Nghiên cứu ảnh hưởng của tinh dầu gừng đến chỉ tiêu vi sinh và chất lượng cảm quan của Bánh ít lá gai theo thời gian bảo quản

TÓM TẮT

Bánh ít lá gai là loại bánh đặc sản rất được ưa chuộng của tỉnh Bình Định, được làm chủ yếu từ bột nếp, lá gai, đậu xanh, dừa và đường với phương pháp chế biến truyền thống. Tuy nhiên, các nghiên cứu đã chỉ ra sự có mặt của một số chủng nấm mốc và vi khuẩn là nguyên nhân gây hư hỏng Bánh ít lá gai. Trong nghiên cứu này, tinh dầu gừng đã được bổ sung vào công thức bánh như một chất kháng khuẩn/kháng nấm giúp kéo dài thời gian sử dụng của bánh. Kết quả khảo sát ảnh hưởng của việc bổ sung tinh dầu gừng đến hạn sử dụng của Bánh ít lá gai thành phẩm cho thấy: bổ sung tinh dầu gừng với nồng độ 4mL/kg bột có thể ngăn ngừa sự phát triển của vi sinh vật, đảm bảo kéo dài hạn sử dụng của Bánh ít lá gai thêm 3 ngày so với bánh không bổ sung tinh dầu gừng. Các chỉ tiêu cảm quan cũng chỉ ra rằng nồng độ tinh dầu bổ sung này phù hợp với thị hiếu của người sử dụng Bánh ít lá gai.

Từ khóa: *Bánh ít lá gai, vi sinh vật, nấm mốc, tinh dầu gừng.*

Research on the effects of ginger essential oil on the microbiological criteria and sensory quality of Ramie leaf rice cake according to storage time

ABSTRACT

Ramie leaf rice cake (RLRC) is a very popular specialty cake of Binh Dinh province, made mainly from glutinous rice flour, ramie leaves, green beans, coconut and sugar with traditional processing methods. However, studies had shown the presence of some strains of mold and bacteria as the cause of RLRC spoilage. In this study, ginger essential oil (GEO) was added to the cake formula as an antibacterial/antifungal agent to help prolong the shelf life of the cake. Survey results on the effects of adding GEO on the shelf life of RLRC show that adding GEO at a concentration of 4mL/kg of flour can prevent the growth of microorganisms, guaranteed to extend the shelf life of RLRC by 3 days compared to cakes without GEO added. Sensory criteria also show that the concentration of this additional essential oil is suitable for the tastes of RLRC consumers.

Keywords: *Ramie leaf rice cake, microorganisms, molds, ginger essential oil.*

1. INTRODUCTION

Ramie leaf rice cake is the well-known traditional specialist of Binh Dinh province. As the other rice cakes in Asia countries, these cakes made from the basic ingredients such as glutinous sticky rice, sugar, and some types of beans, coconuts, or sesame. The common methods for making these cakes include steaming, frying, and boiling. Because of manufacturing process without using the additives and tropical climate, the shel-life of these cakes is so short, about 2 – 3 days[1]. Research by Hoa et al[2] has shown that four fungal strains are frequently present and cause damage to Binh Dinh RLRC, which are *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii* and *Aspergillus fumigatus*.

Thanh et al[3] using extracts of natural compounds from cinnamon, anise, and black cardamom added to RLRC. Research results showed that extracts of natural substances from black cardamom with the addition rate of 5mL/kg of flour can help limit the deterioration of sensory quality and prevent the growth of microorganisms, thereby helping to prolong the storage time of RLRC up to 5 days in normal temperature, 2-3 days longer than traditional production methods. However, the above natural compounds have a strong aroma and affect the taste and smell of this product. Therefore, it is possible to research the use of some other essential oils that are more suitable in prolonging the preservation time, while limiting the change in flavor properties of the cake, and ensuring that not effect the health of consumers.

Ginger *(Z. officinale)* is a species that produces oleoresins and essential oils with chemical compounds of diverse properties. Essential oils have characterized mainly as *gingerols* which have been reported to possess analgesic, anti-inflammatory, anticancer, antipyretic, antioxidant and antibacterial activities. The antifungal activity of *Z. officinale* essential oil is well documented, mainly in filamentous fungi such as *Penicillium spp., Rhizopus sp., A. flavus, A. solani, A. oryzae, A. niger, F. moniliforme, F. verticillioides* and in yeasts such as *Saccharomyces cerevisiae* and *C. albicans*[4-7]

With an eye to extend the shelf life of cakes while still ensuring friendliness to consumers' health, we proceed experiment using ginger essential oil (GEO) as a natural preservation additive and determine its effectiveness to microbiological criteria and sensory quality of RLRC according to storage time.

2. MATERIALS AND METHOD

2.1. Material

The research materials included ramie leaves, glutinous rice, peeled mung beans, shredded coconuts and banana leaves were sourced from

the Binh Dinh Province. Refined sugar was obtained from TTC Bien Hoa-Dong Nai Sugar One-Member Limited Company, Vietnam.

GEO extracted by Hilltown Essential Oil Co. Ltd. (CC43 Ho Van Hue Street, Ward 9, Phu Nhuan District, Ho Chi Minh City, Vietnam).

Chemicals used in microbiological research: NA, PDA, ethanol, NaCl 0.9%.

Equipments used in microbiological research: Benchtop pH meter BP 3001 (USA); quantitative balance; biological safety cabinets; autoclave sterilizer; Petri dishes; implant; alcohol lamp; test tube; volumetric flasks, absorbent and waterproof cotton balls,...

Baking equipment as dough mixer; electric stove,...

2.2. Method

2.2.1. Method of counting total aerobic microorganisms

According to TCVN 4884-1:2015 (ISO 4833-1:2013): Microorganisms in the food chain - Methods for the enumeration of microorganisms. Microbiological analysis techniques were conducted according to Le Van Viet Man[8]

Using the pour plate technique, count the colonies on the agar medium after aerobic incubation at 35 ± 2 ^{0}C for 24 to 48 hours. The number of aerobic bacteria in 1 g or 1 mL of the food product sample tested is calculated from the number of colonies counted on the culture plates according to the dilution concentrations.

Select plates with 15 to 300 colonies on plates of 2 consecutive dilutions. Calculate according to the formula:

$$N = A/(V.D_f)$$

In which:

N: total number of CFU/g (mL) of substrate sample analyzed

A: average number of CFU per petri dish at a certain dilution

V: volume of culture sample per petri dish

D_f: sample dilution

2.2.2. Method for determining antimicrobial ability of ginger essential oil

The antimicrobial activity test of GEO was performed using the agar disc diffusion method as described by Euloge et al (2012) with modifications. Different concentrations of essential oils were mixed into molten PDA medium (45 ^{0}C), shaken well to disperse the essential oils, and the medium was divided into sterile Petri dishes (90 mm in diameter). Fungal rings (5 mm in diameter) were cut from the growing edge of the pure fungal culture (after 3 days) and transferred to the center of the Petri dish containing the essential oil medium, the control dishes (without essential oil) were inoculated according to the same procedure. Then the fungal dishes were incubated in the dark at 28 ± 2 ^{0}C. Colony diameter was measured after 24 hours per time, lasting up to 120 hours. The percentage of inhibition of mycelial growth by essential oils was calculated according to the formula of Philippe et al.[9]

$$\text{Inhibition of mycelial growth (\%)} = \frac{d_c - d_t}{d_c}$$

Where:

d_c: diameter of colony in control sample

d_t: diameter of colony in sample containing essential oil.

2.2.3. Method of investigating the effect of ginger essential oil on the shelf life of Ramie leaf rice cake

The control (without GEO) and added GEO samples of RLRC were maintained at room temperature. The concentration of GEO selected will be based on the results of the survey on the antimicrobial ability of ginger essential oil as presented above. Assuming the inhibitory concentration is *a*, we have the following table of GEO concentration survey

Table 1. Essential oil concentration survey

GEO concentration	*a-2*	*a-1*	*a*	*a+1*	*a+2*
Results	M1	M2	M3	M4	M5

Step: 1 mL

Samples were taken to determine and evaluate the total number of aerobic microorganisms in the cake periodically after each day (24 hours).

2.2.4. Sensory quality assessment method

Assess the sensory quality of RLRC products by scoring method simulating the TCVN 3215-79 standard using the 20-point system. TCVN 3215-79 is a standard using the 20-point system built on a unified scale of 6 levels of 5 points (0 ÷ 5) in which point 0 corresponds to “damaged” product quality, and points from 1 ÷ 5 correspond to decreasing levels of defects. At point 5, the product is considered to have no defects or errors.

In the considered properties, the product has typical and obvious good properties for that indicator. The 20-point system is divided into 6 levels of product quality classification[10].

Table 2. Weight coefficient of indicators (according to TCVN 3215-79)

No	Indicator name	Weight coefficient
1	Status	1.5
2	Color	0.6
3	Odor	0.5
4	Taste	1.4

Table 3. Sensory indicators are applied according to TCCS 01/2017/BILG-ST

No	Indicator name	Requirements
1	Status	Pyramid cake, filling made of coconut and green beans
2	Color	Black
3	Odor	Aroma of ramie leaves
4	Taste	Sweet, rich, fatty

2.2.5. Data collection and processing methods

The reported data are the average values of 3 experimental repetitions. The data were processed and the graph were drawn using Graphpad Prism 8. The average values were analyzed by ANOVA using the Tukey test and the value of $p < 0.05$ indicates a statistically significant difference.

3. RESULT

3.1. The ability of GEO to inhibit the growth of microorganisms under culture conditions

The results of investigating the ability of GEO to inhibit fungal mycelium growth with four pathogen fungal strains *(Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii* and *Aspergillus fumigatus)* after 48 hours are shown below.

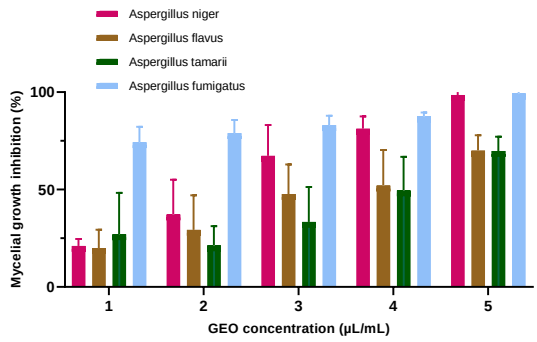

Figure 1. Percentage of mycelial growth inhibition of GEO after 48 hours

The result in Figure 1 shows that GEO has strong inhibitory effect on *Aspergillus fumigatus* and *Aspergillus niger* strains, but weaker inhibition on *Aspergillus flavus* and *Aspergillus tamarii* strains at almost all concentrations.

In addition, the survey result shows that the percentage of mycelium growth inhibition was proportional to the GEO concentration. The higher the concentration is, the stronger the inhibition ability is. The percentage of mycelium growth inhibition of GEO was significantly different ($p < 0.05$), compare the concentration of 3 µL/mL and the concentration of 5 µL/mL. However, the results between the concentration of 4 µL/mL and the concentration of 5 µL/mL showed an insignificant difference ($p > 0.05$). We can rely on this to choose a more optimal concentration of essential oil, ensuring both antifungal effectiveness and the sensory properties of the cake (because high GEO concentration makes the product have a "pungent" and "spicy" smell).

Figure 2. Image of the inhibition survey of GEO at a concentration of 5 mL/L on 2 fungal strains after 48 hours of culture (CB1 - *Aspergillus niger*, CB3 - *Aspergillus tamarii*)

3.2. Effect of GEO on the growth of microorganisms on RLRC during storage

The spoilage index of finished cakes is determined based on the total number of aerobic microorganisms developed on samples over storage time.

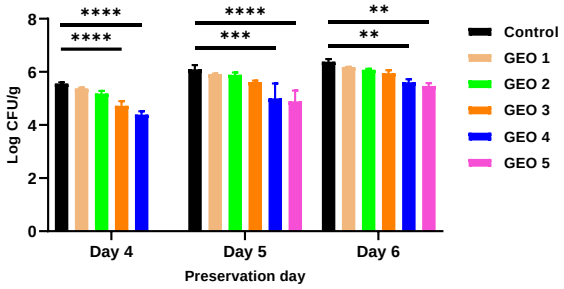

Figure 3. The total number of aerobic microorganisms (Log CFU/g) developed on cake samples over storage time (GEO 1, 2, 3, 4, 5 means the GEO concentration add to the cake with 1, 2, 3, 4, 5 mL/kg of flour, respectively) *(****: $p < 0.0001$, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$)*

Initially, in the first 2 days of preservation period time, there is no damage on the RLRC surface. After that, the control sample (without adding GEO) began to show viscous flow, while the samples using GEO began to gradually harden. In particular, since day 5, the total number of aerobic microorganisms (TNOAM) increased rapidly and exceeded the allowable limit according to TCVN 12941: 2020 standards, with the total number of aerobic microorganisms of the control sample reaching 129.5 x 10^4 (CFU/g), approximately 6.11 logCFU/g.

Under normal conditions, on the 4^{th} day of storage, there was no significant difference of the TNOAM between the samples supplemented with GEO and the control samples (without adding GEO). However, the difference between the experimental samples and the control samples was significant ($p < 0.05$) on the 5^{th} day of storage, which shows that the addition of GEO contributed to limiting the growth of aerobic microorganisms on RLRC according to storage time.

The concentration of GEO added to the cake is inversely proportional to the TNOAM growing in RLRC, in which the GEO concentration of 5mL/kg of flour shows the most obvious difference. The number of microorganisms in this experimental sample was the smallest and did not change much after 6 days of storage (the 6th day was not significantly different from the 4th day). However, there was no significant difference ($p < 0.05$) in the total number of aerobic microorganisms between the sample supplemented with GEO concentration of 5mL/kg and the sample supplemented with GEO 4mL/kg. This shows that adding GEO at a concentration of 4 mL/kg or 5 mL/kg is equally effective in inhibiting the growth of microorganisms. Therefore, to ensure the sensory properties of the product and reduce raw material costs during production, we can choose a lower GEO concentration of 4 mL/kg and still ensure the effectiveness of extending the preservation time of the cake for 3 more days compared to the previous traditional cake (only kept for a maximum of 3 days).

3.3. Effect of GEO on sensory quality of RLRC during storage

To evaluate the influence of GEO on the change in sensory quality of RLRC, a sensory test according to TCVN 3215-79 was performed.

The results of surveying the influence of GEO concentration on the sensory quality of RLRC according to storage time are shown below.

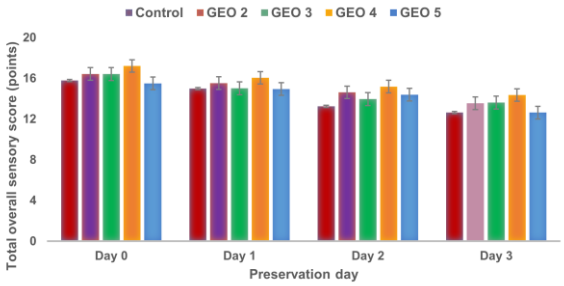

Figure 4. Effect of GEO concentration on the change in sensory quality of RLRC over storage time

The analysis results show that the overall sensory quality of RLRC reflected in the total average sensory score decreased with storage time. What's more there are differences between cake samples on the same day of storage. After 3 days of storing under normal conditions, the control sample without GEO supplementation show the slight decrease of the overall sensory score from 15.77 point, coresponding good grade, to 12.62 point, coresponding average grade. According to the microbiological test results, after 4 days of storage, the TNOAM in the control sample was under safe food standard, hence we did not continue to perform sensory evaluation after the 3rd day of storage.

The cake adding GEO has an aroma in harmony with the typical smell and taste of the traditional RLRC. Especially in the traditional method, people often use ginger in the cake filling as a way to add flavor delicious for cake. Maybe that's why the filling is less damaged than the crust (according to research by Le Duy Thanh[3]).

However, when observing the total sensory score results by day, we found that there was no obvious difference between samples GEO2, GEO3 and GEO4 with additional GEO concentrations of 2, 3 and 4 mL/kg flour, respectively. There is a significant difference between GEO4 and control as well as GEO5, this shows that adding GEO increases the sensory quality of the cake. However, adding a lot of GEO will reduce the sensory quality of the cake, because it is not suitable for consumer tastes. This can be observed via comparing the sensory score of GEO4 and GEO5.

From the above analysis, it shows that, in terms of sensory quality, the cake sample supplemented with GEO concentration of 4 mL/kg of flour has a higher sensory quality than the control sample at the first day of storage. Besides, the sensory score of GEO4 sample decreased slowly over storage time. Therefore, the GEO concentration chosen to add to the traditional RLRC production process is 4 mL/kg of flour.

4. CONCLUSION

The research result indicate that GEO can inhibit all four mold strains that cause RLRC spoilage, in which GEO achieves strong inhibition against *Aspergillus fumigatus* and *Aspergillus niger* strains, weaker inhibition against *Aspergillus flavus* strains and *Aspergillus tamarii* strains at almost all survey concentrations.

The results of surveying the effect of adding GEO on the shelf life of RLRC show that adding GEO concentration of 4mL/kg of flour can prevent the growth of microorganisms, ensuring the extension of the shelf life of the RLRC product adds 3 days compared to cakes without added GEO. And the GEO concentration of 4mL/kg of flour added to the cake meets the tastes of consumers.

Within the scope of the project, we have not yet evaluated the minimum inhibitory concentration of GEO against all four mold strains that cause RLRC spoilage. More surveys are needed to determine the minimum inhibitory concentration of GEO against these four strains.

REFERENCES

1. Sở Khoa học và Công nghệ Bình Định. *Báo cáo kết quả thực hiện dự án khoa học và công nghệ hỗ trợ phát triển các sản phẩm đặc trưng tỉnh Bình Định năm 2015,* Bình Định, 2015.
2. Thi Trong Hoa Vo, Thi Tho Nguyen, Thi Minh Nguyet Nguyen, Thi Mong Diep Nguyen. Survival and Growth of Food Borne Harmful Fungi during Storage of Vietnamese Ramie Leaf Rice Cake, *Asian Journal of Dairy and Food Research*, 2024, Online First.
   doi 10.18805/ajdfr.DRF-383
3. Lê Duy Thanh. *Nghiên cứu sử dụng các hợp chất tự nhiên trong bảo quản Bánh ít lá gai Bình Định*, Báo cáo Đề tài cấp Trường, Bình Định, 2022.
4. Sa-Nguanpuag, K. S. Ginger *(Zingiber officinale)* Oil as an Antimicrobial Agent for Minimally Processed Produce: A Case Study in Shredded Green Papaya, *International Journal of Agriculture & Biology*, **2011**, *13*, 895-901.
5. Noori et al. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets, *Food Control*, **2017,** *84*, 312-320.
6. Sunita Munda, S. D.. Chemical Analysis and Therapeutic Uses of Ginger *(Zingiber officinale Rosc.)* Essential Oil: A Review, *Journal of Essential Oil Bearing Plants*, **2018**, *21*(4), 994-1002.
   doi:10.1080/0972060X.2018.1524794
7. López, E.I.C., Balcázar, M.F.H., Mendoza, J.M.R., Ortiz, A.D.R., Melo, M.T.O., Parrales, R.S. and Delgado, T.H. Antimicrobial Activity of Essential Oil of *Zingiber officinale Roscoe* (Zingiberaceae), *American Journal of Plant Sciences*, **2017**, *8*, 1511-1524.
   doi.org/10.4236/ajps.2017.87104
8. Lê Văn Việt Mẫn, Lại Mai Hương. *Thí nghiệm Vi sinh vật học thực phẩm*, NXB Đại học Quốc gia TP. Hồ Chí Minh, Hồ Chí Minh, 2010.
9. Philippe et al. Chemical Composition and Antifungal activity of Essential oil of Fresh leaves of *Ocimum gratissimum* from Benin against six Mycotoxigenic Fungi isolated from traditional cheese wagashi, *Research Journal of Biological*, **2012**, *1*, 22-27.
10. Hà Duyên Tư. *Kĩ thuật phân tích cảm quan thực phẩm*, NXB Khoa học và kĩ thuật, Hà Nội, 2010.