Properties and Antifungal Effect of Chitosan Film Incorporating Orange Essential Oil on the Shelf Life of Orange Flavored Butter Cake

สมบัติและผลของการต้านเชื้อราของฟิล์มไคโตซานผสมน้ำมันหอมระเหยจากส้มต่ออายุการเก็บรักษาเค้กเนยกลิ่นส้ม

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บทคัดย่อ: การศึกษาสมบัติของฟิล์มไคโตซานผสมน้ำมันหอมระเหยจากส้ม 2 ระดับความเข้มข้น คือ 0.7 และ 1.4 เปอร์เซ็นต์ (v/v) ตามลำดับ เมื่อเปรียบเทียบกับฟิล์มไคโตซานที่ไม่ได้ผสมน้ำมันหอมระเหยจากส้ม พบว่าฟิล์มไคโตซานผสมน้ำมันหอมระเหยจากส้ม 1.4 เปอร์เซ็นต์ มีความยืดหยุ่นมากขึ้นแต่ความแข็งแรงและอัตราการซึมผ่านของน้ำลดลงเป้าหมายของการศึกษานี้คือการใช้ น้ำมันหอมระเหยจากส้มเพื่อยืดอายุการเก็บรักษาเค้กเนยรสส้มโดยศึกษาผลของการยับยั้งการเกิดเชื้อราบนเค้กเมื่อเปรียบเทียบการห่อเค้กเนยรสส้มด้วยฟิล์มไคโตซานที่ไม่ได้ผสมน้ำมันหอมระเหยจากส้มกับฟิล์มไคโตซานที่ผสมน้ำมันหอมระเหยจากส้ม 0.7 และ 1.4 เปอร์เซ็นต์ ซึ่งเค้กทั้งหมดถูกเก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียส เป็นระยะเวลา 10 วัน พบว่าเค้กที่ห่อด้วยฟิล์มผสมน้ำมันหอมระเหยจากส้ม 1.4 เปอร์เซ็นต์ มีเนื้อสัมผัสดีที่สุดและมีการสูญเสียน้ำหนักน้อยที่สุด จากการสังเกตการณ์ปรากฏของเชื้อราบนผิวเค้กทุกวัน พบว่า เค้กที่ห่อด้วยฟิล์มยืด ฟิล์มไคโตซาน ฟิล์มไคโตซานผสมน้ำมันหอมระเหยจากส้มที่ระดับความเข้มข้น 0.7 และ 1.4 เปอร์เซ็นต์ มีอายุการเก็บรักษา 2, 4, 7 และมากกว่า 10 วันตามลำดับ ฟิล์มไคโตซานผสมน้ำมันหอมระเหยจากส้มที่ระดับความเข้มข้น 1.4 เปอร์เซ็นต์ ให้ผลในการยับยั้งการเจริญของเชื้อราได้ดีที่สุด สรุปได้ว่าน้ำมันหอมระเหยจากส้มทำให้ฟิล์มมีคุณสมบัติที่ดีขึ้นสามารถยืดอายุการเก็บรักษาเค้กส้มได้ยาวนานขึ้น และให้ผลทางประสาทสัมผัสเป็นที่ยอมรับ

คำสำคัญ: ไคโตซาน ฟิล์ม น้ำมันหอมระเหยจากส้ม เค้ก การต้านเชื้อรา อายุการเก็บ

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Abstract: This study investigated the properties of chitosan film incorporating orange essential oil at 0.7% and 1.4% (v/v) compared to pure chitosan film. Chitosan film with 1.4% orange essential oil had much better elongation but the strength and water vapor transmission rate were lower. The application of orange essential oil film to extend the shelf life of orange flavored butter cake was the aim of this study. An antifungal effect against mold on orange flavored butter cake was also evaluated. The comparative trials between wrapping pure chitosan film and chitosan film with 0.7% and 1.4% of orange essential oil around the cake were studied at 25°C for 10 days. Cake wrapped by film containing 1.4% orange essential oil had the best texture and least weight loss. The cake surface was observed daily for the growth of mold. The study showed that cake wrapped in stretch film, chitosan film with 0, 0.7 and 1.4% orange essential oil had 2, 4, 7 and more than 10 days shelf life, respectively. Chitosan film with 1.4% orange essential oil provided the best mold inhibition. Therefore, orange essentialoil can improve chitosan film properties and prolong the shelf life of orange cake with acceptable sensory characteristics.

Keywords: Chitosan, film, orange essentialoil, cake, antifungal, shelf life

Introduction

Bakery products normally have a shelf life around 3-4 days when they are unpreserved. Visible growth of fungi is an indicator of the end of product life. Furthermore, fungi are responsible for off-flavor formation and the production of mycotoxins and allergenic compounds (Gutierrez *et al*., 2009). Chemical preservatives can be used to extend the storage life of these products. However, consumer prefers fresh products without chemical preservatives but keeping free from microorganism. Natural antimicrobials are GRAS (Generally Recognized as Safe), however, given the strong flavor and affected the organoleptic qualities of the food (Sangsuwan *et al*., 2008). The application of these antimicrobial agents to food needs to be careful. Citrus essential oil, natural antimicrobials, could be an alternative to chemical additives for use in food industry. Orange essential oil was very effective against *Aspergillus niger*, *A.flavus, Penicillium verrucosum* and *P. chrysogenum* (Viuda-Martos *et al*., 2008).

Active antimicrobial packaging is the packaging that incorporated antimicrobial agents that may delay or prevent the growth of microorganisms on the surface of the products. Release of antimicrobial substances from packaging materials to the food surface may control microbial contamination by reducing the growth rate, extending the lag-phase of the target microorganism, and inactivating microorganism on contact (Han, 2000). Many researchers incorporated essential oils or plant extracts into chitosan film but only some applied antimicrobial films to food products. Zivanovic *et al*. (2005) placed chitosan film and chitosan-oregano essential oil film in contact with inoculated bologna samples and stored 5 days at 10°C. Pure chitosan films reduced *Listeria monocytogenes* by 2 logs CFU, whereas the films with 1% and 2% oregano oil decreased the number of *L. monocytogenes* by 3.6 to 4 logs CFU and *Escherichia coli* by 3 logs CFU. Lee *et al*. (1998) incorporated 1% grapefruit seed extract into low density polyethylene film and used it to package curled lettuce. It was found that the growth rates of

aerobic bacteria and yeast decreased. Pranoto *et al*. (2005) incorporated garlic oil into chitosan film and the activity of the antimicrobial films was tested against food pathogens, *E. coli*, *Staphylococcus aureus, Salmonella typhimurium, L. monocytogenes* and *Bacillus cereus*. Incorporation of 100 µl of garlic oil/g of chitosan had antimicrobial activity against *S.aureus, L. monocytogenes*, and *B. cereus*.

The use of orange essential oil in active film to control mold growth on butter cake is unavailable. Therefore, this research has 3 main objectives. First, to test the properties of antimicrobial film from chitosan incorporating orange essential oil developed for its use with orange flavored butter cake. Second, to evaluate the quality and sensory characteristics of orange flavored butter cake wrapped by film developed. And third, to test the inhibitory effect of film developed against mold.

Materials and Methods

1. Film Preparation

Chitosan with more than 85% deacetylation (Ta Ming Enterprises Co., Ltd, Thailand) 1.5 g was dissolved in 100 ml of 0.5% acetic acid solution at 60°C with continuous stirring for 1 h. Tween 20 was added as an emulsifier. Orange essentialoil (Sigma, St. Louis, USA), 0.7% or 1.4%, was incorporated into the chitosan solution. The film-forming solutions, 33±0.5 g were poured onto glass plates (13.5 cm diameter), and dried at 50°C for 24 h. Dried films were peeled off from the glass plates and conditioned at 25 ±2°C and 50 ±5%RH for 72 h prior to evaluation.

2. Film Properties

2.1 Determination of Tensile Strength and Elongation

Tensile strength and percent elongation were tested by using the Instron Universal Testing Instrument model 5565 (Instron, Canton, MA, USA) following ASTM D638M (ASTM, 1993). Film specimens were cut into rectangular strips (1 cm x 10 cm). The initial grip separation was 5 cm and the cross-head speed was 25 mm/min. Tensile strength was calculated by dividing the maximum load by the cross-sectional area (average thickness × 1 cm) of the initial specimen. The percentage elongation was defined as the percent change in the length (ΔL) of the specimen to its original length (L) between the grips (5 cm). Tensile strength and percent elongation results were obtained from 10 sample replications.

2.2 Determination of Water Vapor Transmission Rate (WVTR)

WVTR was determined gravimetrically according to ASTM E96-95 (ASTM, 1995). Film specimens, approximately 8 cm diameter, were mounted on the aluminum cups containing 10 grams of silica gel. Paraffin was used to fix a film specimen to the wide rim of an aluminum cup. The cups were weighed and then placed in a desiccator containing saturated calcium chloride solution. The relative humidity of the chamber was kept at 35 ±2% and 25 ±1°C throughout the experiment. The water vapor, which passed through the film, was determined by recording the weight gain daily for 5 days. The test was performed in triplicate. WVTR was calculated using the following equation.

$$\text{WVTR} = \frac{W}{t\,A}$$

where $\frac{w}{t}$ = the slope of the plot between weight gain and time, g/day

A = thepermeation area, m^2

3. Cake Quality

Orange flavored butter cake (orange cake) was made with no preservative. Each piece (3.5 cm x 5 cm x 1.5 cm) was wrapped in stretch film, chitosan film, chitosan film incorporating 0.7% and 1.4% orange essential oil and placed on polystyrene trays. All cake treatments were stored at 25±2°C for 8 days.

3.1 Color

The surface color of cake after removal from all film treatments was measured using the Hunterlab color meter Color Quest XE (The Color Management Company, Reston, Virginia, USA) calibrated with a white tile. Multiple readings were taken on 4 pieces from each treatment.

3.2 Texture

Texture of orange cake was measured every 2 days using a Texture Analyzer TA.XT2i (Texture Technologies Corp., Scarsdale, NY, USA). Texture profiles were obtained after setting test speed 2.0 mm/sec, post test speed 2.00 mm/sec, target mode: strain, strain: 40%, time: 5 sec, trigger type: auto (force) and trigger force: 5 g. Cake samples were cut into circle with 4 cm diameter before testing.

3.3 Weight Loss

Weight loss of orange cake was determined by weighing the samples at specific time intervals and plotting weight loss against time. Weight was measured using a balance (±0.01 g) model BP300S (Sartorius, Menlo Park, CA, USA).

3.4 Sensory Evaluation

Evaluations were tested under ambient conditions at about 25°C in a sensory evaluation room using 50 panelists. Samples were labeled with three-digit random codes and placed in small white plastic cups. The order of presentation of the samples was randomized. The acceptability of color, odor, taste, texture and overall quality were evaluated using a nine-point hedonic scale. The variability of acceptance was analyzed by ANOVA and Tukey's-b test ($P<0.05$).

3.5 Shelf Life

Visibility of mold on cake surface was daily observed for 10 days.

Results and Discussion

1. Film Properties

1.1 Tensile Strength and Elongation of Films

Tensile Strength and percentage of elongation of chitosan film without orange essential oil was 32.06 MPa and 4.87%, respectively (Table 1). Film tensile strength reduced while percentage of elongation increased as increasing orange essential oil concentration. Addition of orange essential oil acted as a plasticizer or a lubricant in the film matrix, which increased film flexibility. Since chitosan film alone was very fragile and difficult to apply on food as a wrapper or packaging film. Therefore, incorporation of orange essential oil could provide the advantage to this application. Zivanovic *et al*. (2005) also observed the same trend in which the addition of oregano oil into the chitosan film decreased tensile strength but increased elasticity of the films. While Sánchez-González (2010) reported that the lower tensile strength and percentage of elongation when they added bergamot essential oil (BO) into chitosan film. They explained that this could be explained by discontinuities in the polymer matrix introduced by the BO incorporation and by changes in the polymer chain interactions when oil components are present, which lead to a weak mechanical response. The contradiction could be due to the concentration of essential oil used in chitosan matrix.

Table 1 Tensile strength, elongation and water vapor transmission rate of chitosan film containing different levels of orange essential oil

Orange essential oil concentration, %	Tensile Strength, MPa	Elongation, %	Water vapor transmission rate, $g/m^2.day$
0	32.06 ±2.04[b]	4.87 ±1.70[a]	21.03 ±2.32[a]
0.7	30.98 ±1.78[b]	9.01 ±1.83[b]	18.17 ±0.85[ab]
1.4	26.81 ±0.80[a]	15.16 ±1.89[c]	17.58 ±0.81[b]

*** Each data point of tensile strength and elongation represents the mean ±S.D. of four replications while water vapor transmission rate represents the mean ±S.D. of two replications, respectively. Different superscripts in the same column show significantly different ($P<0.05$)

1.2 Water Vapor Transmission Rate (WVTR) of Films

The incorporation of essential oil from orange into chitosan film was expected to increase the water vapor barrier property due to the hydrophobicity of orange essential oil. WVTR of film with orange essential oil decreased as increasing oil concentration. Incorporation of hydrophobic compounds into hydrophilic polymers causes structural modifications of the polymer matrix, causing an increase in network tortuosity (Redl *et al*., 1996; Callegarin *et al*., 1997). This impedes the transport of molecules through the network (Papadokostaki *et al*., 1997), and reduces water uptake (Vazquez *et al*., 1997). Sánchez-González (2010) also found that bergamot essential oil could also reduce film WVTR of chitosan film, while Pranoto *et al*. (2005) was not observed any different when addition garlic oil to chitosan film. The effect on essential oil on reducing WVTR could depend on the amount of essential oil incorporated into the film.

2. Cake

2.1 Color of Cake

The positive b* value represented the yellowness. As storage, b* value of cake surface was increasing (Figure 1). The inclement in b* value indicated that the color of the cake was becoming more yellow. Cake unwrapped from chitosan film incorporating 1.4% orange essential oil had significantly higher b* value than other treatments. This might be due to the migration of the orange essential oil from to the film which affected the yellowness of the cake. Sangsuwan *et al*. (2008) also reported the migration of vanillin from chitosan/methylcellulose film to fresh-cut cantaloupe and pineapple resulted in more yellowness on fruit's surface. The migration of essential oil from film to food could either provide the inhibition effect on the food surface against microorganisms or adverse effect on sensory characteristics of food (Suppakul *et al*., 2003).

2.2 Texture of Cake

The fresh baked cake had hardness of 1,326 g (Figure 2). During storage, cake hardness increased as time due to moisture loss out of cake surface. On day 8, cake wrapped in chitosan film containing 1.4% orange essential oil had the least hardness which is only 3,087 g while those wrapped in chitosan film had hardness up to 4,609 g. Essential oil in film can reduce cake hardening due to it was hydrophobic. As a result, it lowered water egress out of film which agreed with WVTR value of film, thus maintained cake moisture content and kept

the cake sofer than other treatments.

2.3 Weight Loss of Cake

Orange flavored butter cake tended to lose the weight during storage. Cake unwrapped from chitosan film incorporating 1.4% of orange essential oil had the least weight loss. This is mainly due to the hydrophobicity of orange essential oil present in the film composition lower WVTR value of the film. The film incorporating orange essential oil, which is more hydrophobic than pure chitosan film showed the significant difference in water barrier protection against weight loss (Figure 3).

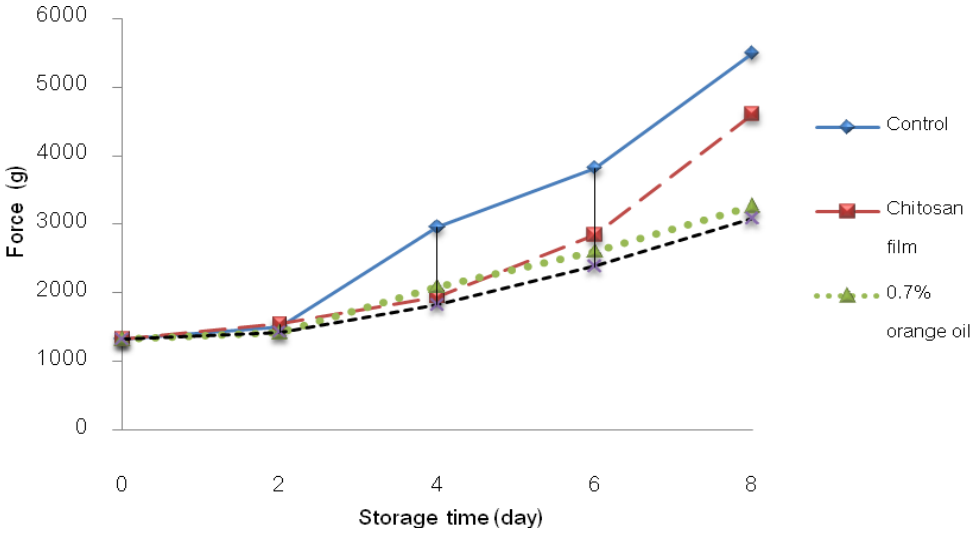

Figure 2 Texture of orange flavored butter cake unwrapped from chitosan film containing different concentration of orange essential oil

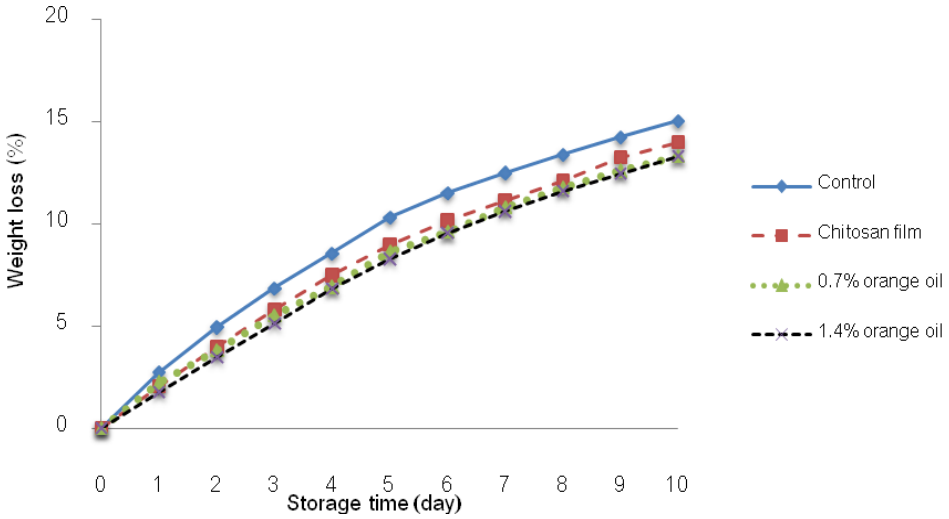

Figure 3 Texture of orange flavored butter cake unwrapped from chitosan film containing different concentration of orange essential oil

2.4 Sensory evaluation of cake

The sensory scores of fresh bake orange flavored butter cake were shown in Figure 4. All attributes had the scores in the range of 6-7 out of 9. On day 3, the sensory characteristics of the cake wrapped with chitosan film, chitosan film incorporating orange essential oil 0.7% and 1.4% were not significantly different between treatments and all scores were greater than 5 which were in sensory acceptable limit (Figure 5). On day 7, the cake wrapped by chitosan film was disregarded due to mold growth. Orange flavored butter cake wrapped by chitosan incorporating 1.4% orange essential oil were not significant different from those wrapped by film containing lower oil concentration but better in texture (Figure 6). Film containing

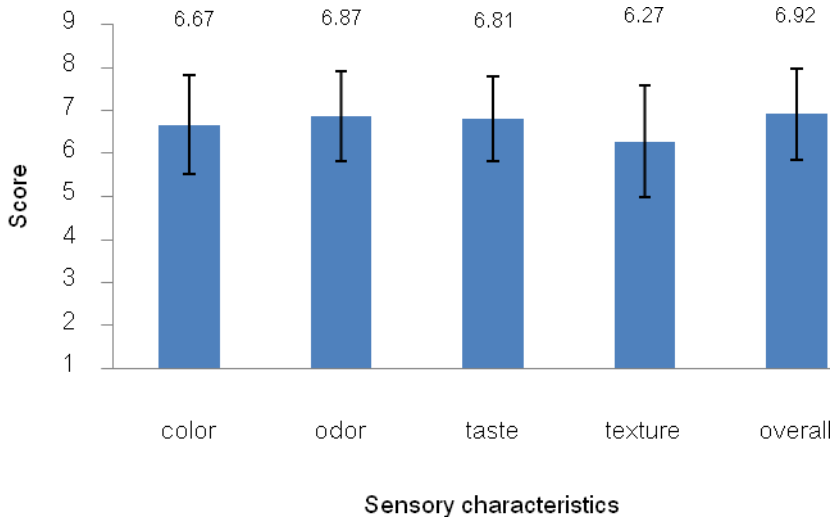

Figure 4 Sensory score of fresh bake orangeflavored butter cake

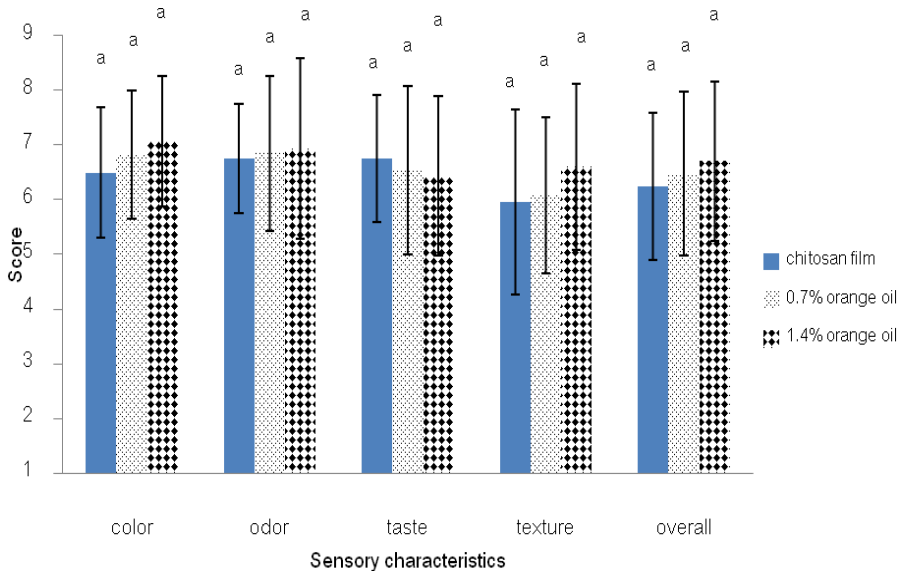

Figure 5 Sensory score of orange flavored butter cake unwrapped from chitosan film containing different concentration of orange essential oil after storage for 3 days

high orange essential oil concentration can protect the moisture loss from cake, thus provide the best cake texture. Cake wrapped in chitosan film containing orange essential oil are still acceptable in all attributes until this day.

2.5 Shelf Life of Cake

Orange flavored butter cake were stored at 25°C for 10 days. The apperance of mold was used to judge the storage life of the cake. Mold incidents were observed on cake wrapped by stretch film on day 3, resulted in 2 days storage life. Orange flavored butter cake wrapped by chitosan film, chitosan film incorporating 0.7% and 1.4% orange essential oil had storage life for 4,7 and more than 10 days, respectively (Figure 7).

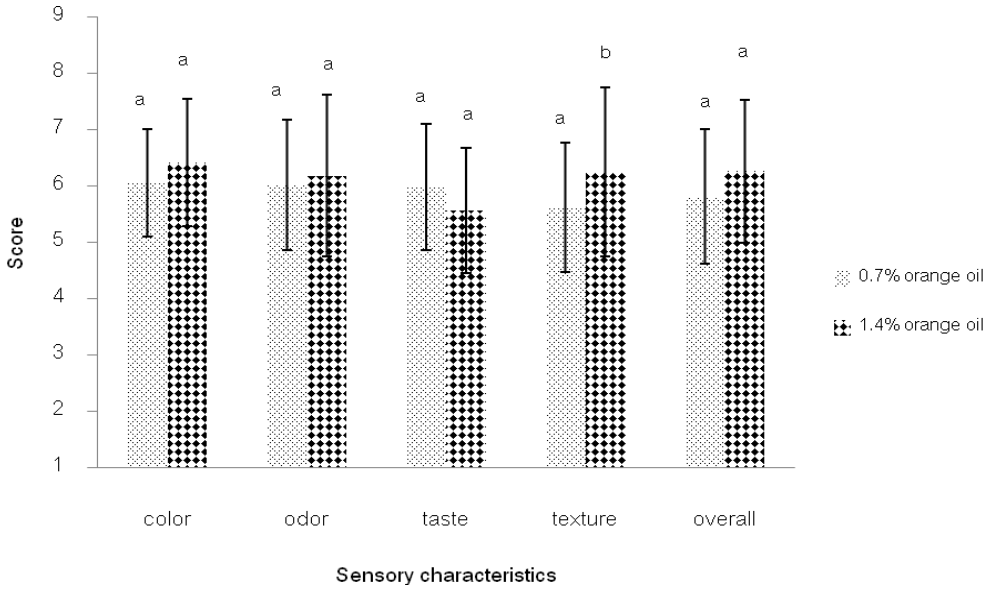

Figure 6 Sensory score of orange flavored butter cake unwrapped from chitosan film containing different concentration of orange essential oil after storage for 7 days

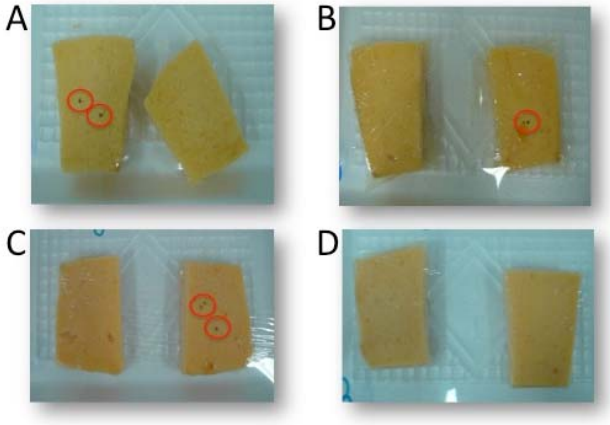

Figure 7 The first date that the visible growth of mold was observed on cake unwrapped from different films (A: stretch film, day 3, B: chitosan film, day 5, C: chitosan film containing 0.7% orange essential oil, day 8 and D: chitosan film containing 1.4% orange essential oil, day 10)

Conclusion

Chitosan film incorporating orange essential oil provided the good inhibition against mold on orange flavored butter cake, thus extending the shelf life of the cake. It can also protect the moisture loss from cake during storage and then keep the best cake texture with acceptable sensory characteristics. Chitosan film incorporating orange essential oil had potential to extend the storage life and maintain the best keeping quality of orange flavored bakery products, fruit cake or other cakes that have the innate flavor compatible with orange aroma.

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