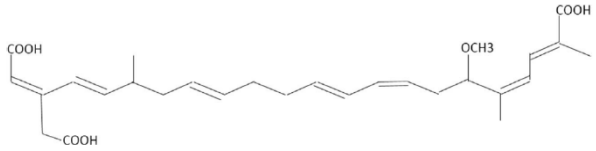

Figure 1. Bongkrekic Acid Structure (Moebius, 2012)

This toxin is produced by the gram-negative, aerobic, rod shaped bacteria *Burkholderia cocovenenans*. Like other species of *Burkholderia* genus, the bacteria is commonly found in the plants and soil. *B. cocovenenans* and the other *B. gladioli* pathovars also produce an electron carrier that generates hydrogen peroxide and subsequent toxicity related to free radicals formation, this toxin named toxoflavin. Its toxicity is relatively mild and secondary to that of bongkrekic acid (Lynch, 2009).

Several studies revealed the lethal dose of this toxin. Deshpande (2002) reported that 1-1.5 mg of bongkrekic acid can be fatal in humans. Another research suggests an oral LD_{50} of 3.16 mg/kg (Liu, 2002). Studies on mice suggest an intravenous LD_{50} of 1.41 mg/kg (Moebius, 2012) and an oral LD_{50} of 0.68-6.84 mg/kg (Hu, 1984). Bongkrekic acid causes lethal food poisoning which is associated with hyperglycemia, research (Kiranadi, 1991) showed that bongkrekic acid is a potent inhibitor of the mitochondrial ATP/ADP translocase, inhibits glucose-induced electrical activity in the pancreatic beta-cell through the stimulation of ATP-sensitive potassium channel (K-ATP-channel) activity.

DETECTION OF BONGKREKIC ACID

The contaminated food of bongkrekic acid can be either detected by the presence of *B. cocovenenans* or bongkrekic acid itself. Moleculer identification of 16S rDNA is the most commonly used method for the identification of *B. cocovenenans*. However it is reported that sometimes it identified falsely as other *Burkholderia* pathovars for *B. cocovenenans* (Lynch, 2009). Commercial test kits for example the Biologic GN2 System can be used also for the identification. Other methods such as capillary electrophoresis-single strand conformation polymorphisms (CE-SSCP), probe-based cell fishing, or microarray analysis have the ability to be used

Table 1. Bongkrekic Acid Outbreaks in Indonesia

Outbreak location	Year	Number affected	Deaths
Java	1895	Unknown	Unknown
	1951-1975	7216	850
	1975	1036	125
	1977	400	70
	1983	450	42
	1988	200	14
Magelang regency	2007	30	10
Banjarnegara	2013	4	1

Source : summarized by Anwar (2017)

Table 2. Optimal conditions for proliferation of *B. cocovenenans* and bongkrekic acid production

Factor	Growth of *B. cocovenenans*	Bongkrekic acid production
Temperature	30-37 ºC	22-30 ºC
pH	>5.5	6.5-8.0
NaCl	<6%	<1.5-2%

Source : summarized by Anwar (2017)

for identification of *B. cocovenenans*. Above all, the most reliable method might be the multiplex PCR protocol (Lynch, 2009).

The presence of bongkrekic acid in food samples can be tested using rapid thin-layer chromatographic procedure (Soedigdo, 1977), while the bongkrekic acid in environmental samples can be quantified using chromatography-mass spectroscopy and high-pressure liquid chromatography (Hu, 1984).

EPIDEMIOLOGY IN INDONESIA

Several outbreaks had been occurred in the country of origin of the toxin. Table 1 shows the places of bongkrekic acid outbreak in Indonesia. All of it happened in the Island of Java. During the first time of bongkrekic acid discovery to 1975, high number of affected people were recorded. Near thousand casualties because of the bongkrekic acid outbreaks in this period. Outbreaks still happened several times after 1975 but the number was decreased significantly. Further studies of the toxin made it possible to reduce the casualties. Recent outbreaks of bongkrekic acid in Indonesia occurred in 2007 and 2013, both of them were also in Java Island. The development of food technology and food safety in Indonesia had saved great amount of people from outbreak.

CONTAMINATION PREVENTION

Research and studies of the toxin and its producer had created ways to prevent its lethal effect to the human. Summarized in table 2, strategies can be managed to minimize the contamination and production of bongkrekic acid in food. Optimal growth of *B. cocovenenans* is in slightly above room temperature, 30-37^{o}C. Storage of the food is recommended below this temperature to decrease the chance of contamination from the bacteria of bongkrekic acid producer. Attention should be made too because the optimal temperature for *B. cocovenenans* to produce bongkrekic acid is slightly below room temperature, 22-30^{o}C. Further decrease of the storage temperature for the food to prevent the production of this toxin is suggested.

Growth of the *B. cocovenenans* is optimum at pH of above 5.5 and it produces bongkrekic acid optimally at pH around 6.5-8.0. This indicates the recommended acidity of the food to be 5.5 or less in order to reduce the production of bongkrekic acid. While the salinity (NaCl concentration on the substrate) for the optimum growth of *B. cocovenenans* is 6% or less and it optimally produce bongkrekic acid in salinity of 1.5-2% or less. These optimal conditions for the production of the bongkrekic acid are similar condition for the production of common tempe (Deshpande, 2002).

Another research conducted by Garcia *et al.* (1999) indicated that the concentration and type of lipid in the substrate is critical for bongkrekic acid formation. This may explain why bongkrekic acid intoxication is limited to certain foods. Thus the fat content of the food should be decreased to prevent the production of bongkrekic acid.

REGULATORY STANDARD

Standard for tempe bongkrek or bongkrekic acid contamination is not found in Indonesian documentation. Indonesian National Standardization Body (BSN) have created SNI 3144:2009 for soya bean tempe standard. Table 3 shows the requirements and limits for the production of tempe in Indonesia. Microbial toxins are not mentioned specifically, only the numeration of coliforms and *Salmonella*.

Codex Alimentarius also have created Regional Standard For Tempe (CAC, 2017). In this document, tempe shall comply with the maximum levels (MLs) of the General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995). Tempe bongkrek or

bongkrekic acid also not mentioned in Codex documents.

Table 3. Quality requirements for soya bean tempe production (BSN, 2009)

Criteria	Unit	Requirement
Odor	-	Normal, unique
Color	-	Normal
Taste	-	Normal
Water content (w/w)	%	Max. 65
Ash content (w/w)	%	Max. 1.5
Fat content (w/w)	%	Min. 10
Protein content (N x 6.25) (w/w)	%	Max. 2.5
Crude fiber content (w/w)	%	Max. 2.5
Cadmium (Cd)	mg/kg	Max. 0.2
Lead (Pb)	mg/kg	Max. 0.25
Tin (Sn)	mg/kg	Max. 40
Mercury (Hg)	mg/kg	Max. 0.25
Arsenic (as)	mg/kg	Max. 0.25
Coliform	MPN/g	Max. 10
Salmonella sp.	-	Negative/25 g

CONCLUSION

Bongkrekic acid was first discovered and named after the local Indonesian food, tempe bongkrek. This toxin, produced by bacterium *B. cocovenenans*, had already caused several outbreaks in Indonesia since early 1990s. Studies of the bacteria and toxin itself had developed the strategies to prevent the outbreaks. Supported by the hygiene and technologies in parts of the world, bongkrekic acid could be considered under control in the perspective of food safety today.

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