Food and Applied Bioscience Journal, 2020, 8(1): 33-42

Effectiveness of bergamot juice on the survival of *Bacillus cereus* and quality of Thai steamed pumpkin cake

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Abstract

This work aimed to assess the effects of bergamot juice on the qualities of Thai pumpkin cake after steaming. The percentages of bergamot juice, such as 25% (w/w), 50% (w/w), and 100% (w/w), were applied as a new prototype of natural preservative ingredient in the pumpkin cake. The results revealed that the hardness, adhesiveness and springiness, and pH values of all steamed pumpkin cakes containing bergamot juice, were affected. Surprisingly, the survivals of *B. cereus*, yeasts and molds were decreased in 25−100% (w/w) bergamot juice treated pumpkin cakes compared to those of a control treatment after steaming. However, the sensory preferences of steamed pumpkin cake containing 50% (w/w) bergamot juice were the highest acceptable product, based on the highest score of appearance, flavor, texture, color and overall acceptability. This scientific evidence could be used as a prototype of natural preservative agent from bergamot juice for further steamed dessert production.

Keywords: Steamed pumpkin cake, Bergamot juice, *Bacillus cereus* survival, Quality

1. Introduction

Steamed pumpkin cake (Kanom Fak Thong) is an Asian steamed dessert product. It was made from flour, coconut milk and sugar, and then steamed. However, its product quality has a fairly short shelf life because of the survival of food−borne pathogens and microbial spoilages, which are the microbiological criteria of food quality after steaming (Asianinspirations, 2019; Pal *et al.,* 2014). Due to consider the *B. cereus* characteristics, it is gram positive motile rods, which form endospores in the middle of the cells. It can survive at high temperature stress used in many cooking techniques, especially steaming process. This food−borne pathogen is the greatest concern in cereal based ready−to−eat foods. In addition, the *B. cereus* strains produce at least two types (emetic and enteric) of enterotoxins which cause food poisoning (Griffiths and Schraft, 2017; Nsw food authority, 2009; Pal *et al.,* 2014).

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Food and Applied Bioscience Journal, 2020, 8(1): 33-42

Food safety objective (FSO) is a goal that food processing steps provide a safe suitable food product to consumers. In the present, the natural preservative agents can be applied in food products for inhibiting food-borne pathogens and microbial spoilages. Many researchers revealed that Thai herbs and spices have been recognized by their alternative approach to preserve food products (Jarriyawattanachaikul *et al.,* 2016; Perni *et al.,* 2009; Wanangkarn *et al.,* 2018; Wanchaitanawong *et al.,* 2005).

Bergamot (*Citrus bergamia*) is a typical citrus fruit of the Southeast Asia. Its essential oil can be extracted from peel and has been used in the pharmaceutical industry because of its antimicrobial activity. It could inhibit food-borne pathogens, such as *Escherichia coli* O157:H7*, Campylobacter jejuni, Pseudomonas putida, Salmonella enterica, Listeria innocua, Staphylococcus aureus, Listeria monocytogenes* and *B. cereus* (Cordery *et al.,* 2018; Fisher and Phillips, 2006; Mandalari *et al.,* 2007). Although the essential oil from bergamot has the most effective activity to inhibit food-borne pathogens, but the extraction steps have high cost and complicated procedures. Indeed, Gattuso *et al.* (2006) recommended that their knowledge of the flavonoid content is the starting point for bergamot juice exploitation in food applications. Filocamo *et al*. (2015) reported that bergamot juice was effective in vitro against *Helicobacter pylori*. Recently, Da Pozzo *et al.* (2018) presented that bergamot juice exerts antioxidant and antisenescence effects, making it useful for further application.

At present, no previous articles have reported the effect of bergamot juice on the microbial quality of Thai steamed pumpkin cake. The aim of this study was to demonstrate that the bergamot juice could decrease the survival of *B. cereus* in Thai pumpkin cake after steaming. In addition, we based our assessment on the main of product qualities, such as texture, color and sensory evaluation.

2. Materials and methods

2.1 Preparation of steamed pumpkin cakes

The fresh bergamots (*Citrus bergamia*) were purchased from a local Sura-Nakhon market in Nakhon Ratchasima province, Thailand. It was cleaned thoroughly with tap water before being sliced. Its juice of crude solution was squeezed by mechanical machine (Severin, Germany) and then filtered by sterile gauze. Afterward, the steamed pumpkin cakes were prepared. The formulas of steamed pumpkin cake are shown in Table 1, which their compositions were mixed. The steaming temperature was set at 100°C, their samples were steamed in a steamer for 20 min and then cooled at ambient temperature for 10 min. The physicochemical characteristics, microbiological qualities and sensory evaluation were then tested.

Food and Applied Bioscience Journal, 2020, 8(1): 33-42

Table 1 Formulation of steamed pumpkin cake with the different amounts of bergamot juice

Ingredients (g)	Treatments			
	Control	F1	F2	F3
Rice flour	90	90	90	90
Tapioca flour	70	70	70	70
Sugar	100	100	100	100
Salt	2	2	2	2
Steamed pumpkin	200	200	200	200
Coconut milk	100	75	50	0
Bergamot juice	0	25 (25%w/w)	50 (50%w/w)	100 (100%w/w)

The % w/w of this experiment means the bergamot juice was used for the steamed pumpkin cake preparation, based on a weight between bergamot juice and coconut milk.

2.2 Physicochemical characteristic measurements

2.2.1 Color

To measure the color of samples, the surface of each sample was evaluated using a colorimeter (Color Quest XE) according to Hunter's color value system. The parameters L* (brightness), a* (redness/greenness), and b* (yellowness/blueness) were measured (Modified from Akesowan, 2007).

2.2.2 Texture analysis

The samples were cut at a uniform size (2×2×2 cm). Textural properties (hardness, adhesiveness, and springiness) of samples were measured using a texture analyzer (BROOKFIELD, USA CT3, USA). The texture profile was set with the two-bite compression test using the following operation conditions: No. 3 4.77 mm probe; maximum weight, 2 kg; distance, 33%; table speed, 120 mm/min; 2 bites (Modified from Chaiya and Pongsawatmanit, 2011).

2.2.3 Water activity and pH measurement

The water activity (aw) was measured using an Aqua Lab CX−2 instrument at room temperature. To determine the pH value of each sample, 10 g of sample was mixed with 90 mL of distilled water in a 250 mL beaker and homogenized for 1 min. The pH of the solution was measured after 15 min.

2.3 Microbiological analyses

The microbiological analyses were carried out from FDA−BAM (2001). The total aerobic bacterial plate counts were enumerated using the spread plates of Standard Plate Count Agar (Hi−Media), and incubating the samples at 37°C for 24 h. Meanwhile, the yeasts

Food and Applied Bioscience Journal, 2020, 8(1): 33-42

and molds were counted using the spread plates of Potato Dextrose Agar (Hi–Media), and incubating the samples at 25°C for 48–72 h. In addition, the *B. cereus* was counted using the spread plates of Mannitol Egg Yolk Polymyxin Agar (Hi–Media), and incubating the samples at 37°C for 24 h. These microbial results were expressed as log CFU/g.

2.4 Sensory evaluation

The sensory evaluation was modified from Chaiya and Pongsawatmanit (2011). The trained panelists of 30 individuals (20–30 years of age) participated in this test and analyzed the appearance, flavor, texture color, and overall acceptability of samples using the 9–point scale method (strong dislike = 1 and strong like = 9). The panels received five samples (2×2×2 cm) on a white plate with a three–digit random number and served in a randomized order. During the panel session, water was provided to panelists to minimize any residual effect before testing a new sample.

2.5 Statistical analysis

The results were presented as the mean ± standard deviation (SD). IBM SPSS statistics 22 (Armonk, New York, U.S.A) was used to perform all statistical analysis. One–way analysis of variance (ANOVA) was determined, which was followed by Duncan's multiple range test (DMRT) with an overall significance level set at 0.05.

3. Results and discussion

3.1 Effect of bergamot juice on physicochemical qualities of steamed pumpkin cakes

The appearance of steamed pumpkin cakes is shown in Fig 1. The physicochemical parameters, including color, texture profiles, a_w and pH values, were evaluated as shown in Fig 2–4, Fig 2A–C reveal that the higher values of L*, a* and b* were found in the steamed pumpkin cake samples containing the bergamot juice compared to a control treatment ($P<0.05$), which was due to the consequence of the bergamot juice characteristic. As shown in Fig 3A–C, the hardness and adhesiveness gradually decreased ($P<0.05$), whereas the springiness significantly increased ($P<0.05$), while the level of bergamot juice increased. This might be interpreted that the moisture content and its internal distribution from bergamot juice ingredient are the primary factor. Its texture properties of flour products were affected during processing (Widjajaseputra, 2012). Likewise, the existed capacities of flour can absorb large amount of water in order to bind the starch molecules. Thus, the considerable amount of starch can raise its viscosity followed by the swollen starch granule (Prameswari *et al.*, 2018). Fig 4A shows that the a_w values of all steamed pumpkin cakes containing the bergamot juice were not significantly different compared to the control treatment ($P>0.05$). Meanwhile, the pH values decreased ($P<0.05$) due to the increase in the level of bergamot juice (Fig 4B).

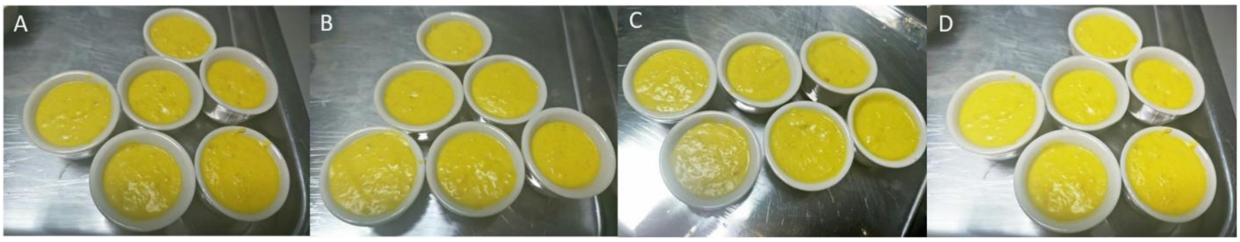

Fig 1 The appearance of steamed pumpkin cakes

(A) Control (no bergamot juice)

(B) 25% (w/w) bergamot juice

(C) 50% (w/w) bergamot juice

(D) 100% (w/w) bergamot juice.

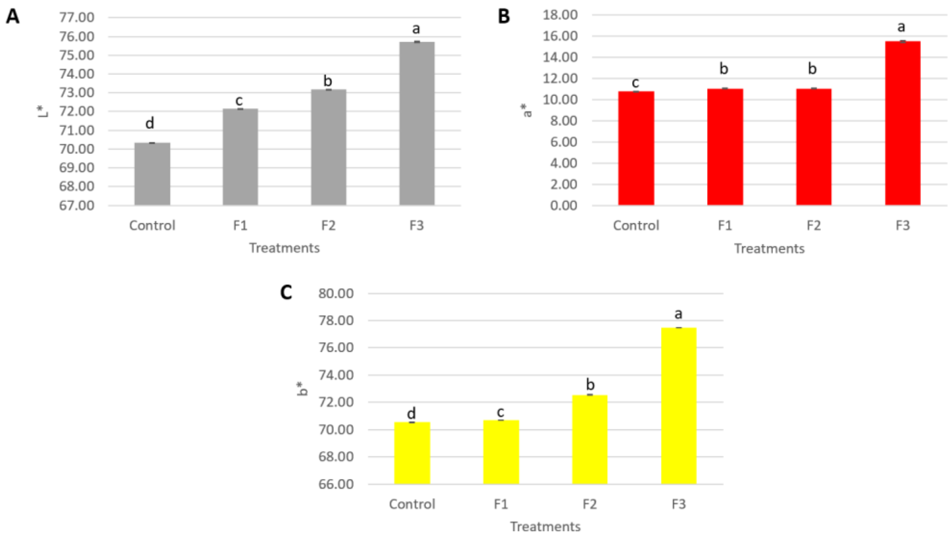

Fig 2 The color values, (A) L*, (B) a* and (C) b* of steamed pumpkin cakes with various treatments; Control = no bergamot juice; F1=25% (w/w) bergamot juice; F2=50% (w/w) bergamot juice; and F3=100% (w/w) bergamot juice. Different superscripts ($^{a-d}$) indicate difference ($P<0.05$) amongst treatments.

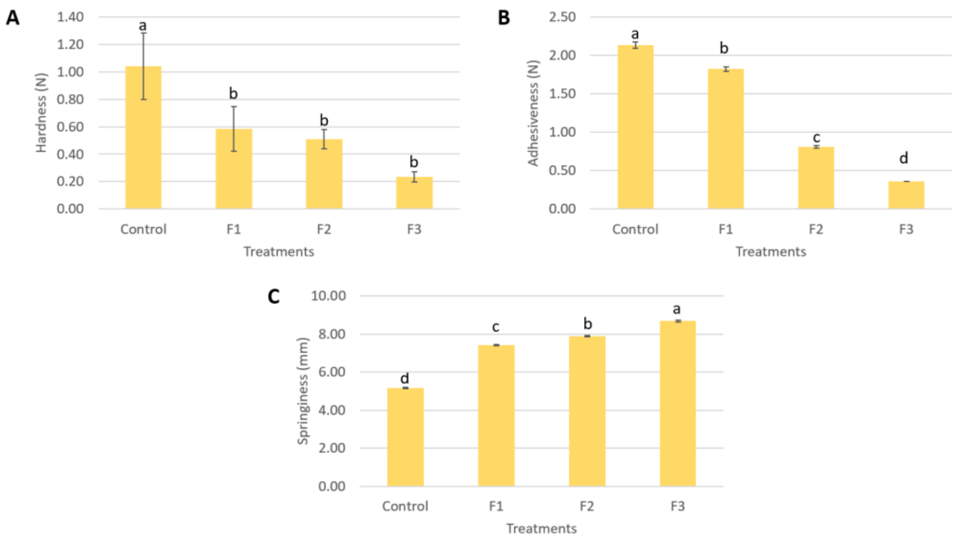

Fig 3 Texture profile analysis, (A) hardness, (B) adhesiveness and (C) springiness of steamed pumpkin cakes with various treatments; Control = no bergamot juice; F1=25% (w/w) bergamot juice; F2=50% (w/w) bergamot juice; and F3=100% (w/w) bergamot juice. Different superscripts ($^{a-d}$) indicate difference (P<0.05) amongst treatments.

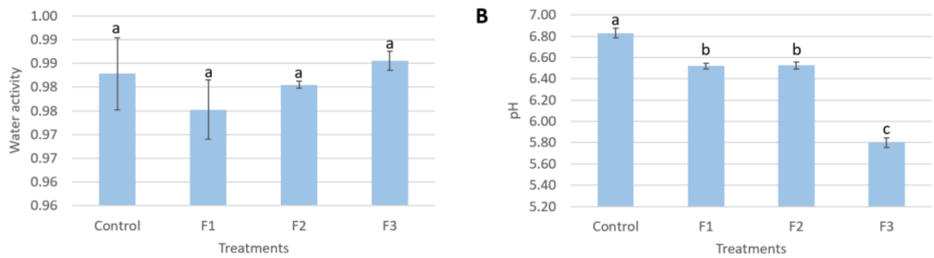

Fig 4 (A) water activity and (B) pH values of steamed pumpkin cakes with various treatments; Control = no bergamot juice; F1=25% (w/w) bergamot juice; F2=50% (w/w) bergamot juice; and F3=100% (w/w) bergamot juice. Different superscripts ($^{a-d}$) indicate difference (P<0.05) amongst treatments.

3.2 Effect of bergamot juice on microbiological qualities of steamed pumpkin cakes

The microbiological qualities of steamed pumpkin cakes are represented in Table 2. The total viable count of 25−100% (w/w) bergamot juice treated pumpkin cake was considered, they were not significantly different from the control treatment (P>0.05). It indicated that the product characteristics (Fig 4), including pH, and a_w values, were approximately 5.8−6.8 and >0.60, which were relevant to the growth potential of mesophilic and aerobic microorganisms (Doyle *et al*., 2019). In addition, Table 2 reveals that the bergamot juice had an antimicrobial effect in steamed pumpkin cakes. The survivals of *B. cereus*, yeasts and molds were decreased. However, the total viable counts of all steamed pumpkin cake samples remained, which were not significantly different compared to the control treatment (P>0.05). These results were relevant to microbiological quality guide for ready−to−eat foods (Nsw food authority, 2009), while they recommend that the potential hazard of *B. cereus* is approximately >4 log CFU/g. Considering the Thai Community product standard (No.1531/2552), they recommended that the total viable count, yeast and mold, and *B. cereus* are ≤ 6, ≤ 4, ≤ 2 log CFU/g, respectively. The microbiological qualities of all steamed pumpkin cake samples from this study were relevant to Thai Community product standard of Thai desserts. The bergamot juice had presented the significant polyphenol compounds, especially flavonoids, neohesperidin and hesperetin (aglycone), neoeriocitrin and eriodictyol (aglycone), naringin and naringenin (aglycone) (Filocamo *et al*., 2015; Gattuso *et al*., 2006). We believe that these components played an important role in *B. cereus* survival and had a severe effect on the level of microbial food spoilages after steaming.

Table 2 Microbiological quality of steamed pumpkin cakes with various treatments

Microorganisms	**Treatments**			
(log CFU/g)	**Control**	**F1**	**F2**	**F3**
Total viable count	1.50±2.12[a]	1.40±0.42[a]	1.30±0.14[a]	1.05±0.07[a]
Yeast and mold count	2.10±0.14	<2	<2	<2
B. cereus	2.74±0.37	<2	<2	<2

Control = no bergamot juice; F1=25% (w/w) bergamot juice; F2=50% (w/w) bergamot juice; and F3=100% (w/w) bergamot juice. The same superscripts in the same row indicate non difference (P>0.05) amongst treatments

3.3 Effect of bergamot juice on sensory properties of steamed pumpkin cakes

All steamed pumpkin cakes containing the bergamot juice were sensory evaluated and compared to the control sample as shown in Table 3. Regarding to all sensory preferences, steamed pumpkin cake containing the 50% (w/w) bergamot juice were the highest acceptable sample. Its appearance, flavor, texture and overall acceptability were not significantly different as compared with the control treatment (P>0.05).

Food and Applied Bioscience Journal, 2020, 8(1): 33-42

Table 3 Sensory preference scores for steamed pumpkin cakes with various treatments

Sensory preferences	Treatments			
	Control	F1	F2	F3
Appearance	7.30±0.59[a]	6.87±0.82[b]	7.53±0.82[a]	3.93±0.96[c]
Flavor	7.43±0.73[a]	7.00±0.74[b]	7.33±0.84[ab]	4.27±0.86[c]
Texture	7.03±0.76[a]	6.90±0.84[a]	7.10±0.84[a]	4.25±0.94[b]
Color	7.13±0.82[b]	7.03±0.81[b]	7.70±1.02[a]	4.37±0.79[c]
Overall acceptability	7.30±0.53[ab]	7.10±0.76[b]	7.60±0.72[a]	4.44±0.85[c]

Control = no bergamot juice; F1=25% (w/w) bergamot juice; F2=50% (w/w) bergamot juice; and F3=100% (w/w) bergamot juice. Different superscripts ([a-c]) in the same row indicate difference ($P<0.05$) amongst treatments.

4. Conclusion

The present study revealed the effect of bergamot juice on the qualities of Thai steamed pumpkin cake. As results, the 50% (w/w) bergamot juice could be applied as a natural preservative agent due to the decrease in hazardous *B. cereus,* and yeasts and molds after steaming. Furthermore, the sensory preferences of steamed pumpkin cake containing 50% (w/w) bergamot juice were the highest acceptable product.

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Food and Applied Bioscience Journal, 2020, 8(1): 33-42

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