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Combination of humectants with potassium sorbate and sodium benzoate to inhibit *Curvularia clavata* contamination in Thai Fermented Fish Spicy Dip (Nam Phrik Pla Ra)

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Abstract Samples of Thai fermented fish spicy dip (nam phrik pla ra) had a high total viable count and high yeast and mold counts of 4.6 and 2.2 log CFU/g, respectively, that exceeded the standard values set by the Thai Industrial Standards Institute (TISI) of less than 4 and 2 log CFU/g, respectively. Sample isolation and identification revealed that *Curvularia clavata* (isolate 518) was the highest contaminating species. Glucose syrup (5%) should be used in chili paste production to reduce its a_w and to extend the shelf life of nam phrik pla ra (5 days) and not exceed the standards set by the TISI. *C. clavata* growth was inhibited by adding humectants and preservatives to the sample. The shelf life of nam phrik pla ra containing 5% glycerol with 500 mg/kg potassium sorbate was 7 days, while adding 5% glucose syrup with 500 mg/kg potassium sorbate could prolong the shelf life to longer than 14 days.

Keywords: *Curvularia clavata*, Fermented fish spicy dip, Glucose syrup, Potassium sorbate, Sodium benzoate

Introduction

Thai fermented fish spicy dip (nam phrik pla ra) is one of the chili pastes made in the central region of Thailand. It is well-known as a "one tambon one product" (commonly known as OTOP) from Nakhon Sawan, Uthai Thani and Singburi provinces (Info Systech, 2015). Currently, nam phrik pla ra, which is produced by a local community enterprise (Ban Suan Lok Champ) in the Uthai Thani province, is popular with consumers because it is a cultural condiment using famous ingredients from the province, especially fermented fish made from the Giant goramy (*Osphronemus goramy*). It is recognized with a Thai geographical indication (Thai GI) for Uthai Thani province

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(Department of Intellectual Property, 2016). Moreover, nam phrik pla ra has a unique flavor and high consumer acceptability because the taste is not as sour as other types of chili paste, resulting in higher market growth.

However, nam phrik pla ra has been categorized as being in the group of high water activity (a_w) chili pastes. This ready-to-eat food has an a_w value higher than the standard (>0.85; Thai Industrial Standards Institute, 2003) and together with the high pH value of this food, can result in rapid deterioration by microorganisms (Jay, 1996; Ray, 2001). It has been known for a long time that fungi are the major cause of spoilage in chili paste products. Several fungi, such as *Aspergillus flavus*, *A. niger*, *Rhizopus* sp. or *Penicillium* sp. are not only a major cause of chili paste spoilage but can also be the cause of foodborne diseases leading to consumer illnesses (Jay, 1996; Mahakarnchanakul *et al*., 2011; Toorisut, 2014). Unfortunately, these fungal pathogens can cause diseases in humans because they survive the mild heating process involved in preparing chili paste.

Curvularia species are found in the soil and in plant tissues (Zakaria *et al*., 2011; Bindu and Harikumar, 2016) and have been reported as being plant pathogenic fungi (Yeasmin and Shamsi, 2013; Chen *et al*., 2014b; Zhong, 2016). It has also been found that these fungi are opportunistic pathogens of humans and can cause skin infections (Gugnani *et al*., 1990; Madrid, 2014). At least eight species of this genus (*Curvularia aeria, C. geniculata, C. lunata, C. brachyspora, C. clavata, C. inaequalis, C. pallescens* and *C. verruculosa*) have been reported to cause diseases in humans ranging from mild skin and nail infections to severe invasive disease, depending on the route of infection and the immune status of the host (Kamalam *et al*., 1992; Ebright *et al*., 1999; de Hoog *et al*., 2000; Madrid, 2014). Thus, if some of the raw materials used to make nam phrik pla ra (especially plants with underground heads such as finger root, shallots or lemongrass) are not cleaned, they might transfer *Curvularia* contamination and subsequent survival into the finished product.

The survival of *Curvularia* spp. in nam phrik pla ra after processing or during storage might occasionally be the source of foodborne diseases, and the product might become a public health risk. Although TISI has not set criteria for *Curvularia* spp. in such products, those products that were not of acceptable quality could constitute a high potential public health risk. In Thailand, there is no information about the contamination of *Curvularia* spp. and the survival of this fungus in nam phrik pla ra chili paste that contains only humectants or humectants and preservatives. Therefore, the main purpose of the present study was to evaluate the microbial contamination and safety of traditional fermented fish spicy dip (nam phrik pla ra) produced by a local community enterprise (Ban Suan Lok Champ) in Uthai Thani province, Thailand. Further, the

survival of *Curvularia* spp., which is a major cause of spoilage in nam phrik pla ra, was investigated in the presence of humectants or preservatives or both. The current food safety data for traditional chili paste was reviewed to develop good practice production guidelines (including raw material sorting and cleaning) and sanitation of food processing (including personal hygiene standards) for other local community enterprises in Thailand. The study aimed to bring awareness to Thai consumer to be alert regarding food safety issues and especially microbiological hazards from fungal contamination which may not only lead to food spoilage but also to foodborne illness.

Materials and methods

Sample collection

Samples of Thai fermented fish spicy dip (nam phrik pla ra) (500 g) and raw materials (fermented fish, finger root, lemongrass, dried chili and shallots) were collected three times from the local community enterprise (Ban Suan Lok Champ) in Uthai Thani province, Thailand from June 2019 to March, 2020. This province is well known for the production of Thai fermented fish spicy dip in the central region of Thailand, especially fermented fish made from Giant goramy (*Osphronemus goramy*) that is classified under the Thai geographical indication (Thai GI) system.

Investigation of characteristics and microbial status of samples

The physical and chemical characteristics and the microbial status of the samples and raw materials were investigated within 12 h of collection. All samples were analyzed in triplicate. The color of samples was determined using a Hunter Lab Colorflex 4510 meter (Colorflex®, Hunter Association Laboratory, Inc., USA) and expressed as L* (lightness), a* (red-green), b* (yellow-blue) values. Water activity (a_w) was evaluated using an a_w meter (Series 3TE, Aqua lab, Charpa Techcenter Co., Ltd., USA) and the moisture content was determined according to AOAC method 925.45 (AOAC, 2000). pH was measured using a pH meter (PB-10, Sartorius, Scientific Promotion Co., Ltd., Germany).

For samples, the total viable count and the presence of yeast and mold and of *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus* and *Salmonella* spp. were determined following the standard methods of the American Public Health Association (Downes and Ito, 2001). The populations of aerobic spore-formers were determined using the pour plate technique with tryptone glucose extract agar (Merck, Germany). A 50 g amount of each

sample was weighed and transferred to 450 mL of sterile 0.1% peptone water, and then preheated at 80 ℃ for 30 min before the next serial dilution. After incubation at 35 ℃ for 48 h, both surface and subsurface colonies were counted (Stevenson and Segner, 2001). The populations of proteolytic and non-proteolytic anaerobic spore-formers were enumerated according to Scott *et al.* (2001) and Tassanaudom *et al.* (2017) using a preheating step in a stirred water bath at 80 ℃, holding for 10 min and at 60 ℃ for 30 min respectively, before serial dilution. The plating technique used tryptose sulfite cycloserine agar (Merck, Germany) with egg yolk emulsion was used. Inoculated plates were incubated at 37 ℃ for 24 h in an anaerobic jar using 2.5 L AnaeroPack-Anaero (Mitsubishi Gas Chemical Company, Inc., Japan). Typical colonies with a black color (resulting from the reduction of sulfite, which precipitates as iron sulfide) and with or without the opaque zone were enumerated.

Isolation and identification of fungi from Thai fermented fish spicy dip

A modified protocol (Downes and Ito, 2001; Domsch *et al*., 2017) was used to isolate spoilage fungi from the Thai fermented fish spicy dip samples. Dichloran rose bengal chloramphenicol (DRBC) agar (Merck, Germany) was used to screen the different fungal colonies using a surface plating technique (incubated at 27°C for 5−7 days). Different morphological colonies were cultured using spore inoculation onto the surface of potato dextrose agar (PDA) (Merck, Germany), acidified with tartaric acid (10%), and incubated at 30 ℃ for 5−7 days. The macroscopic (color of mycelium and spores) and microscopic (septate or non-septate hyphae and spore type) appearance of isolated colonies was recorded.

Isolated colonies were identified by the Clinical Research Laboratory, Center Institute of Health Sciences, Department of Medical Sciences, Ministry of Public Health, Thailand. After identification of fungal species, the most common species in the isolates were used to study the survival of the fungi in the presence of various humectants and preservatives.

Effect of humectants in Thai fermented fish spicy dip on growth of Curvularia clavata

Cultivation and spore preparation

A spore suspension of *Curvularia clavata* was cultured on PDA at 30 ℃ for 10 days. Spore recovery was carried out by rinsing with 5 mL of sterile distilled water and 1−2 drops of 0.1% Tween 80 solution. A sterilized glass spreader was used to scrape the surface of the fungal growth throughout the plate. The spore suspension was filtered through glass wool (Supply Chem,

USA) in a 50 mL sterile centrifuge tube until the fungus on the plate was completely drained. The glass wool was washed with sterile distilled water 3−4 times. The spore suspension was then centrifuged at 5,000 rpm for 10 min, the supernatant separated and then centrifuged with 20 mL of distilled water. After the second centrifugation, the sediment (spores) was adjusted to final volume with 5 mL of sterile distilled water. The concentration of spores was determined under a microscope using a hemocytometer (Neubauer, Germany) (Malo *et al*., 2007). Finally, the spore suspension was diluted to 1×10^4 spores/mL with sterile distilled water for further study.

Preparation of sample with humectants

The original Thai fermented fish spicy dip (pH 5.3–5.5, a_w 0.92–0.94) was prepared by adding either 5% glucose syrup (food grade, Food Dee Dee, Thailand) or 5% glycerol (Merck, Germany). One batch of dip was prepared by mixing all ingredients (minced pork or fermented fish made from Giant goramy, sliced finger root, sliced lemongrass, sour tamarind juice, ground dried chilies and sliced shallots, with a total weight of 1,705 g) into a pan on a gas stove at 60 ℃. The ingredients were mixed and stirred continuously for 30 min (at which time the ingredients were slightly oiled and clumped together). Either 5% of glucose syrup or 5% glycerol were added at this stage. Then, the mixture was cooled while stirring continuously for about 5 min.

Artificial inoculation in sample

C. clavata was artificially inoculated into the dip following the protocol of Toorisut (2014). In brief, 200 μL of 1×10^4 spore/mL spore suspension was inoculated into the sample with or without humectants (see below). A 60 g sample of the dip was spread into the bottom of a 200 g glass container. Then, 100 μL (10 drops) of spore suspension were dropped on the surface of the first layer of the sample. This first layer was covered with another 60 g of sample and a further 100 μL of spore suspension was added to the surface, which was then covered with another 60 g layer. A 1.5 cm space was left between the top of the third layer and the lid of the glass container. All treatments were stored at room temperature (32±1 ℃) for 4 weeks and the populations of fungi were determined.

Total fungus, yeast and mold count

The fungal population of both the dip samples and the individual raw materials, including yeasts and molds, was assessed. An amount of 25 g from

each sample was diluted in 225 mL of 0.1% sterile peptone water (Merck, Germany) and placed in a paddle blender (stomacher) for 1 min. Serial dilutions of 1×10^{-1} to 1×10^{-5} were prepared. Total counts of yeasts and molds were determined on DRBC agar (Merck, Germany) by spreading 0.1 mL of diluents on the agar and incubating at 25 ℃ for 5 days before counting the number of yeasts and molds (Downes and Ito, 2001).

Survival of Curvularia clavata in Thai fermented fish spicy dip including both humectants and preservatives

Preparation of dip samples with humectants and preservatives was performed using the protocol outlined above for the preparation of samples with humectants. Three preservatives—500 mg/kg of sodium benzoate (food grade, Food Dee Dee, Thailand), 500 mg/kg of potassium sorbate (food grade, Food Dee Dee, Thailand) and a mixed treatment of sodium benzoate and potassium sorbate (ratio 250:250 mg/kg)—were added to the dip samples containing humectants.

Artificial inoculation of *C. clavata* into samples was performed using the same protocol as outlined above. The populations of yeasts and molds were determined after storage at room temperature (32 ±1 ℃) for 4 weeks.

Results

Characteristics and microbial status of commercial samples and ingredients

To evaluate the microbial contamination and safety of the traditional chili paste (nam phrik pla ra) produced by a local community enterprise (Ban Suan Lok Champ), the physical and chemical characteristics and microbial status of both samples and raw materials were investigated. The a_w, moisture content and pH values were in the ranges 0.80−0.97, 30.2−87.7 %wb and 5.0−6.1, respectively (Table 1).

The total viable count and yeast and mold count of the paste samples (4.6 and 2.2 log CFU/g, respectively, Table 2) were higher than the standard values of the Thai Industrial Standards Institute which limit these counts to less than 4 and 2 log CFU/g in a 1 g sample, respectively (Thai Industrial Standards Institute, 2003). *Clostridium perfringens* (for which the standard specifies that no trace of this pathogen may be found in samples of 0.1 g), aerobic spore formers and proteolytic anaerobic spore formers were found in samples in the ranges 1.3−1.8, 1.7−2.5 and 2.5−2.6 log CFU/g, respectively. However, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and non-proteolytic anaerobic spore formers were not detected.

Table 1. Physical and chemical characteristics of fresh Thai fermented fish spicy dip samples and raw materials taken from a local community enterprise in Uthai Thani province, Thailand

Parameter	Thai fermented fish spicy dip	Raw material				
		Fermented fish	Finger root	Lemongrass	Dried chili	Shallot
L*	32.30±2.67	37.77±4.85	46.56±0.68	48.62±0.61	44.04±0.90	55.45±2.57
a*	12.84±0.40	10.37±0.16	4.48±0.19	5.44±1.58	6.26±0.86	-0.52±0.06
b*	21.60±6.37	17.53±4.07	24.12±1.58	22.89±0.63	23.41±6.17	10.69±0.43
a_w	0.932±0.005	0.792±0.029	0.939±0.012	0.945±0.004	0.869±0.004	0.969±0.027
Moisture content (%wb)	54.94±6.09	53.18±2.90	87.69±2.28	86.63±1.21	30.17±0.35	84.69±0.34
pH	5.38±0.31	4.96±0.34	6.06±0.32	5.41±0.62	4.98±0.56	5.71±0.61

Note: Results expressed as mean values ± standard deviation, determined in triplicate.

Table 2. Microbial status of Thai fermented fish spicy dip samples and raw materials taken from a local community enterprise in Uthai Thani province, Thailand

Microbial type (log CFU/g)	Thai fermented fish spicy dip	Raw material				
		Fermented fish	Finger root	Lemongrass	Dried chili	Shallot
Total viable count	4.60±0.42	3.79±0.07	8.10±0.07	6.18±0.44	6.25±0.41	7.33±0.61
Yeast and mold	2.23±0.47	2.33±0.63	6.96±0.16	4.27±0.31	4.79±0.17	4.90±0.31
Escherichia coli	<10	<10	6.13±0.02	5.15±0.55	3.61±0.35	4.18±0.34
Staphylococcus aureus	<100	3.38±0.56	4.84±0.13	4.30±0.70	3.87±0.70	2.02±0.28
Salmonella spp.	-	-	-	-	-	-
Clostridium perfringens	1.22±0.07	2.47±0.07	0.67±0.58	0.47±0.40	0.23±0.40	0.47±0.40
Aerobic spore formers	2.07±0.42	1.61±0.75	3.67±0.16	2.14±0.09	1.18±0.00	1.43±0.12
Proteolytic anaerobic spore formers	2.53±0.06	2.28±0.26	0.80±0.17	<10	<10	<10
Non-proteolytic anaerobic spore formers	<10	1.28±0.76	0.23±0.40	0.23±0.40	0.57±0.51	<10

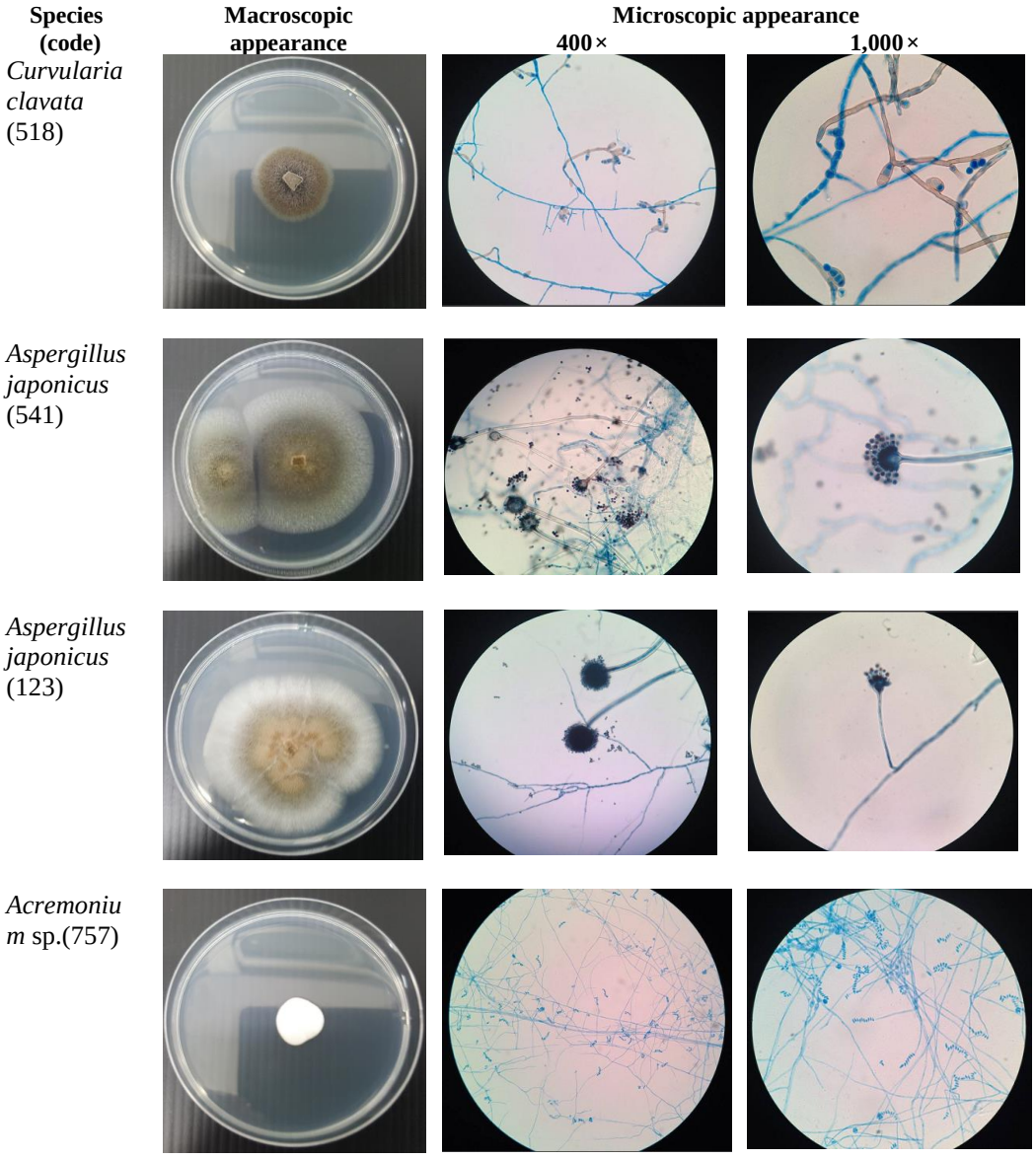
Note: Results expressed as mean values ± standard deviation, determined in triplicate.

- indicates undetectable in 25 g of sample using qualitative determination with enrichment medium; < 10 indicates undetectable in 25 g of sample at 1×10^{-1} dilution using the pour plate technique; < 100indicates undetectable in 25 g of sample at 1×10^{-1} dilution using the spread plate technique.

Isolation and identification of fungi from Thai fermented fish spicy dip

Several typical colonies of contaminated fungi from the paste samples were isolated and characterized using DRBC agar for screening the different colonies and mycelia. When selected and purified on PDA plates, eight

different morphological isolates could be classified based on their macroscopic and microscopic appearance (Figure 1). Further results from the identification (Figure 1) revealed that isolate 518 (*Curvularia clavata*) was the most prevalent species in the sample, occurring in about half of all isolates (20 isolates). *Aspergillus japonicas* was identified in 30% of the isolates and other species, such as *Acremonium* sp., *Aspergillus flavus*, *Penicillium citrinum* and *Aspergillus niger*, were found in 20% of the isolates.



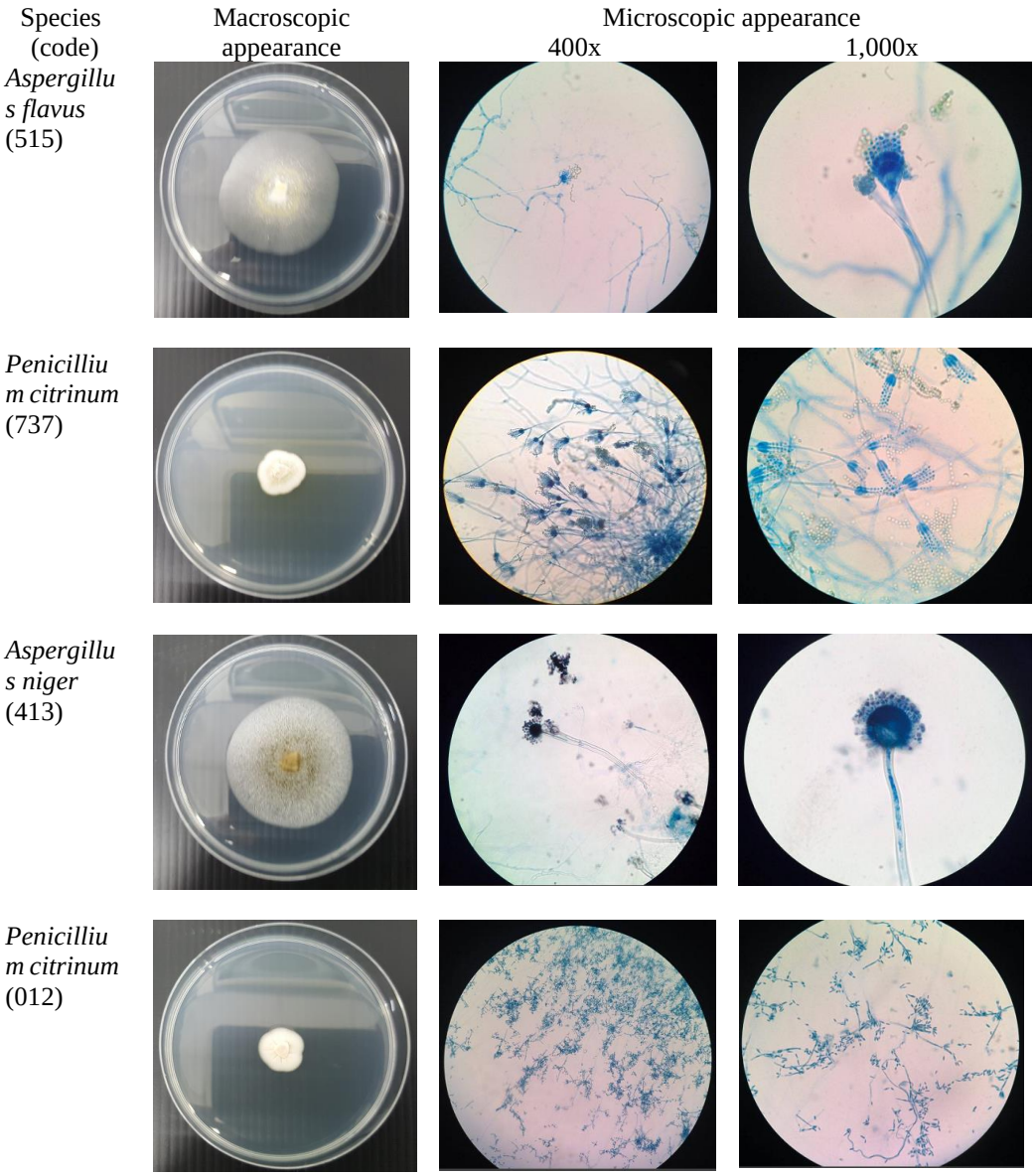


Figure 1. Macroscopic and microscopic appearance of various fungal species isolated from Thai fermented fish spicy dip samples

Effect of humectants in Thai fermented fish spicy dip on growth of Curvularia clavata

The survival was monitored of *C. clavata*, which is a major cause of spoilage isolated from nam phrik pla ra, with either 5% glucose syrup or 5%

glycerol. During storage at room temperature (32±1 ℃) for 4 weeks, there was a continuous increase in the number of fungi with the increase in the range 5.3−6.8 log CFU/g. In paste samples containing 5% glucose syrup, the increase in the amount of *C. clavata* tended to be lower than where 5% glycerol had been added (Figure 2).

Adding 5% glucose syrup to paste that had not been artificially inoculated with *C. clavata* could extend the storage life for up to 5 days. The amount of fungus (1.7 log CFU/g) in the sample did not exceed the standard criterion (less than 2 log CFU/g). Although the addition of 5% glucose syrup to the inoculated sample only extended storage to 3 days, that was longer than the control (containing no humectants). In fact, in the control sample, the amount of fungus exceeded the standard criteria from the first day of storage. The a_w values of samples with either 5% glucose syrup or 5% glycerol were constant (0.89−0.91) during storage. In contrast, the pH values reduced from 5.1 to 4.8 and 5.1 to 4.5, respectively. However, adding more than 5% glucose syrup or 5% glycerol to nam phrik pla ra would not be possible because it would make the sweet taste less acceptable to the consumer (data not shown).

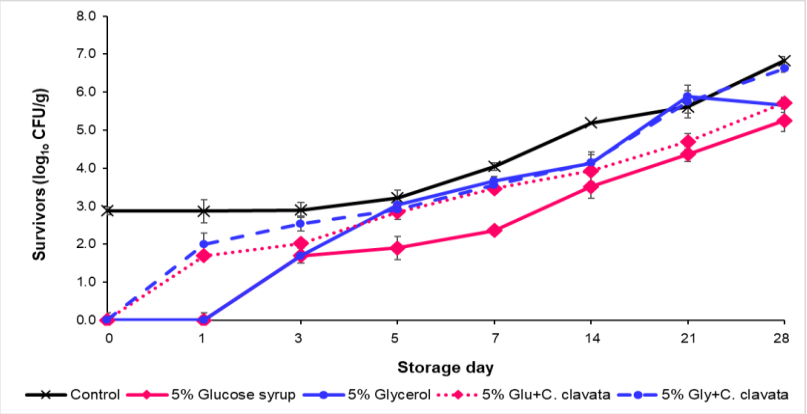
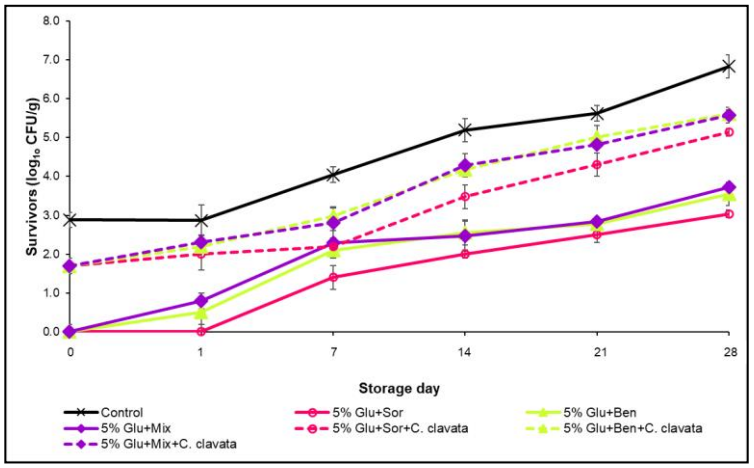


Figure 2. Effect of glucose syrup and glycerol to inhibit fungal growth in nam phrik pla ra during storage at 32±1 ℃ for 4 weeks
Note: Glu = glucose syrup, Gly = glycerol, Control = nam phrik pla ra (paste) without added humectants and not artificially inoculated with C. clavata, + C. clavata = artificially inoculated with C. clavate.

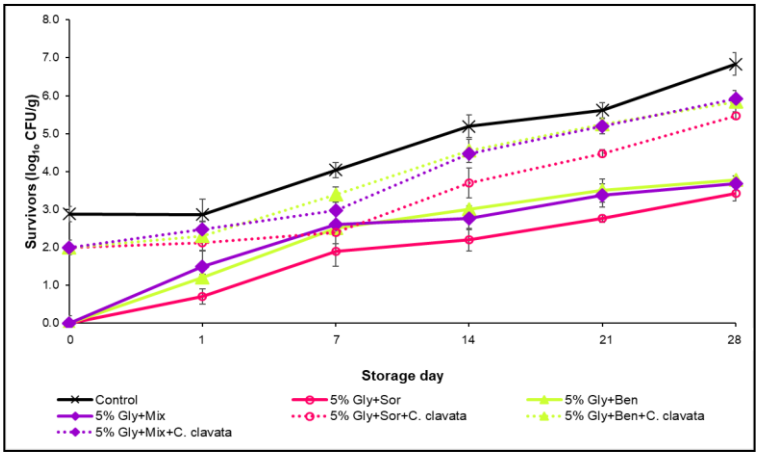
Survival of Curvularia clavata in Thai fermented fish spicy dip treated with humectants and preservatives

The use of either 5% glucose syrup or 5% glycerol in combination with either 500 mg/kg sodium benzoate or 500 mg/kg potassium sorbate or a combined treatment were compared in the absence of *C. clavata* innoculation.

Potassium sorbate was found to be the best preservative for extending the shelf life when used with either humectant, but especially with glucose (14 days), for samples stored at room temperature (32±1 ℃) (Figure 3). The occurrence of yeast and mold did not exceed the standard (2 log CFU/g). Adding 500 mg/kg sodium benzoate with the combined humectants was effective in inhibiting the growth of *C. clavata* but the samples could not be stored for more than 7 days without spoilage.



(a)



(b)

Figure 3. Effect of combination with humectants and preservatives to inhibit fungal growth in nam phrik pla ra during storage at 32±1 ℃ for 4 weeks

Note: (a) glucose syrup added, (b) glycerol added, Glu = glucose syrup, Gly = glycerol, Control = nam phrik pla ra (paste) without added humectants, preservatives and not inoculated with *C. clavata*, + Sor = mixed with potassium sorbate, + Ben = mixed with sodium benzoate, + Mix = blended with sodium benzoate and potassium sorbate, + *C. clavata* = artificially inoculated with *C. clavata*.

When the nam phrik pla ra was inoculated with *C. clavata* spores, which contained 5% glucose syrup, the fungus was able to grow rapidly in the sample with sodium benzoate, the mixed treatment and with potassium sorbate giving rates of 3.0, 2.8 and 2.2 log CFU/g, respectively after 7 days of storage. When 5% glycerol was used as the humectant, the amounts of fungus were higher in the sodium benzoate, mixed treatment and potassium sorbate samples at 3.4, 3.0 and 2.4 log CFU/g, respectively. These results revealed that the best conditions for inhibiting *C. clavata* in nam phrik pla ra in this study were 5% glucose syrup combined with 500 mg/kg potassium sorbate.

Discussion

Characteristics and microbial status of commercial samples and ingredients

Only dried chilies had lower values than the other raw materials and the paste. The high values of these parameters suggested only a short shelf life for this product. Our previous research revealed that high water activity in the chili paste group (fresh chili paste, nam phrik ta dang and nam phrik pla ra) restricted storage to less than 2 days at room temperature (30−35 ℃) in order to retain quality and to avoid food safety issues (data not shown). Similarly, it has been shown that microorganisms can grow rapidly under such storage conditions and are a major cause of product spoilage (Jay, 1996; Ray, 2001).

The high numbers of a range of microorganisms in the samples identified a potential safety risk with this product. In addition, all the raw materials (fermented fish, finger root, lemongrass, dried chili and shallot) also contained high numbers of several of the microbial organisms. These results indicated that the nam phrik pla ra produced by the local community enterprise requires quality improvement. This study also identified several types of fungi, that due to a short cooking time at a low temperature, it could possibly be re-contaminated during the cooling process before packing as the survival of fungi and spores may have been encouraged. The fungi identified were not only a major cause of chili paste deterioration but were also a potential cause of foodborne diseases leading to consumer illnesses.

Isolation and identification of fungi from Thai fermented fish spicy dip

The *Curvularia* genus consists of about 100 species. Their morphology is characterized by the production of sympodial conidiophores with tretic, terminal and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar. Conidia are often curved at an asymmetrically swollen intermediate cell, but species with straight conidia

also have been described (Sivanesan, 1987). Conidiophores are brown and erect, producing ellipsoidal, brown, usually curved conidia with three or four transverse septa. Species can be differentiated on the basis of the rDNA internal transcribed spacer (ITS) region and glyceraldehyde-3-phosphate dehydrogenase gene sequences (de Hoog, 2007).

C. clavata is a fungus that can be found in soil and in plant tissues (Bindu and Harikumar, 2016; Zakaria *et al*., 2011). It is reported as being a pathogen in plants causing such diseases as burning germs in gerbera (Yeasmin and Shamsi, 2013), and leaf spot disease in pineapple (Zhong *et al*., 2016). In addition, this mold can be an opportunistic pathogen of humans (Madrid *et al*., 2014) and can cause skin infection (Gugnani *et al*., 1990). *Curvularia* species may cause allergic sinusitis, which can disseminate to the brain of immunocompetent patients (Ebright *et al*., 1999). Other manifestations include subcutaneous infections following traumatic implantation, such as keratitis. Colonies of *Curvularia* are black in color, expanding, and hairy (Tanabe *et al*., 2010; Moody *et al*., 2012).

The isolation of these fungi from the samples of nam phrik pla ra showed that it is possible that this type of mold is attached to the raw materials used to make the paste, especially to plants with underground organs such as finger root, shallot or lemongrass. Thus, if the washing process for the raw materials was not thorough, it might have led to fungal contamination and consequently their survival in the finished product. Importantly, if the other properties of the food product such as the a_w, moisture content and pH were suitable for the growth of the fungi, food spoilage would be accelerated. Most fungi can grow rapidly at an a_w value of more than 0.8, except for xerophilic fungi which are able to grow at an a_w value of 0.65−0.75 (Barbosa-Carnovas *et al*., 2017). In addition, the pH and temperature can affect the growth of *Curvularia* spp. Chen *et al*.(2014b) reported that the optimum temperature and pH for mycelial growth of *Curvularia clavata* that causes leaf spot and burn in *Curcuma wenyujin* (in the same family (Zingiberaceae) as finger root) were 30 ℃ and 5−8, respectively. Similar results were found by Lal *et al*. (2014) studying the growth and sporulation of *Curvularia lunata*, which caused black spot disease, with the optimum temperature reported as 28 ℃.

Although TISI has not set criteria for *Curvularia* spp. in such kinds of paste product, the levels found were not acceptable and could constitute a potential public health risk. This finding is the first intensive report of *C. clavata* being a contaminant in traditional fermented fish spicy dip. Thus, further investigation of *C. clavata* and *Curvularia* spp. in these products is warranted.

Effect of humectants in Thai fermented fish spicy dip on growth of Curvularia clavata

One of the strategies used to extend the shelf life of several products is the incorporation of humectants. Humectants are substances that attract water; they can retain water in foodstuffs, reduce the a_w and improve the texture and mouthfeel (Nabors, 2002; Taoukis and Richardson, 2007; Sorapukdee *et al*., 2016). A reduction in a_w directly affects the growth and death rate of microorganisms. However, this response depends on the type of humectant and on the nature of the microorganism (Gliemmo *et al*., 2006). Fungi can cause spoilage of chili paste products during shelf life if the product acquires moisture from the environment or if there is a high a_w in the initial product. Thus, a_w reduction of nam phrik pla ra through the addition of a humectant has been shown to inhibit the growth of *C. clavata*.

For example, among several types of humectant, both glucose syrup and glycerol are categorized as binding free water and decreasing the a_w of a solution or a product (Brown, 2008). Glucose syrup is usually used to reduce the a_w in chili paste products in Thailand (Toorisut, 2014). Although no reports have been found on the use of glycerol in chili paste, it can be used in many intermediate-moisture food products (Taoukis and Richardson, 2007; Sorapukdee *et al*., 2016). From the result of the current study, the a_w value of nam phrik pla ra with 5% glucose syrup was lower than from using glycerol and in the control samples. These results indicated that the use of either 5% glucose syrup or 5% glycerol might be suitable for controlling the spoilage of nam phrik pla ra by *C. clavata*. The current results are similar to those reported by Chen *et al*. (2014a) who found that the a_w values of jerky treated with glycerol were significantly lower than those where sorbitol was used and in the control groups. Thus, using humectants, particularly glucose syrup and glycerol, reduced *C. clavata* growth in nam phrik pla ra.

Survival of Curvularia clavata in Thai fermented fish spicy dip treated with humectants and preservatives

Preservatives such as sorbic and benzoic acids and their salts are usually added to food products to inhibit the development of microorganisms and to enhance shelf stability (Leistner, 2000). According to the Thai Food Act, B.E. 2522 (1997) and the Thai Food and Drug Administration (2016), the maximum amounts of benzoic and sorbate that can be added to chili paste are 500 mg/kg or 500 ppm (calculated in benzoic acid equivalents) and 1,000 mg/kg or 1,000 ppm (calculated in sorbic acid equivalents), respectively. Where both types of preservatives are used, the total amount of preservative

must not exceed one of these maximum allowable amounts (Thai Food and Drug Administration, 2013; Thai Food and Drug Administration, 2016). Thus, using 500 mg/kg potassium sorbate in nam phrik pla ra would comply with the Thai Food Act and Thai FDA regulations. Potassium sorbate is one of the preservatives commonly added in foods to prevent food spoilage by microorganisms, especially fungal spoilage. This preservative is reported to have lower toxicity to mammals, to impart a lower after-taste sensation and to be more inhibitory for controlling the growth of spoilage yeast and mold than sodium benzoate (Deuel *et al*., 1954; Dryden and Hills, 1959; Gliemmo *et al.*, 2016). However, the effectiveness of a preservative in inactivating microbial growth depends on the amount of acid available to break down microbial cells which is determined by the pH of the food and the pKa value of each acid.

The nam phrik pla ra in the current study had a pH of 5.4, while the pKa values of benzoic acid and sorbic acid are 4.19 and 4.75, respectively (Jorge and Guillermo, 1992). Normally, the pKa of the acid is lower than the pH of the food system, and the acid can be in both the dissociated and undissociated forms. With pH 4.0, benzoic acid is 66% in the undissociated form, while sorbitol is 84% (Jorge and Guillermo, 1992). Consequently, the undissociated form of acid can penetrate the membrane layer and break down within the microbial cells resulting in destruction of those cells (Guynot *et al*., 2005). At pH 5.4, nam phrik pla ra, has a higher occurrence of the undissociated form of sorbic acid than for benzoic acid. The pH within the microbial cell would be reduced rapidly by the sorbic acid, resulting in the inhibition of glycolysis. Because these undissociated acidic molecules are lipophilic molecules which can be soluble in the lipid phase, they can pass into the membrane layer to the cytoplasm and break down within the cell. H^+ ions and negative ions are generated by the breakdown of undissociated acidic molecules (Guynot *et al*., 2005). These H^+ ions cannot pass through the cell membrane and accumulate within the cell causing cell death (Brul and Coote, 1999).

To date, there have been few studies into the contamination and hazards of *C. clavata* in nam phrik pla ra and other types of chili paste in Thailand that are prepared using mild heating. Our results indicated that nam phrik pla ra has a potential risk as a source of the foodborne pathogen *C. clavata*. Consequently, methods should be developed to enhance the quality of the product and to improve hygiene during production to ensure consumer safety.

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