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Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality

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Abstract

The antimicrobial and antioxidant effects of two spice extracts and their combination on raw chicken meat during storage for 15 days at 4 °C were studied. Raw chicken meat was treated with BHT (positive control), rosemary (RO), cloves (CL), and their combination, and the results were compared to those obtained for raw chicken meat without any additive (negative control). The antioxidant and antimicrobial activities of spice extracts were determined. The total phenolic and total flavonoid contents of rosemary was lower than those of cloves. Cloves exhibited a higher DPPH radical scavenging activity than that of rosemary. However, the ferrous ion-chelating effect of rosemary was significantly higher than that of cloves. The pH, instrumental colour (CIE L^*, a^*, b^*), total viable counts (TVC), lactic acid bacteria (LAB) counts, Enterobacteriaceae counts, *Pseudomonas* spp. counts and 2-thiobarbituric acid reactive substances (TBARS) were determined at 3-day intervals over a period of 15 days. The bacterial counts of T-RO-CL samples were lower than those of control samples during storage. T-RO-CL samples maintained significantly ($P < 0.05$) higher L^*, a^* and b^* values during storage. The TBARS values of T-RO-CL samples were the lowest among the samples. These results demonstrate that spice extracts are highly effective against microbial growth and lipid oxidation and show potential as a natural antioxidant in raw chicken meats.

Keywords: Spice extracts; Antimicrobial; Antioxidant; Raw chicken meat; TBARS values

1. Introduction

Chicken meat is favoured by consumers around the world because of its desirable nutritional qualities, such as a low fat content and a relatively high concentration of polyunsaturated fatty acids [1]. Fresh meat products are usually marketed at refrigerated temperatures (2–5 °C). Lipid oxidation and microbial growth may occur during refrigeration storage. Spoilage of fresh poultry meat is a financial burden to producers and requires the development of new methods to extend the shelf-life and overall safety/quality of the meat, which is the main problem faced by the poultry processing industry [2].

Lipid oxidation, which is initiated in the unsaturated fatty acids fraction in subcellular membranes, is a major cause of the deterioration and reduced shelf-life of meat products [3]. Lipid oxidation may generate changes in meat quality parameters such as colour, flavour, odour, texture, and even nutritional value [4]. In addition, meat and poultry products have frequently been found to be contaminated with microorganisms during the butchering and manufacturing process. These microorganisms produce undesirable quality changes in meats, especially in relation to lactic acid bacteria, a major bacterial group associated with meat spoilage [5].

Many synthetic preservatives, such as butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT) and tertiary butylhydroquinone (THBQ), are currently being used to reduce microbial growth and thereby extend the shelf-life of meat. Because of the increasing consumer demand for "healthier" meals (free of conventional chemical preservatives), the use of natural preservatives and environmentally friendly technologies has been suggested [6]. In recent years, much attention has been

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focused on extracts from herbs and spices, which have been used for centuries to improve the sensory characteristics and shelf-life of foods [7]. Unlike synthetic compounds, natural preservatives obtained from spices are rich in phenolic compounds and they can enhance the overall quality of food by decreasing lipid oxidation and microbial growth.

Cloves and rosemary, which are commercially cultivated in China, are important aromatic spices. They are generally used as condiments to enhance the sensory quality of foods in China. In addition to their health benefits, which have been widely studied [8,9], the extracts from cloves and rosemary have been found to possess great antioxidant and antimicrobial activity [10–12]. Furthermore, to the best of our knowledge, the antioxidant and antimicrobial effects of cloves or rosemary extracts, singly or combined, on fresh chicken breast meat have not been investigated. Thus, the objective of the present work was to determine the effects of cloves and rosemary, applied individually and/or in combination, on pH, microbiological analysis, colour, thiobarbituric acid reactive substances (TBARS) and sensory analysis during storage at 4 °C.

2. Materials and methods

2.1. Materials

Dried cloves (*Eugenia caryophyllata*) and rosemary (*Rosemarinus officinalis*) were purchased from a local traditional Chinese pharmacy (Luoyang, China).

2.2. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), trolox, 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulonic acid) (ABTS), Folin-Ciocalteu's reagent (FCR), sodium carbonate (Na_2CO_3), gallic acid, sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), quercetin, butylated hydroxytoluene (BHT), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Plate Count Agar, Violet Red Bile Glucose Agar, Buffered Peptone Water, de Man Rogosa & Sharpe Agar, and Glutamate Starch Phenol Red Agar were purchased from Yongxin Biological Technology Co., Ltd. (Yixing, Jiangsu, China). Methanol and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.3. Preparation of spice extracts

Aliquots (50 g each) of powdered and dried spices were mixed into 400 mL of 95% (v/v) ethanol for 12 h in enclosed flasks with constant shaking (100 rpm). After filtration with Whatman No. 2 filter paper, the residue was re-extracted with an additional 200 mL of 95% ethanol for an additional 12 h and then filtered. The combined filtrates were concentrated in a rotary evaporator (RE 52AA, Yarong Biochemical Analysis Co., Ltd., Shanghai, China) (50 °C) with a vacuum pump, and the extracts were freeze dried. Dried extracts were placed in sealed bottles and stored at 4 °C before use. The extracts were dissolved in 95% ethanol for analysis of antioxidant and antimicrobial properties and were dissolved in distilled water (1%, w/v) for application on chicken meat products. The extraction yields of the spice ethanolic extracts were calculated using the following equation:

$$\text{Yields} \quad (\%) = \frac{\text{weight of extracted sample}}{\text{weight of initial sample}} \times 100$$

2.4. Analysis of spice extracts

2.4.1. Antioxidant activity

2.4.1.1. DPPH radical scavenging activity. For the DPPH radical scavenging activity (RSA) assay, the procedure of Hatano et al. [13] was followed. Briefly, aliquots of 0.5 mL of the DPPH$^{\bullet}$ solution (50 mg/mL) was mixed with 4.5 mL of methanol, and 0.1 mL of spice extracts at various concentrations (0.1–10.0 mg/mL) was added. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and the absorbance was read at 517 nm using a UV–vis spectrophotometer (Carry 100 UV-VIS, Agilent Technologies, Santa Clara, CA, USA) against a blank. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\text{Scavenging activity} \quad (\%) = \left(1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right) \times 100.$$

The EC_{50} value (mg/mL) was calculated as the concentration at which the DPPH radical scavenging activity was 50%.

2.4.1.2. Metal ion-chelating assay. The assay for metal chelation (Fe^{2+}) was carried out according to the method of Wang and Xiong [14]. Briefly, 1 mL of 20 μmol/L $FeCl_2$ was mixed with 2 mL of 50 μmol/L ferrozine, which produces a chromophore that absorbs strongly at 562 nm. After the addition of 0.5 mL of spice extracts (0.1–10.0 mg/mL), the colour change was measured spectrophotometrically at 562 nm. The ability of extracts to chelate ferrous ion was calculated as follows:

$$\text{Chelating activity} \quad (\%) = \left(1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right) \times 100$$

The EC_{50} value (mg/mL) was calculated as the concentration at which the chelating activity was 50%.

2.4.2. Total phenolic content

The Folin-Ciocalteu reagent assay was used to determine the total phenolic content of extracts [15]. A 0.1-mL aliquot of the extract was mixed with 0.1 mL of Folin-Ciocalteu reagent (previously diluted three-fold with distilled water) and allowed to react for 3 min, and then 0.3 mL of 2% sodium carbonate (Na_2CO_3) solution was added. The mixture was allowed to stand for another 2 h before the absorbance was measured at 760 nm. Gallic acid was used as the standard for the calibration curve. The total phenolic content was expressed in mg gallic acid equivalents (GAE) per gram of sample (mg/g). All determinations were performed in triplicate.