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Antifungal effects of clove oil microcapsule on meat products

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ABSTRACT

Clove oil exhibits antimicrobial activities against a wide range of pathogenic microorganisms. Microencapsulation technology can be used to improve its stability and water solubility. The actual application of clove oil microcapsule on the antifungal preservation of meat products, such as cooked fish, cooked chicken, cooked pork, and cooked beef, was investigated in this study. Results showed that clove oil microcapsule displayed good antiseptic effects on these meat products at an effective fungicidal concentration of above 0.070%. Even if the meat was boiled for 0.5 h, the efficiency of clove oil microcapsule increased to 0.080%. Clove oil microcapsules had a trong heat resistance and high inhibition effect on meat products, and their disease indices and variation rates of mold spores also decreased. As a novel alternative, clove oil microcapsule had practical applications as a preservative in meat products, especially in foods requiring heat processing.

1. Introduction

With the development of economic globalization and world food trade, the problem of meat product safety becomes a serious concern worldwide. Meat contamination not only affects the health of the people but also causes a decline in export trade of meat products in China. The demand for nontoxic, natural preservatives has increased in recent years, and the search for new alternatives to preserve foods is of great interest to food industry (Guillard, Issoupov, Redl, & Gontard, 2009; Shim et al., 2011; Tajkarimi, Ibrahim, & Cliver, 2010).

Natural antimicrobials attract increasing attention in food industry. The use of essential oils from plants as native antimicrobial agents has increased (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Bassolé & Juliani, 2012). Clove (*Syzygium aromaticum*) is an important aromatic spice. Clove not only contains many kinds of biological active compositions but also has highly effective and comprehensive antibacterial functions (Sebaaly, Jraij, Fessi, Charcosset, & Greige-Gerges, 2015). Its essential oil contains mainly phenylpropanoids, such as eugenol, β-caryophyllene, and α-humulene organic compounds (Guillard et al., 2009; Gülçin, Elmastaş, & Aboul-Enein, 2012). Clove has light yellow or orange color, produces an agreeable aroma, and has a wide spectrum of medicinal properties, such as antimutagenic, anti-inflammatory, and analgesic effects (Chen et al., 2017). Remarkably, clove has strong antimicrobial activities against a wide range of pathogenic microorganisms (Ghosh, Mukherjee, & Chandrasekaran, 2012). Clove oil possesses enormous commercial potential and can be used as a preservative, colorant or spice in food products (Aguilar-González, Palou, & López-Malo, 2015).

In terms of its application, the use of essential oil in natural liquid state gains extensive attention (Mejía-Garibay, Palou, & López-Malo, 2015). Clove oil had an excellent inhibitory effect on *Listeria monocytogenes* in meat and cheese (Menon & Garg, 2001). Moreover, the essential oils of clove and ginger were found to improve the immune response against *Streptococcus agalactiae* in tilapia farming (Brum et al., 2017). Furthermore, chitosan combined with clove oil exhibited increased antifungal properties on *Penicillium digitatum* and effectively contributed to control the green mold on citrus (Shao et al., 2015). Linear low-density polyethylene surface was chemically modified by chromic acid treatment and coated with clove essential oil, and the developed film could be used as active packaging for fresh chickens (Mulla et al., 2017). However, this modified film is difficult to dissolve in water. Active compounds present in this film are also volatile, and it easily runs out of its activity. Moreover, this film is unstable and is vulnerable to oxidants, light, and heat. A great concern is its inapplicable usage in food thermal processing and will lose its activity entirely at high temperatures (Xu, Wu, Li, & Li, 2006).

Microencapsulation technology has been extensively used in various food processing fields. This technology can embed compounds and protects these ingredients against deterioration, volatile losses, or premature interaction with other ingredients. With this technology, tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix. Wall material acts as a physical and permeability barrier for core material (Wang, Lu, Lv, & Bie, 2009; Wang, Lu, Wu, & Lv, 2009; Wang, Shao, Wang, & Lu, 2012; Wang, Shao,

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Zhou, et al., 2012). A few reports were available about how to improve its solubility and stability in previous studies (Wang, Shao, Zhou, et al., 2012; Wang et al., 2014). Clove oil microcapsule was successfully obtained by spray drying, and its solubility and stability could be improved by microencapsulation. Furthermore, the structure of its microcapsule was confirmed by spectrum analysis, such as Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy (SEM), and proton nuclear magnetic resonance spectrometries (^{1}H NMR and ^{1}H ROESY NMR) (Zhu, Zhang, Zhu, & Ding, 2016). To date, despite the several works on clove oil, its application, and its microencapsulation, the actual application of its microcapsule was still not studied.

In this work, based on preceding studies, the objective was to evaluate the preservative effects of clove oil microcapsule. To the best of our knowledge, this research is the first study to determine its actual preservative effects on several meat products, especially similar products requiring thermal processing. Moreover, the results would provide basis and references for practical meat production.

2. Materials and methods

2.1. Materials

Cooked fish, cooked chicken, cooked pork, and cooked beef without any additives were purchased from Suguo Supermarket (Nanjing, China). Clove oil as core material (99% of purity) was obtained from China Aroma Chemical Co., Ltd. (Hangzhou, China). Clove oil microcapsule was prepared in our laboratory. Sucrose ester (99% of purity) was used as the emulsifier, purchased from Shen Zhen Jiang Yuan Trading Co., Ltd. (Shenzhen, China). Porous starch (98% of purity) was from Chongqing Taiwei Bioengineering Ltd. (Chongqing, China). β-Cyclodextrin (98% of purity) was from Shangdong Binzhou Zhiyuan Biotechnology Ltd. (Binzhou, China). Deionized water was used and purified by a Mill-Q water purification system from Millipore (Bedford, MA, USA). Chemicals and reagents were of analytical grade from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), except those which were specified.

2.2. Preparation of clove oil microcapsule

β-Cyclodextrin and porous starch were adopted as wall materials, and they (mass ratio of core to wall material, 10%; mass ratio of porous starch to β-cyclodextrin, 1:5) were dissolved in deionized water (above 60 °C) to obtain an aqueous solution. After preheating and dissolving in alcohol (solution concentration of 40%), clove oil sample (volume ratio of clove oil to alcohol, 1:2) was dripped into the aqueous solution. Then, the aqueous solution was added with sucrose ester as an emulsifier (volume ratio of sucrose ester to clove oil, 1:3) and stirred to form a coarse suspension at 60 °C for 1.5 h. The solution was then fed into a Model YC-105 Spray Dryer (Pilotech Instrument & Equipment Co., Ltd., Shanghai, China) to obtain particles with an encapsulation efficiency of 92.3 ± 3.6% and a yield of 84.5 ± 2.0% under the following conditions: inlet gas temperature of 170 °C, feed flow rate of 40 mL/min, and drying air flow of 60 m^3/h. For the YC-105 spray dryer, its outlet gas temperature was not a controllable parameter. Clove oil particles were collected and stored at 4 °C.

2.3. Encapsulation efficiency

The encapsulation efficiency *(EE)* is a key indicator for microcapsules. EE is calculated in Equation (1) as follows (Wang, Shao, Zhou, et al., 2012; Wang et al., 2014):

$$EE(\%) = \frac{C_T - C_S}{C_T} \times 100\%, \quad (1)$$

where C_T (total clove oil) refers to the internal and surface oil content of the microcapsules, whereas C_S (surface clove oil) is the unencapsulated oil content at the surface of the microcapsules.

C_T was obtained by dispersing the microcapsules (5.0 g) in petroleum ether (10 mL). Microcapsules were sonicated in a Model Scientz-IID ultrasonic cell disruptor (Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China) at 70% amplitude and frequency of 20 kHz for 10 min. They were extracted by Soxhlet extraction for 5 h at room temperature. The clove oil solution was dried in a Model GXZ-9240 MBE electrothermal blowing dry oven (Shanghai Boxun Co., Ltd., Shanghai, China) at 80 °C for 12 h, and its weight was measured as the content of the total clove oil.

C_S was achieved as follows. To keep them from deteriorating, the microcapsules (5.0 g) were dispersed in petroleum ether (10 mL) with slight stirring. After 10 min, the solvent was filtered from the solution and then was dried in the above dry oven with the same operational parameters. Its weight was monitored as the content of the surface clove oil.

2.4. Yield of clove oil microcapsule

The yield of clove oil microcapsule (Y) is the ratio of the final content of microcapsule solids after spray drying (C_a) and the initial content of raw microcapsule solids (C_b) illustrated as Equation (2) as follows (Wang, Ye, et al., 2012; Wang et al., 2014):

$$Y(\%) = \frac{C_a}{C_b} \times 100\%. \quad (2)$$

2.5. SEM imaging

The surface morphology of clove oil microcapsule was examined by SEM (JSM-6400, Jeol, Japan) at an acceleration voltage of 20 kV. Dry microcapsule was fixed on metal stubs with double-sided tape and coated with gold by a gold sputter coater in a high-vacuum evaporator, which produced high quality microscopic images at the preset magnification.

2.6. Preservative effects of clove oil microcapsule on meat products

After cutting into small squares (20 × 20 × 20 mm), cooked fish was placed in 11 sterile Petri dishes filled with clove oil microcapsule solutions over a range of concentrations (0.100, 0.090, 0.080, 0.070, 0.060, 0.050, 0.040, 0.030, 0.020, 0.010, and 0 wt%, with water as solvent). After immersing in solutions at 27 °C for 0.5 h (Wang et al., 2014), these squares were fetched out, drained, and cultivated in a Model HN−60B biochemical incubator (Beijing Hengnuo Li Xing Technology Co., Ltd., Beijing, China) at 37 °C with a relative humidity of 75% for different times (24, 48, 72, and 96 h). Mildew levels and mold spores of meat products were inspected every other day. Experiments for cooked chicken, cooked pork, and cooked beef were carried out, according to the above operation steps for cooked fish.

2.7. Preservative effects of heat-treated clove oil microcapsule on meat products

For investigating the influence of thermal processing on the preservative effects of clove oil microcapsule, its solutions at the above concentrations (0.100, 0.090, 0.080, 0.070, 0.060, 0.050, 0.040, 0.030, 0.020, 0.010, and 0 wt%, with water as solvent) were boiled for 30 min and then cooled to 27 °C. Similar to the process mentioned above, cooked meat product (20 × 20 × 20 mm) was placed in 11 sterile Petri dishes filled with the solutions. After immersing for 0.5 h, the squares were then drained and placed in the above biochemical incubator under the same conditions. Mildew levels and mold spores of meat products were also investigated every other day.