The Journal of Applied Science **Vol. 21 No. 2 [2022]: 245412**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2022.02.002
ISSN 1513-7805 Printed in Thailand ISSN 2586-9663 (Online)

Research Article

Incorporation of Natural Colourants into Thai Steamed Cupcakes

Pimonpan Suwan and Thitikorn Mahidsanan*

Institute of Interdisciplinary Studies, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000 Thailand

***E-mail**: thitikorn_mahi@hotmail.com, thitikorn.ma@rmuti.ac.th

Received: 08/09/2021; Revised: 29/08/2022; Accepted: 30/08/2022

Abstract

Thai steamed cupcake has a short shelf-life at room temperature. To resolve the problem, this research aimed to reveal the potential effects of powdered natural colourants, including turmeric, roselle and green tea-based ingredients for controlling the quality of Thai steamed cupcake product during storage at chilled temperature (4 °C). After production at 0 day, the sensory preferences in appearance and texture of all steamed cupcakes containing powdered natural colourants were not significantly different from the control treatment, which were moderately appreciated ($P>0.05$), but the odour, flavour, and overall acceptability of turmeric steamed cupcakes were lower than those of other treatments ($P<0.05$). This indicated that the sensory characteristics of turmeric steamed cupcakes should be further improved. The texture analysis and colour measurement of all steamed cupcakes treated with powdered natural colourants at the storage period of 0-2 days showed that the hardness and fracturability increased, while the colour values changed. Additionally, the microbiological parameters of all steamed cupcakes treated with powdered natural colourants met the standard of ready-to-eat bakery products based on the storage period of 2 days at 4 °C, whereas the control treatment had an unpleasant odour after 2 days. This investigation implies that the application of powdered turmeric, roselle and green tea could be used as a promising approach of natural colouring and preservative for controlling the quality of steamed cupcake product in Thailand.

Keywords: Thai steamed cupcake, Turmeric, Roselle, Green tea, Product quality

Introduction

Steamed cupcake product, called Khanom Pui Fai, is a traditional street dessert in Thailand. Its characteristics are fluffy and light due to expanding like cotton wool after steaming (Pakditawan, 2016). However, the problem of short shelf-life and food-borne pathogens remains, especially *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*, which might be found during storage at ambient temperature because of intrinsic and extrinsic factors. To consider the risk hazard of each food pathogen, *B. cereus* spores can germinate and grow out rapidly after heat treatment and produce emetic and enteric toxins. Many strains of *S. aureus* produce staphylococcal enterotoxins, while certain strains of *E. coli* are used as a faecal contamination index of food sanitation (Angelidis et al., 2006; Dlubala et al., 2021; Olaimat et al., 2021).

For this reason, the microbiological quality must be taken into account based on the standard requirement of ready-to-eat food product. Artificial food colouring is generally used to improve the appearance of desserts, and it has raised concerns due to adverse effects on consumer health because certain chemicals are produced from highly toxic substances, and can cause human diseases and mutations, which should be limited in the food ingredients (Ahmed et al., 2021; Kobylewski and Jacobson, 2012). Currently, the application of plants for implementing various health care treatments and food additives have been adapted (Mulat et al., 2020). A part of plant could be applied for development of food products. For example, turmeric (*Curcuma longa*), roselle (*Hibiscus sabdariffa* L.), and green tea (*Camellia sinensis*), were scientifically used as bioactive ingredients in foods due to antimicrobial activity and antioxidant capacity (Azeez and Lunghar, 2021; Lim et al., 2020; Rashidinejad et al., 2021). Even though the essential oil extracted from medicinal plants is generally used for controlling food-borne

pathogens, the extraction procedures have high value and are inconvenient. Previous publications revealed that juice extracted from plants could be applied for food and dessert products to improve the microbiological and physicochemical quality (Gattuso et al., 2006; Lertnirundon and Mahidsanan 2020). Boonkum and Mahidsanan (2021) demonstrated that turmeric and peppermint could be prepared as either food ingredients or natural colourants to control the microbiological safety of Flatbread Roti product. However, parts of turmeric, roselle and green tea have not been thoroughly investigated in steamed cupcake quality, while the cold temperature might be used as a promising hurdle effect during storage.

Thus, to extend the shelf-life of steamed cupcake product, the objective of this study was to investigate the potential effects of turmeric, roselle and green tea-based natural colourants for controlling the quality of Thai steamed cupcake product during storage at chilled temperature. Significant product qualities including water activity, colours, texture, and microbiological criteria were assessed to represent shelf-life for further development of steamed dessert products.

Materials and methods

Preaparation of three powdered natural colourants and Thai steamed cupcakes

Three plant powders including turmeric, roselle and green tea, were obtained from Suranakhon market, Nakhon Ratchasima province, Thailand. The preparation steps were followed according to Boonkum and Mahidsanan (2021). Each powdered material (200 g) was dissolved in hot water (1000 g) and then filtered by sterile gauze. Afterward, each formulation of Thai steamed cupcake product was prepared (Table 1). Those treatments were steamed at 100 ^{o}C for 7 min and then cooled at ambient temperature for 10 min. Each sample was packed in polypropylene box and stored at chilled temperature (4 ^{o}C)**.** Sensory evaluation was conducted at 0 day. Meanwhile, the parameters of product quality, including microbiological analysis and physicochemical characteristics were assessed at 0, 2, 4 and 6 days.

Table 1 Formulation of Thai steamed cupcake product with the different natural colourants

Ingredients (g)	Treatments			
	Control	Turmeric steamed cupcake	Roselle steamed cupcake	Green tea steamed cupcake
Cake flour	200	200	200	200
Sugar	175	175	175	175
Egg	100	100	100	100
Water	100	100	100	100
Lemon juice	5	5	5	5
Jasmine flavour	2.5	2.5	2.5	2.5
Pasteurised milk	51.5	51.5	51.5	51.5
Baking powder	2.5	2.5	2.5	2.5
Foaming agent & emulsifier (UFM SP)	10	10	10	10
Turmeric suspension	-	10	-	-
Roselle suspension	-	-	10	-
Green tea suspension	-	-	-	10
Water	10	-	-	-

Sensory evaluation

The hedonic test was performed to evaluate the degree of overall liking for each sample at 0 day. Untrained consumers were recruited from the undergraduate students at Rajamangala University of Technology Isan, Nakhon Ratchasima. Thirty consumers received four samples and were asked to rate them according to degree of liking on a 9-point hedonic (strong dislike = 1 and strong like = 9). Samples were placed on plates and identified with random three-digit numbers. Panellists evaluated the samples in a testing area and were instructed to rinse their mouths with water between samples to decrease any residual effect.

Colour measurement

To analyse the colour values, the surface of each sample was evaluated using Chroma meter model CR-410 (Konica minolta, Japan). The parameters L* (brightness), a* (redness/greenness), and b* (yellowness/blueness) were recorded.

Texture analysis

The samples were prepared to a size 5×5×5 cm. Textural properties (hardness, fracturability and stringiness) of samples were examined using a texture analyser model CT3 (BROOKFIELD, USA). The texture profile was set with the two-bite compression test using the following operation conditions: No. TA11/1000 25.4 mm dia.; maximum weight, 2 kg; distance, 33%; table speed, 1.00 mm/min; 2 bites.

Measurement of pH and water activity (a_w)

To measure the pH value of each sample, 10 g of sample was diluted with 90 mL of distilled water in a 250 mL beaker and homogenised for 1 min. The pH of the solution was measured after 15 min. The a_w was measured using a Lab Master-aw neo machine (Novasina, Switzerland) at ambient temperature.

Microbiological detection

The microbial load, including total viable count, yeasts and moulds, *S. aureus B. cereus*, *Salmonella* spp. and *E. coli*., were quantified according to FDA-BAM (2001).

Statistical analysis

For each analysis, triplicate experiments were performed. The results were expressed 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 performed, which was followed by Duncan's multiple range test (DMRT) with an overall significance level set at 0.05.

Results and discussion

Sensory characteristics of steamed cupcakes

The product appearances and sensory characteristics of steamed cupcakes with various treatments are presented in Table 2 and 3, respectively. On day 0, the sensory preference scores of each treatment depended on their powdered natural colourants characteristics. In respect to appearance and texture of all steamed cupcakes containing powdered natural colourants, they were not significantly different from the control treatment ($P>0.05$), and those features were moderately appreciated (6.30-7.33). By contrast, the odour, flavour, and overall acceptability of turmeric steamed cupcakes was classified as neither like nor dislike level, which were lower than other treatments ($P<0.05$). This abnormal score may be due to the pungent smell, especially ar-turmerone (2-methyl-6-(4-methylphenyl)-2-hepten-4-one) (Sharifi-Rad et al., 2020).

Physicochemical characteristics of steamed cupcakes during storage

pH and a_w are the major intrinsic factors in limiting or controlling the microbial growth of food products. Therefore, the microbial activity affects certain undesirable quality changes and alters food characteristics (Tapia et al., 2020). Considering all steamed cupcake products during storage for 4 days at 4 ^oC, the pH values of all treatments were approximately 6.93-7.53, while the a_w values were about 0.90-0.93 (data not shown). At the storage period of 0-2 days (Figure 1B and C), a decrease in the a* of steamed cupcakes containing turmeric, roselle and green tea powder was observed ($P<0.05$), while the b* of steamed cupcakes containing roselle and green tea powder decreased rapidly ($P<0.05$). This phenomenon might be interpreted that the colour quality of natural colourants-based food product was affected by light in a polypropylene box, while its percentage of light transmittance was approximately 85-90 (Omnexus SpecialChem, 2021). Thereby, World Health Organization (1998) suggests that natural colourants requiring protection from light should be preserved in a light resistant packaging. However, Figure 1A shows that the L* of all steamed cupcakes containing powdered natural colourants was not significantly different during storage for 4 days ($P>0.05$). On day 6, all samples were not examined because they had physical spoilages, especially an unpleasant odour was observed. Meanwhile, texture profiles of each treatment were considered during storage. The hardness (Figure 2A) and fracturability (Figure 2B) of all steamed cupcakes containing powdered natural colourants increased due to an increase

in the storage time up to 2 days, whereas the stringiness (Figure 2C) of roselle and green tea steamed cupcake was not significantly different during storage for 0-4 days (P>0.05). According to other studies, these textural changes could be a result of the changes in product moisture (water loss) and the starch retrogradation, resulting in a rapid recrystallisation of amylose, which causes the hardness characteristic, slow recrystallization of amylopectin as well as staling process of cakes and bread (Culetu et al., 2018; Marchetti et al., 2021).

Table 2 Physical characteristic of all steamed cupcakes during storage at chilled temperature

Samples	Storage time (Days)			
	0	2	4	6
Control				
Turmeric steamed cupcake				
Roselle steamed cupcake				
Green tea steamed cupcake				

Table 3 Sensory evaluation of steamed cupcakes with various treatments

Sensory preferences	Control	Turmeric steamed cupcake	Roselle steamed cupcake	Green tea steamed cupcake
Appearance	7.20±1.83^{a}	6.63±2.11^{a}	6.30±1.91^{a}	6.57±2.05^{a}
Colour	7.20±1.60^{a}	6.67±1.92^{ab}	6.23±1.81^{b}	6.83±1.46^{ab}
Odour	6.60±1.50^{a}	5.57±2.01^{b}	6.57±1.63^{a}	6.13±1.74^{ab}
Flavour	7.10±1.30^{a}	4.97±2.13^{b}	6.67±1.69^{a}	6.66±1.54^{a}
Texture	7.33±1.24^{a}	6.57±1.74^{a}	7.30±1.32^{a}	7.33±1.32^{a}
Overall acceptability	7.50±1.11^{a}	5.87±1.94^{b}	6.90±1.21^{a}	7.20±1.03^{a}

Different letters in the same row indicate significant difference (P<0.05).

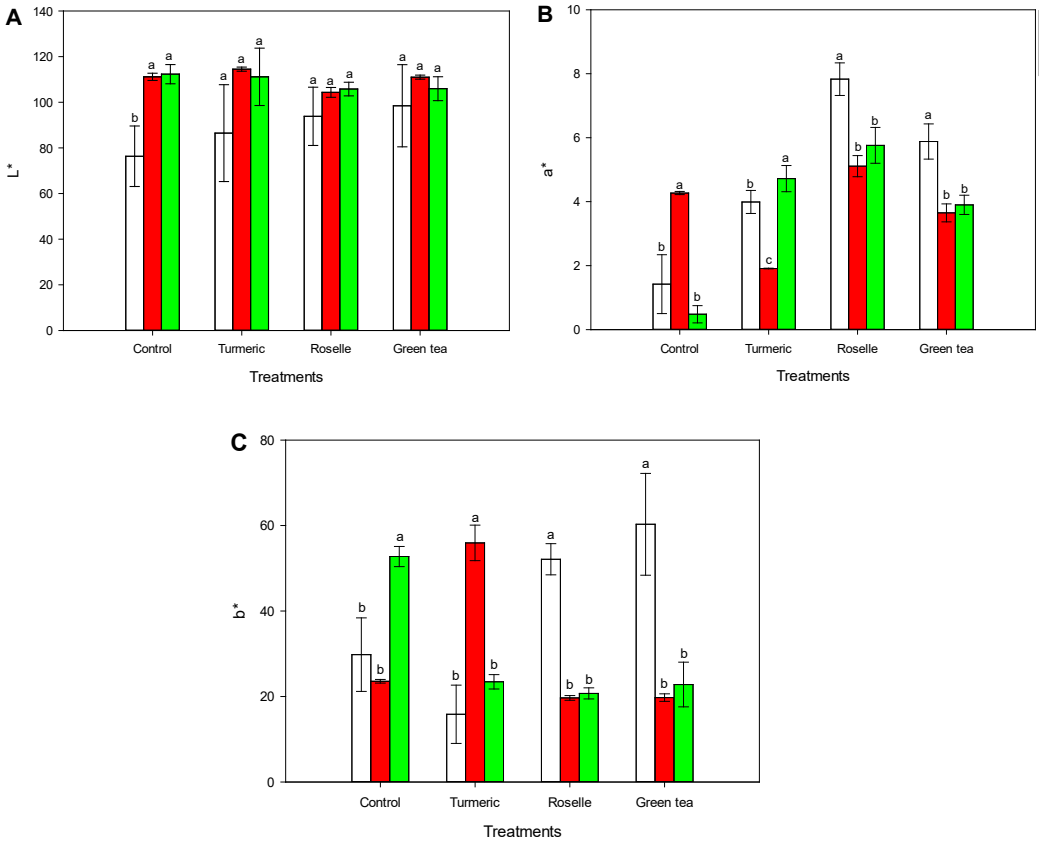

Figure 1 The colour values, (A) L*, (B) a* and (C) b* of steamed cupcake with various treatments during storage; white bar = day 0; red bar = day 2; and green bar = day 4. Different letters indicate different (P<0.05) within the same natural colourant treatments during storage.

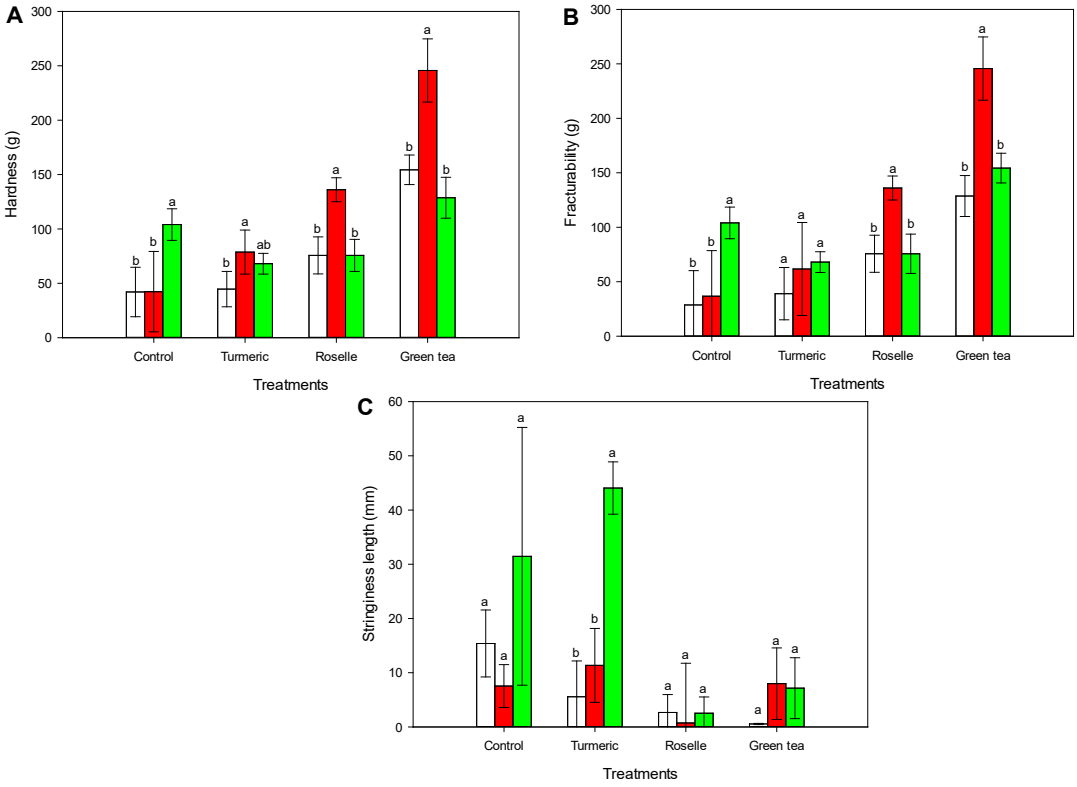

Figure 2 Texture profile analysis, (A) hardness, (B) fracturability and (C) stringiness of steamed cupcake with various treatments during storage; white bar = day 0; red bar = day 2; and green bar = day 4. Different letters indicate different ($P<0.05$) within the same natural colourant treatments during storage.

Microbiological quality of steamed cupcakes during storage

As a matter of fact, steamed cupcakes had a short shelf-life because the water activity and pH were 0.90-0.93 and 6.93-7.53, respectively. These conditions cause the growth of microorganisms, especially a group of mesophiles and/or food-borne pathogens. Based on the standard of microbiological safety in ready-to-eat bakery products (Centre for Food Safety, 2014; Thai Community product standard, 2009), the microbial combination criteria, such as total viable count, yeasts and moulds, *S. aureus, B. cereus, E. coli*, and *Salmonella* spp., were considered during storage (Table 4). The *E. coli* and *Salmonella* spp. were not found in all samples. *S. aureus* was found to be <100 CFU/g in all steamed cupcakes containing natural colourant powder, which was relevant to standard of ready-to-eat bakery product. On day 2, the viable count of yeasts and moulds in the control treatment was 2.01×10^4 CFU/g which met an unsatisfactory level, while the number of *S. aureus* was about 2.50×10^2 CFU/g which met the limit level. In addition, an unpleasant odour was found in the control treatment after day 2. The total viable counts and *B. cereus* of turmeric, roselle and green tea steamed cupcakes were approximately $<10^4$ and $<10^3$ CFU/g, respectively, which satisfied the criteria of microbiological safety for 2 days, but the day 4 had an unsatisfactory level. However, all steamed cupcakes containing powdered natural colourants had physical spoilage at the storage period of 6 days. The consequence of steamed cupcakes treated with powdered natural colourants was achieved, resulting in controlling hazardous food-borne pathogens and extending the product shelf-life. Many studies have confirmed that the chemical composition of turmeric, roselle, and green tea is rich in bioactive compounds with antimicrobial properties. These medicinal plants could be applied as colouring agents and natural preservative in food production to enhance the preservative functions. Turmeric contains curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin. Green tea is a rich source of epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, catechin, gallocatechin gallate,

catechin gallate, and gallocatechin. Roselle consists of phenolics, flavonoids and anthocyanins in which generally posssess antimicrobial activity (Lim et al., 2020; Naz et al., 2010; Radi et al., 2017; Siripatrawan and Noipha, 2012).

Table 4 Microbiological quality of steamed cupcake with various treatments during storage

Microbiological parameters	**Storage time (Days)**		
	0	**2**	**4**
Total viable count (CFU/g)			
control (untreated sample)	2.00×10^2	1.00×10^2	Not examined
Turmeric steamed cupcake	2.00×10^2	<100	<100
Roselle steamed cupcake	4.60×10^2	1.50×10^2	1.00×10^4
Green tea steamed cupcake	<100	3.40×10^2	4.80×10^6
Yeasts and Molds (CFU/g)			
control (untreated sample)	2.50×10^2	2.01×10^4	Not examined
Turmeric steamed cupcake	1.00×10^2	4.00×10^2	4.50×10^2
Roselle steamed cupcake	1.40×10^3	2.50×10^2	1.00×10^2
Green tea steamed cupcake	2.50×10^2	1.00×10^2	1.50×10^2
***S. aureus* (CFU/g)**			
control (untreated sample)	2.00×10^2	2.50×10^2	Not examined
Turmeric steamed cupcake	<100	<100	<100
Roselle steamed cupcake	<100	<100	<100
Green tea steamed cupcake	<100	<100	<100
***B. cereus* (CFU/g)**			
control (untreated sample)	<100	4.50×10^2	Not examined
Turmeric steamed cupcake	<100	<100	2.85×10^3
Roselle steamed cupcake	1.00×10^2	5.00×10^2	2.55×10^2
Green tea steamed cupcake	7.00×10^2	<100	1.00×10^7
***E. coli* (MPN/g)**			
control (untreated sample)	<0.03	<0.03	Not examined
Turmeric steamed cupcake	<0.03	<0.03	<0.03
Roselle steamed cupcake	<0.03	<0.03	<0.03
Green tea steamed cupcake	<0.03	<0.03	<0.03
***Salmonella* spp.**			
control (untreated sample)	Not detected	Not detected	Not examined
Turmeric steamed cupcake	Not detected	Not detected	Not detected
Roselle steamed cupcake	Not detected	Not detected	Not detected
Green tea steamed cupcake	Not detected	Not detected	Not detected

Not examined indicates that the sample was not examined because of spoilage.

Conclusion

The results of this study showed that the parameters of microbiological safety of all steamed cupcakes treated with powdered natural colourants met the standard of ready-to-eat of bakery product based on the storage period of 2 days at 4 ^{o}C, whereas a control treatment had a spoilage characteristic of unpleasant odour within 2 days. The present investigation demonstrated that the application of turmeric, roselle and green tea powder is a promising approach for controlling the physical and microbiological quality of steamed cupcakes. Part of these plants could be further applied as a natural colouring agent for substituting the use of artificial colouring for steamed cupcake product in Thailand. However, sensory characteristics of turmeric steamed cupcake should be improved, while antioxidant activity measurement should be evaluated in further study.

Acknowledgement

This work was supported by Rajamangala University of Technology Isan, Nakhon Ratchasima. We would also like to thank Miss. Martha Eromine for English language editing of this manuscript.

References

Ahmed, M. A., Al-Khalifa, A. S., Al-Nouri, D. M., & El-Din, M. F. S. (2021). Dietary intake of artificial food color additives containing food products by school-going children. *Saudi Journal of Biological Sciences*, *28*(1), 27-34.

Angelidis, A. S., Chronis, E. N., Papageorgiou, D. K., Kazakis, I. I., Arsenoglou, K. C., & Stathopoulos, G. A. (2006). Non-lactic acid, contaminating microbial flora in ready-to-eat foods: A potential food-quality index. *Food microbiology, 23*(1), 95-100.

Azeez, T. B., & Lunghar, J. (2021). Antiinflammatory effects of turmeric (Curcuma longa) and ginger (Zingiber officinale). *Inflammation and Natural Products,* 127-146.

Boonkum, N., & Mahidsanan, T. (2021). Effect of turmeric juice and peppermint juice on the quality of Flatbread Roti. *Thai Science and Technology Journal*, Accepted manuscript. (in Thai)

Centre for Food Safety. (2014). *Microbiological guidelines for food. For ready-to-eat food in general and specific food items*. https://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf

Culetu, A., Duta, D. E., & Andlauer, W. (2018). Influence of black tea fractions addition on dough characteristics, textural properties and shelf life of wheat bread. *European Food research and technology, 244*(6), 1133-1145.

Dlubala, A., Boguslawska-Was, E., & Daczkowska-Kozon, E. (2021). Prevalence, virulence genes, and genetic diversity of *Bacillus cereus* isolated from convenience food. *Acta Scientiarum Polonorum Technologia Alimentaria*, *20*(1).

FDA-BAM. (2001). *In FDA's bacteriological analytical manual*. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

Gattuso, G., Caristi, C., Gargiulli, C., Bellocco, E., Toscano, G., & Leuzzi, U. (2006). Flavonoid glycosides in bergamot juice (*Citrus bergamia* Risso). *Journal of Agricultural and Food Chemistry, 54*(11), 3929-3935.

Kobylewski, S., & Jacobson, M. F. (2012). Toxicology of food dyes. *International journal of occupational and environmental health, 18*(3), 220-246.

Lertnirundon, N., & Mahidsanan, T. (2020). Effectiveness of bergamot juice on the survival of Bacillus cereus and quality of Thai steamed pumpkin cake. *Food and Applied Bioscience Journal, 8*(1), 33-42.

Lim, H. W., Seo, K. H., Chon, J. W., & Song, K. Y. (2020). Antimicrobial activity of *Hibiscus sabdariffa* L.(Roselle) powder against food-borne pathogens present in dairy products: Preliminary study. *Journal of Dairy Science and Biotechnology, 38*(1), 37-44.

Marchetti, L., Acuña, M. S., & Andrés, S. C. (2021). Effect of pecan nut expeller meal on quality characteristics of gluten-free muffins. *LWT, 146*, 111426.

Mulat, M., Khan, F., Muluneh, G., & Pandita, A. (2020). Phytochemical profile and antimicrobial effects of different medicinal plant: current knowledge and future perspectives. *Current Traditional Medicine, 6*(1), 24-42.

Naz, S., Jabeen, S., Ilyas, S., Manzoor, F., Aslam, F., & Ali, A. (2010). Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. *Pak J Bot, 42*(1), 455-62.

Olaimat, A. N., Ghoush, M. A., Al-Holy, M., Hilal, H. A., Al-Nabulsi, A. A., Osaili, T. M., & Holley, R. A. (2021). Survival and growth of *Listeria monocytogenes* and *Staphylococcus aureus* in ready-to-eat Mediterranean vegetable salads: Impact of storage temperature and food matrix. *International Journal of Food Microbiology,* 109149.

Omnexus SpecialChem. (2021). *Transparency*. Transmission of visible light. https://omnexus.specialchem.com/polymer-properties/properties/transparency

Pakditawan, S. (2016). *Thai steamed cupcakes*. Khanom Pui Fai. https://www.sirinyas-thailand.de/2016/01/04/thai-steamed-cupcakes-khanom-pui-fai/

Radi, M., Firouzi, E., Akhavan, H., & Amiri, S. (2017). Effect of gelatin-based edible coatings incorporated with Aloe vera and black and green tea extracts on the shelf life of fresh-cut oranges. *Journal of Food Quality,* 2017.

Rashidinejad, A., Boostani, S., Babazadeh, A., Rehman, A., Rezaei, A., Akbari, S., & Jafari, S. M. (2021). Opportunities and challenges for the nanodelivery of green tea catechins in functional foods. *Food Research International*, *142*, 110186.

Sharifi-Rad, J., El Rayess, Y., Abi Rizk, A., Sadaka, C., Zgheib, R., Zam, W., & Martins, N. (2020). Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Frontiers in pharmacology*, *11*.

Siripatrawan, U., & Noipha, S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food hydrocolloids, 27*(1), 102-108.

Tapia, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. *Water activity in foods: Fundamentals and applications*, 323-355.

Thai Community product standard. (2009). *Thai desserts (No.1531/2552)*. https://tcps.tisi.go.th/pub/tcps1_52.pdf

World Health Organizatio. (1998). *Quality control methods for medicinal plant materials*. World Health Organization. https://books.google.co.th/books?hl=en&lr=&id=ZjizDwAAQBAJ&oi=fnd&pg=PT3&dq=Quality+control+methods+for+medicinal+plant+materials.+World+Health+Organization&ots=yRqYJedPFb&sig=C5rpQYtI92MhVEkVX_P8N3ogTmg&redir_esc=y#v=onepage&q=Quality%20control%20methods%20for%20medicinal%20plant%20materials.%20World%20Health%20Organization&f=false