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Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods

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ABSTRACT

This study evaluated the minimum inhibition concentration (MIC) and minimum lethal concentration (MLC) of cinnamon and clove essential oils against mold growth on green bean cake and finger citron crisp cake, and also examined the effects of these two essential oils and their application methods on the shelf life of the baked products in normal and vacuum packages by accelerated storage test. The results showed that the MIC of cinnamon and clove essential oils against molds were 0.21–0.83 and 0.21–1.67 μL/mL, respectively and the MLC were 0.42–0.83 and 0.83–1.67 μL/mL, respectively. In normal package cinnamon and clove essential oils could prolong the shelf life of green bean cake 9–10 and 3–4 days, respectively and could prolong the shelf life of finger citron crisp cake 5–6 and 2–3 days, respectively. And in vacuum package they were 15–16, 8–9, 10–12 and 7–9 days, respectively in turn.

1. Introduction

Mold is the major microbial agent that causes mildew in baked foods (Nielsen & Rios, 2000). Food spoilage caused by molds not only results in huge economic loss, but is also harmful to human health owing to toxic secondary metabolites excreted by molds such as citrinin, aflatoxin, and requefortine. These toxins are produced during the growth of molds on the food substrate (Filtenborg, Frisvad, & Thrane, 1996), and therefore, it is particularly important to prevent the growth of molds as well as maintain safety and nutrition of baked foods.

Currently, the commonly used preservatives in baked foods mainly include organic acids and their salts (Yao & Xu, 2014). However, because of their toxic effects on human body, the use of organic acids and their salts has been restricted (Ju, Wang, Qiao, Li, & Li, 2017; Wilson & Bahna, 2005). With the growing demand for safe and natural products without chemical preservatives, there has been an increase in exhaustive investigations by food authorities and researchers to assess the feasibility of using mild preservation techniques and improve the quality and safety of products, while simultaneously maintaining their good nutritional and organoleptic properties (Goni et al., 2009). Nowadays, natural biological preservatives are mainly extracted from edible spices, and the essential oils from these spices have been found to be effective preservatives (Burt, 2004). Essential oils contain active components, and most of them have been noted to have a wide spectrum of antimicrobial activity against food-borne pathogens and spoilage bacteria (Gutierrez, Barry-Ryan, & Bourke, 2008; Lucera, Costa, Conte, & Del Nobile, 2012). Currently, cinnamon and clove essential oils are the most widely used natural preservatives (Patel, 2015; Rieger & Schiffman, 2014; Uçak, Özogul, & Durmuş, 2011). Cinnamon essential oil is mainly composed of cinnamaldehyde, and has good inhibitory effect on many food spoilage microorganisms. Eugenol, the major active ingredient of clove oil, has antibacterial and insecticidal effects (Matan et al., 2006). Both cinnamon and clove essential oils are natural preservatives and flavor substances, which are safe to consume (Kordali et al., 2005; Pezo, Salafranca, & Nerin, 2006). As the natural antibacterial agents, these oils satisfy the current requirements of "natural, safe, and healthy" preservatives (Ishlak, Günal, & AbuGhazaleh, 2015; Kim & Rhee, 2016; Matan et al., 2006). Besides, the use of appropriate amount of essential oils does not affect the flavor of the product, and can also increase consumers'appetite to a certain extent (Goni et al., 2009). Therefore, essential oils are allowed as preservatives in most of the countries, including the European Union, the USA, China, etc (Zhang et al., 2017).

In recent years, the microbial inhibitory effect of cinnamon and clove essential oils has been widely researched. Mulla et al. (2017) demonstrated that the linear low density polyethylene (LLDPE) surface chemically modified by chromic acid and coated with clove essential oil films exhibited strong antimicrobial activity against *Salmonella typhi*

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and *Listeria monocytogenes* in a packed chicken sample refrigerated for 21 days. Khaleque et al. (2016) reported that high concentrations of cinnamon and clove essential oils could inhibit *L. monocytogenes* in ground beef meat and improve the safety of ground beef products. Zhang et al. (2017) found that cinnamon essential oil could inhibit the growth of *Aeromonas* spp. and *Lactococcus* spp., and based on sensory analysis, revealed that cinnamon essential oil could extend the shelf life of vacuum-packed common carp fillets by about 2 days. However, there are only a few studies on the antimicrobial effect of essential oils on baked foods and their shelf life. Therefore, in the present study, the inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods surface were investigated based on the inhibition zone diameter (IZD) as the evaluation index. Subsequently, the effects of these two essential oils as well as their application methods on the shelf life of baked foods were examined, which has an important guiding significance in extending the shelf life of baked foods.

2. Materials and methods

2.1. Materials

Baked foods (cake, bread, green bean cake and finger citron crisp cake) were purchased from a local super market (Wuxi City, Jiangsu Province, China). including cake (flour 60%, eggs 15%, cream 10%, sugar 8%, milk 5%), bread (flour 50%, water 10%, sugar 10%, eggs 10%, cream 5%), green bean cake (flour 25%, mung beans 45%, sugar 15%, peanut oil 10%) and finger citron crisp cake (flour 60%, water 15%, sugar 8%, eggs 5%, cream10%). Cinnamon essential oil (containing 50–60% cinnamaldehyde and 4–7% eugenol) and clove essential oil (containing 70–80% eugenol as the major ingredient) were provided by Zhengzhou Flavours and Fragrances Industry Co., Ltd. (Henan province, China). Nutrient agar was obtained from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Polypropylene (PP) packing bags were purchased from Xiongxian Shengda color printing factory (Hebei Province, China).

2.2. Separation, purification, and identification of molds

The mold colonies on the baked foods were cultured on potato dextrose agar (PDA, natural pH, QingdaoHope Bio-Technology Co., Ltd., China) under aseptic conditions at 28 °C for 2–5 days to achieve pure cultures (Lopez, Sanchez, Batlle, & Nerin, 2005). When necessary, plate streaking was repeated until pure culture was obtained. Finally, six mold strains were obtained and marked as A, B, C, D, E, and F. The colony characteristics of the six strains cultivated using two different culture methods (spot culture and moist chamber culture) were observed and recorded (Knight et al., 1996). Besides, the mycelium characteristics were observed under the microscope (Metallographic BMM-202, China).

2.3. Analysis of the bacteriostatic effects of essential oils

2.3.1. Preparation of bacterial suspension

Establishment of A-X curve and regression equation for the six mold strains: After activation (28 °C, 48 h), standard single spore suspensions of the six mold strains were prepared by serial dilution, and their absorbance (A) at 560 nm was determined by using UV spectrophotometer (Shimadzu UV-2450, Japan). Furthermore, the mold spores numbers (X) per mL of each standard single spore suspension were calculated by using the blood count plate. The absorbance value (A) was linearly fitted with the corresponding mold spores numbers (X), and correlation coefficients (R^2) were calculated from regression equations. The F-test showed that the A-X curve had significant relativity. Subsequently, standard bacterial suspensions of various bacteria were used as control. The bacterial spores were washed with sterile water, diluted, and their turbidity was compared under sterile conditions. The concentrations of the bacterial suspensions were determined based on the A-X curve, and were maintained at 10^5 CFU/mL (Thompson, 1989).

2.3.2. Measurement of bacteriostatic activity by filter paper method

A total of 0.5 mL of the bacterial suspension was uniformly inoculated onto the surface of PDA agar in the plates (containing 15 mL of PDA that was melted and cooled to 50 °C) priorly, and the PDA agar containing bacteria was obtained.

Gas diffusion test: The two essential oils were diluted (cinnamon essential oil diluted with glycerol as the diluent and clove essential oil was diluted with acetone as the diluent) to obtain concentrations of 200, 150, 100, and 50 μL/mL, respectively. Subsequently, 6 μL of each concentration of the essential oils was respectively added to sterile filter paper discs, which were then placed on the center of inner surface of the plate cover and located above the PDA agar containing bacteria, and then incubated for 4–5 days at 28 °C. During culture the EO volatilized in the space between plate cover and PDA agar containing bacteria. The IZD was measured, and the assay was performed in triplicate. The control comprised filter paper discs with diluents alone.

Solid diffusion test: The procedure was the same as that employed for gas diffusion test. However, sterile filter paper discs with different concentrations of the two essential oils were respectively placed onto the surface center of PDA agar containing bacteria and incubated for 4–5 days at 28 °C (Wilkinson, Hipwell, Ryan, & Cavanagh, 2003). During culture the EO permeated into PDA agar mainly. The IZD was measured, and the assay was performed in triplicate. The control included filter paper discs with diluents alone.

2.3.3. Measurement of minimum inhibitory concentration and minimum lethal concentration

The two essential oils were first diluted to 50.000, 25.000, 12.500, 6.250, 3.125, and 1.562 μL/mL, respectively, by twofold dilution method. Then, 1 mL of each diluted sample was mixed with 14 mL of PDA culture medium and poured into Petri dish under sterile environment. The control comprised PDA medium with 1 mL of diluent. Subsequently, 1 mL of the prepared mold suspensions was evenly inoculated onto the PDA plates and incubated for 48 h at 28 °C. The assay was performed in triplicate. The growth of the strain was observed, and the lowest concentration of the essential oil at which there was no strain growth was selected as the minimum inhibitory concentration (MIC). To determine the minimum lethal concentration (MLC), the plates without strain growth were observed for another 48 h, and the lowest concentration at which no growth was detected was considered as the MLC.

2.4. Sensory evaluation

Without changing the original packaging style, the essential oils were added to the inner package of the green bean cake and finger citron crisp cake by point addition and coating method, respectively (Xu, 2008). The point addition was drop the essential oils to the inner surface of packaging material, and use the vapor of essential oils to inhibit the mold growth, and coating method was coat the essential oils to the surface of cakes and use the contact of essential oils with cakes directly to inhibit the mold growth. To ensure the added concentration was enough for inhibition molds, the amounts of added essential oils were calculated based on their MIC values and the space volume of packing bags (the space volume of packing bag = the volume of packing bag − the volume of green bean cake or finger citron crisp cake). The added amount of essential oil was decided depending on the functional absolute volume of essential oils, the volume of experimental samples and the space volume of packing bag. In this paper the adding amount of essential oil were 7.2 μL and 8.4 μL in the package of green bean cake and finger citron crisp cake, respectively. According to the sense of smell and taste of the product, 12 students in reading were