Research Note: Preservative effect of compound spices extracts on marinated chicken

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ABSTRACT The antimicrobial effect of 21 nature spices essential oils (**EOs**) on marinated chicken was investigated and response surface analysis was applied to obtain the optimal combination. Cassia bark EO, cinnamon EO, tea tree EO, and angelica EO had the best antibacterial effect. Their inhibition zone diameters (**IZD**) were 23 mm, 21 mm, 15 mm, and 12 mm, and their minimal bactericidal concentrations (**MBC**) were 1.25 μL/mL, 1.25 μL/mL, 10.00 μL/mL, 20.00 μL/mL. Using the Box-Behnken Design model, with the minimum total number of spoilage bacteria as the evaluation index, the optimal mixture was cassia bark EO 2.40 μL/mL, cinnamon EO 1.00 μL/mL, tea tree EO 3.50 μL/mL, and angelica EO 9.00 μL/mL. Compared with the control group, the total number of colonies was reduced by 1.3 log unites at the 12th sampling day, and the protein degradation process was slowed down owing to the preservative addition. These results indicate the potential application of nature extracts in chicken and other meat preservation.

Key words: preservation, nature extract, spices essential oil, compound, chicken

2022 Poultry Science 101:101778
https://doi.org/10.1016/j.psj.2022.101778

INTRODUCTION

Meat products account for a growing proportion of people's daily diet, especially chicken products, which are favored for their high protein, low fat, low cholesterol, and low calorie characteristics (Utama et al., 2019). Marinated chicken is the outstanding representative of traditional Chinese meat products. However, high nutrition leads to susceptibility to microbial contamination at all stages of processing and distribution, and this problem is exacerbated by the underdevelopment of the cold chain systems. So that a large number of microorganisms breed in the products, shorten the shelf-life, and ultimately leading to food insecurity and economic losses.

Meat preservation technologies mainly include preservative, nuclear radiation, high-pressure treatment, low-temperature, and air conditioning packaging, among which adding preservative agent has advantages of obvious effect, easy operation and low cost, widely used in practical production. However, potential harm of chemical preservatives to human health has been gradually discovered, and the development of new, safe, efficient, and economical natural preservatives has become the general trend of the food industry (Flores and Toldrá, 2021).

Spice is the general terms of the natural plant raw materials with typical flavor, which can be used for food conditioning, mainly to promote the aroma, seasoning, color, and other functions(Sun et al., 2019). In addition, spices exacts were employed as antiseptics to inhibit or kill the microbe of food, showing higher safety compared with chemical preservatives. Total polyphenol from *Allium senescens L.* seeds were proved to be effective in inhibiting lipid and protein oxidation and reducing color deterioration of dry sausages (Qin et al., 2021). Incorporation of specific spice essential oils (exp. oregano, coriander, thyme, and clove) as preservative agents in meat eliminates many pathogens and autochthonous spoilage flora (Tsigarida et al., 2000). However, higher concentration of single EO would be unacceptable sensorially (Karam et al., 2020). Thus, mixed preservatives that contain more than 2 factors are frequently preferred, because they commendably avoid sensory decline and the development of a range of bacteria (Yu et al., 2019).

In this study, 21 nature extracts, including pepper EO, rosemary EO, tea tree EO, fennel EO, piper nigrum EO, ginger EO, cumin EO, aniseed EO, tangerine peel EO, capsicum EO, angelica EO, peppermint EO, nutmeg EO, coriander EO, cassia bark EO, cinnamon EO, myrcia EO, amomum tsao-ko EO, clove EO, salvia EO, and garlic EO, were selected as the candidates of

Received September 13, 2021.
Accepted February 1, 2022.

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preservative. The IZD, minimum inhibitory concentration (**MIC**) and MBC of the EOs were determined to screen out the extracts with antimicrobial effect on spoilage bacteria. Subsequently, response surface methodology was applied to obtain the best combination as a preservative. And then, the antibacterial effect of compound nature preservative was evaluated. Our research provides potential support for exploring natural preservatives and ensuring food safety.

MATERIALS AND METHODS

Materials

Chicken carcass, chicken stew bag (orange peel, arene, cumin, dried ginger, cinnamon, aniseed, angelica, prickly ash, myrcia), salt, cooking wine, soy sauce, hallot, ginger and garlicused used in this study were brought form Beiguo supermarket, Shijiazhuang. EOs was brought from Hebei Chenguang Biotech Group Co., Ltd., China. Reagents for culture medium were brought from Beijing Land Bridge Technology Co., LTD, China.

Culture Medium Ingredients

Sodium chloride-peptone-soy broth medium (g/1,000 mL distilled water): tryptone 17.0, soybean peptone 3.0, NaCl 100, K_2HPO_4 2.5, sodium pyruvate 10.0, glucose 2.5, pH 7.1−7.5.

Plate count agar medium (g/1,000 mL distilled water): tryptone 5.0, yeast extract 2.5 glucose 1.0, Agar 15.0, pH 7.0 ± 0.2.

Luria-Bertani broth medium (g/1,000 mL distilled water): tryptone 10.0, yeast extract 5.0, NaCl 10.0, pH 7.0−7.5.

Preparation of Putrefaction Liquid

Two hundred gram of chicken was washed and marinated for 3 h with 20 mL cooking wine, 10 g shallot, 10 g ginger, 10 g garlic, 10 mL soy sauce. Then the marinated chicken was blanched for 5 to 10 min in 300 mL water with 10 mL cooking wine and removed foam. Next, 5 g chicken stew bag, 8 mL soy sauce, 8 g shallot, 10 g ginger, 10 g garlic, 10 mL soy sauce, and 6 g salt were added, simmered for 15 min. The obtained samples were used for subsequent experiments.

Marinated chicken samples were stored in sealed packages at 30°C for 20 h to accelerate spoilage. Twenty-five grams of samples were cut into pieces and put in the aseptic homogenizing bags with 225 mL normal saline to homogenize. One milliliter of sample homogenate was slowly injected into the preservation tube with porcelain beads and place at −80°C for later use. A porcelain bead was removed from the seed preservation tube using sterilized tweezers, and slowly put into a test tube containing 10 mL of sodium chloride-peptone-soy broth medium. The putrefying bacteria liquid was obtained after culturing at 30°C for 20 h.

Screening of Spice Extracts With Antibacterial Activity

The putrefying bacterial solution was gradient diluted and added to the plate count agar medium which was sterilized and cooled to about 50°C, so that the concentration of live bacteria in the culture medium was 10^5 to 10^6 CFU/mL respectively. A sterilized oxford cup was used to drill holes in the test plates. Thirty microliter of spice extract was injected into the hole, followed by prediffusion at 4°C for 2 h, and culture at 30°C for 24 h. IZD was measured as the following formula:

IZD (mm) = bacteriostatic circle diameter (mm) - pore diameter (6 mm).

Determination of MIC and MBC

Serial dilutions the spice extracts to different concentrations with Tween 80. Mixture of 0.8 mL Luria-Bertani broth medium, 0.1 mL diluted spice extracts (final concentration was 50.00−0.625 μL/mL) and 0.1 mL putrefying bacteria liquid (final concentration of live bacteria was 10^5−10^6 CFU/mL) were added to marked tubes, and incubated at 30°C for 24 h. The growth of bacteria was judged by the turbidity of the culture. If there is no obvious turbidity below a certain tube, the concentration of the spice extract in this tube is the MIC value (Zhu et al., 2004). Further, 100 microliter bacterial suspension from the aseptically tube was spread evenly on a solid plate, and incubate at 30°C for 24 h to observe whether there is bacterial growth. The minimum concentration of aseptic growth is the MBC value.

Optimization Compounded System

The evaluation of the antibacterial effect of a mixed natural preservative was performed with Box-Behnken Design mode. Different concentrations of cassia bark EO, cinnamon EO, tea tree EO, and angelica EO were the main factors (X, independent variable), and −1, 0, +1 were used to represent the low, medium and high levels of the independent variable concentrations. Among that, the value of the MBC was chosen as high level, while the value 0 was chosen as the low level. One hundred microliter of the mixture of 0.8 mL Luria-Bertani broth medium, 0.1 mL spice extracts with different ratio and 0.1 mL putrefying bacteria liquid (final concentration of live bacteria was 10^5−10^6 CFU/mL) was evenly coated on the pate count agar medium, and incubated at 30°C for 24 h. The total number of colonies (**TNC**) was used as the evaluation index to optimize the compounded system.

The Effect of Compound Spices Extracts on the Preservation of Marinated Chicken

Samples were immersed into the compound spice EO for 30 min, put into the sterile sealed bags and stored 4°C, respectively. Samples were taken on d 0, 1, 3, 5, 7, 9, 12. On the sampling days, 2 samples from each group were removed from storage in order to perform microbiological and pH sensorial analysis. Here, the date of chicken products was delivered on the zero day, and the compound spices extracts were combined with marinated chicken products on the first day.

Analytical Methods

PH Determination Measurements of pH were made using a digital pH meter (Testo 205 pH meter, Lenzkirch, Germany), which was calibrated using standard buffers of pH 4.0 and 9.18 at 25°C. The probe of pH meter was inserted in a parallel direction to the surface of 1.5-cm thick patty and the pH values were recorded. For each sample, 3 readings were taken from different locations and averaged.

Microbiological Analysis

Ten gram of peeled sample was prepared aseptically, and homogenized in a sterile stomacher bag containing 90 mL of 0.1% sterile peptone water (Oxoid) (IUL, Barcelona, Spain). Decimal dilutions in 0.1% sterile peptone were made. The TNC were determined on plate count agar medium following the method of the part "Optimization Compounded System", incubating at 30°C for 48 h.

Statistical Analysis

All tests were repeated 3 times. Correlation analysis was obtained by SPSS 19.0, response surface design and analysis was conducted by Design expert 8.0, and Origin 2021 was used for drawing.

RESULTS AND DISCUSSION

Screening of Spice Extracts With Antibacterial Activity

IZD was used to screening the spice extracts with antibacterial activity. Of the selected EOs, cassia bark EO, cinnamon EO, tea tree EO, cumin EO, angelica EO, clove EO, rosemary EO, and garlic EO had varying degrees of bacteriostatic effect. The other EOs selected in the experiment did not produce bacteriostatic circle. The MIC and MBC of the spice extracts with antibacterial activity were measured (Table 1). Cassia bark EO, cinnamon EO, tea tree EO and garlic EO were the top four antibacterial extractives, while the top 4 bactericidal extractive were cassia bark EO, cinnamon EO, tea tree EO, and angelica EO. Although garlic EO had good bacteriostatic effect, the bactericidal effect was poor. Taking MIC and MBC values into consideration, cassia bark EO, cinnamon EO, tea tree EO, and angelica EO were selected as the compound experimental objects in subsequent experiments.

Table 1. IZD, MIC, and MBC of spices extract on the putrefaction liquid.

Spices extract [a]	IZD (mm)	MIC (μL/mL)	MBC (μL/mL)
Rosemary EO	4	40	-[b]
Tea tree EO	15	10	10
Cumin EO	14	40	-[b]
Angelica EO	12	20	20
Cassia bark EO	23	1.25	2.5
Cinnamon EO	21	1.25	2.5
Clove EO	8	40	-[a]
Garlic EO	3	10	50

Abbreviations: IZD, inhibition zone diameters; MBC, minimum inhibitory concentration; MIC, minimal bactericidal concentrations.
[a]Only the spices extracts with antibacterial effect are shown in this table.
[b]At the experimental concentration, the bactericidal effect cannot be reached.

Optimization Compounded System

Design-Expert software was used to fit the data, and the 4-factor regression equation of cassia bark EO (A), cinnamon EO (B), tea tree EO (C), and angelica EO (D) relative to the value of TNC (M) was as follows:

$$M= +1603.06667 -749.85333 \times A -824.92000 \times B -46.48000 \times C -16.35667 \times D +194.56000 \times AB +5.16000 \times AC +3.80000 \times AD +6.92000 \times BC +1.64000 \times BD +0.28000 \times CD +92.47467 \times A^2 +117.91467 \times B^2 + 1.70467 \times C^2 +0.19117 \times D^2.$$

ANOVA results for the experimental models showed that the $P_r > F$ value of the total model <0.001, which means the equation model is extremely significant. The lack of fit value was 0.0872 (>0.05), suggesting there is little deviation between the predicted value and the actual value, which belongs to the normal error range, and it is feasible to simulate real 4-factor and 3-level analysis by using this equation. The R^2 value and R^2_{Adj} value of the entire model were 0.9574 and 0.9433, respectively, suggesting that the polynomial model fit the experimental data primely (Resendiz-Moctezuma et al., 2021). Therefore, the best natural preservative ratio for the antibacterial effect of marinated chicken can be selected through this model.

The optimal formula of compound natural preservative obtained by regression equation was: cassia bark EO 2.43 μL/mL, cinnamon EO 1.04 μL/mL, tea tree EO 3.46 μL/mL, and angelica EO 9.00 μL/mL. In consideration of the feasibility of practical operation, the formula was modified to: cassia bark EO 2.40 μL/mL, cinnamon EO 1.00 μL/mL, tea tree EO 3.50 μL/mL, and angelica EO 9.00 μL/mL.

The Effect of Compound Spices Extracts on the Preservation of Marinated Chicken

The Effect of Compound Spices Extracts on the TNC The effect of compound spices extracts on the TNC of marinated chicken was given in Figure 1A. In summary, after treatment with nature compound spices extracts, TNC of the experimental group were always less than those of the control group ($P < 0.05$). And the

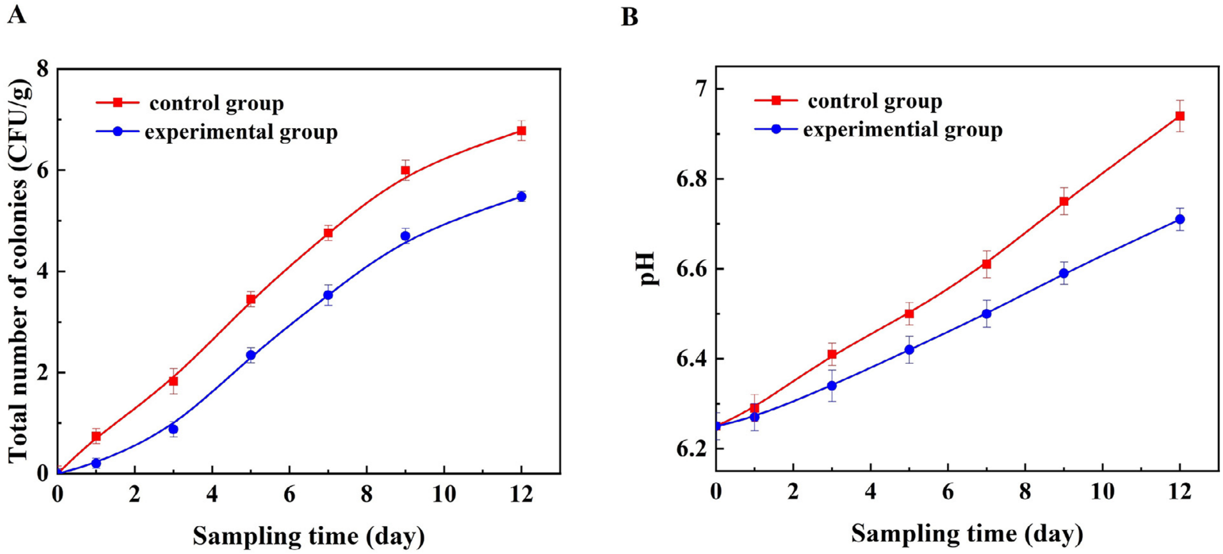

Figure 1. Time profiles of pH and total number of colonies with and without compound spices extracts addition. Red square: the control group, no preservative; blue circle: the experimental group, compound spices extracts worked as preservative.

final TNC reduced by 1.3 log unites after the compound spices extracts addition, which were 6.78 log units for the control group and 5.48 log units for the experimental group, respectively. Besides, almost no lag period was observed in the samples without preservatives, while a lag phase was observed in those samples treated with nature compound spices extracts. Thus, compound spices extracts exerted positive effects on extending lag phase and reducing the growth rate of spoilage microorganisms.

The Effect of Compound Spices Extracts on the pH

The pH value of the 2 groups of samples showed a gradual upward trend (Figure 1B). This phenomenon is attributed to that the chicken samples corrupted gradually over time, and their proteins are constantly decomposed under the action of microorganisms, which mainly produces small alkaline nitrogen-containing molecules (ammonia, trimethylamine, etc.), causing the increased pH value (Riebroy et al., 2007). Further, the pH value and rising speed of the experimental samples were lower than those of the control samples ($P < 0.05$), indicating that the compound spices extracts had a certain effect in slowing down the protein degradation process and extending shelf-life of marinated chicken.

Briefly, this study aimed to explore nature alternatives of preservative for marinated chicken. Thus, the antimicrobial effect of 21 nature spices extracts on marinated chicken was investigated and response surface analysis was applied to obtain the optimal combination. The optimal mixture was cassia bark EO 2.40 μL/mL, cinnamon EO 1.00 μL/mL, tea tree EO 3.50 μL/mL, and angelica EO 9.00 μL/mL. In the case of compound spices extracts addition, the TNC was reduced by 1.3 log unites at the 12th sampling day and the protein degradation process was slowed down, compared with the control group. Our research provides potential support for exploring natural preservatives and ensuring food safety.

ACKNOWLEDGMENTS

This study was supported by Subsidized Funds of Poultry Deep Processing Technology Innovation Center Construction of Hebei province (207790537H), Performance Subsidy Funds of Poultry Deep Processing Technology Innovation Center Construction of Hebei province (20567656H) and General research fund projects of Hebei University of Economics and Business (2019YB07).

DISCLOSURES

In this study, we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Flores, M., and F. Toldrá. 2021. Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products - invited review. Meat Sci. 171:108272.
Karam, L., R. Chehab, T. M. Osaili, and I. N. Savvaidis. 2020. Antimicrobial effect of thymol and carvacrol added to a vinegar-based marinade for controlling spoilage of marinated beef (Shawarma) stored in air or vacuum packaging. Int. J. Food Microbiol. 332:108769.
Qin, L., J. Yu, J. Zhu, B. Kong, and Q. Chen. 2021. Ultrasonic-assisted extraction of polyphenol from the seeds of *Allium senescens L.* and its antioxidative role in Harbin dry sausage. Meat Sci. 172:108351.
Resendiz-Moctezuma, C., S. D. Rezac, M. J. Miller, and M. J. Stasiewicz. 2021. Enabling cost-effective screening for antimicrobials against Listeria monocytogenes in ham. J. Food Protect. 84:802–810.
Riebroy, S., S. Benjakul, W. Visessanguan, and M. Tanaka. 2007. Effect of iced storage of bigeye snapper (*Priacanthus tayenus*) on

the chemical composition, properties and acceptability of Som-fug, a fermented Thai fish mince. Food Chem. 102:270–280.

Sun, W., M. H. Shahrajabian, and Q. Cheng. 2019. Anise (*Pimpinella anisum L.*), a dominant spice and traditional medicinal herb for both food and medicinal purposes. Cogent Biol. 5:1673688.

Tsigarida, E., P. Skandamis, and G. J. E. Nychas. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. J. Appl. Microbiol. 89:901–909.

Utama, D. T., H. S. Jeong, J. Kim, F. H. Barido, and S. K. Lee. 2019. Fatty acid composition and quality properties of chicken sausage formulated with pre-emulsified perilla-canola oil as an animal fat replacer. Poult Sci. 98:3059–3066.

Yu, H. H., M. W. Song, Y. J. Song, N. K. Lee, and H. D. Paik. 2019. Antibacterial effect of a mixed natural preservative against *Listeria monocytogenes* on lettuce and raw pork loin. J. Food Protect. 82:2001–2006.

Zhu, X., H. Zhang, and R. Lo. 2004. Phenolic compounds from the leaf extract of Artichoke (*Cynara scolymus L.*) and their antimicrobial activities. J. Agric. Food Chem. 52:7272–7278.