Anti-bacteria Effect of Active Ingredients of \textit{Cacumen Platycladi} on the Spoilage Bacteria of Sauced Pork Head Meat

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Abstract: Extraction and anti-bacteria effect of active ingredients of \textit{Cacumen Platycladi} were studied in this paper. Extraction combined with ultrasonic was adopted. The optimum extraction condition was determined by single factor test; the anti-bacteria effect of active ingredients and minimum inhibitory concentration (MIC) were valued by Oxford-cup method. The results indicated that kaempferol was the active ingredients of \textit{Cacumen Platycladi} whose optimum extraction condition for ethanol concentrations were sixty-five percent and twenty minutes with ultrasonic assisted extraction.; the active ingredients of \textit{Cacumen Platycladi} had anti-bacteria effect on \textit{Staphylococcus}, \textit{Proteus}, \textit{Bacillus} and \textit{Serratia} and MIC was 0.5 g/mL, 0.5 g/mL, 0.0313 g/mL and 0.0625 g/mL. The active constituent of \textit{Cacumen Platycladi} is kaempferol which has obvious anti-bacteria effect and can be used to prolong the shelf-life of Low-temperature meat products.

1. Introduction

Low-temperature meat products are processed with the sterilization condition of 68–72 °C which can retain mostly nutritive value and flavour with short shelf life [1,2]. Sauced pork head meat is a kind of Low-temperature meat products with special flavour and short shelf life about 10 days which is popular with public. Previous researches of Sauced pork head meat have demonstrated that \textit{Staphylococcus}, \textit{Proteus}, \textit{Bacillus} and \textit{Serratia} are the main microorganisms leading to rot [3-5]. Therefore the preservatives used in Low-temperature meat products have got more attention on prolonging shelf life in recent years [6].

The food preservatives are classified as natural and chemical, with the improvement of people’s life level, Consumers prefer the natural, safe, efficient preservatives to chemical preservatives which is harmful [7]. At present, species [8,9] and Chinese herbal medicine [10,11] were mostly used as natural bacteriostatic agent. In this paper, the anti-bacteria effect of active ingredients of \textit{Cacumen Platycladi} was investigated which is a medicinal plant in China. The previous researches showed that volatile oils, flavonoids, tannin, organic acid and inorganic elements were the main chemical substances of...
Cacumen Platycladi [12,13]. And volatile oils and flavonoids have different antibacterial effects [14,15]. Kaempferol is a kind of flavonoid which is the main chemical substance of Cacumen Platycladi [16-19]. In this paper, anti-bacteria effect of kaempferol was studied aiming to extend the shelf-life of Sauced pork head meat.

2. Material and Methods

2.1. Sample
Cacumen Platycladi purchased from Mengzheng parity pharmacy in Beijing, China was smashed by Pulverizer (Tianjin Tasite Instruments Co., Ltd, China) and 40 meshes.

2.2. Test Strains
All the test strains including Staphylococcus (X12), Proteus (T4), Bacillus (X10) and Serratia (N11) were separated from putrid Sauced pork head meat.

2.3. Extraction of Active Substances
Five grams of sample were soaked in ethanol for 15 hours and extracted with ultrasonic (Kunshan Ultrasonic Instruments Co., Ltd, China) of 480 W and 50 °C. Finally, the supernatant was taken to evaporate until paste after centrifuged with 3000 rpm for 5 minutes and then preserved at 4°C until tested.

2.3.1. Standard Curve of Kaempferol. Standard solution of kaempferol(97%; Aladdin Industrial Corporation, Shanghai, China) was prepared for 0.0500 mg/mL. Ultraviolet absorption wavelength was valued by spectrum scanning standard solution. Standard curve was drawn by detecting absorbance values of standard solution of different concentration [20].

2.3.2. Concentration of Ethanol. Five grams of sample were extracted with 125 mL ethanol [21] of 60%, 65%, 70%, 75% and 80% respectively with ultrasonic for 30 minutes. Finally, the optimum concentration of ethanol was determined by the yield of kaempferol.

2.3.3. Time of Ultrasonic Extraction. Five grams of sample were extracted with 125 mL ethanol of optimum concentration [21], extraction time with ultrasonic were 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes respectively. Finally, the optimum extraction time was determined by the yield of kaempferol.

2.3.4. Determination of the Yield of Kaempferol. The paste of 0.00125 g/mL was suspended with 70% ethanol whose yield of kaempferol was determined by standard curve.

2.4. Bacteriostatic Experiments
All the strains preserved at -80°C were thawed at room temperature and mixed with sterile nutrient broth of 50 mL which was prepared by dissolving of 19 g/L in water, next the sterile nutrient broth (Beijing land bridge technology CO., LTD, China) inoculated with strains was incubated for 12 h at 37 °C which was repeated two times.

The sterile nutrient agar (Beijing land bridge technology CO., LTD, China) which was prepared by dissolving of 33 g/L in water was inoculated with bacterial suspension of 1×10⁹ CFU/mL diluted by plate counts method [22] (1 mL bacterial suspension in 100 mL agar medium) and poured into sterile petri dishes. Two sterile Oxford cups were placed on the surface of agar medium and marked with C and CK (C: antibacterial solution; CK: blank control), then 100 μL sterile water and antibacterial solution which was prepared by dissolving the paste of 1 g/mL in sterile water were added into Oxford cups respectively. Finally all the plates were incubated for 16~18 h at 37°C. Thereafter the anti-bacteria effect of active substances was determined by measuring the diameter of inhibition zone in the plates.
2.5. Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined by Oxford-cup method [23]. The sterile nutrient agar prepared by dissolving of 33 g/L in water was inoculated with bacterial suspension (1 mL bacterial suspension in 100 mL agar medium) and poured into sterile petri dishes. Eight sterile Oxford cups marked numbers were placed on the surface of agar medium symmetrically and steadily, next 100 μL antibacterial solution of different concentrations were added into Oxford cups respectively and sterile water was blank control. All plates were incubated for 16~18h at 37°C. The lowest concentration that had no inhibition zone compared with blank control was regarded as MIC.

2.6. Statistic Analysis

Data analysis and charts were made by Microsoft Office Excel 2007, significant difference analysis was valued by IBM SPSS Statistic 21.

3. Results and Discussions

![Graph 1](image1.png)

**Figure 1.** Effect of concentration of ethanol on Kaempferol yield.

![Graph 2](image2.png)

**Figure 2.** Effect of ultrasonic extraction time on Kaempferol yield.

Figure 1 demonstrated that the concentration of ethanol had an obvious impact on the yield of kaempferol (P < 0.05), with the increase of concentration, the kaempferol yield increased first and then decreased. The highest yield of Kaempferol was 2.19 mg/g with 65% ethanol. Kaempferol is a kind of flavonols and slightly dissolved in water, easily dissolved in hot ethanol, existed in *Cacumen Platycladi* with free form mainly and kaempferol-7-O-glucosides[24]. As is showed in figure 1, the reason that kaempferol yield decreased when the concentration of ethanol over 65% was that higher ethanol concentration restrained the hydrolysis of kaempferol-7-O-glucosides. Finally, the concentration of ethanol used in extraction was determined with 65%.

Figure 2 indicated that ultrasonic time had no obvious impact on the yield of kaempferol (P > 0.05), so ultrasonic extraction time was 20 minutes and the yield of Kaempferol was 2.31 mg/g. Compared to
ethanol extraction, ultrasonic can break cells and obtained higher yield. As is shown in Figure 1 and Figure 2, the extraction condition of active substances of *Cacumen Platycladi* was determined as follows: solid-liquid ratio of 1:25, 65% ethanol, temperature of 50°C, ultrasonic time of 20 minutes, power of 480W.

![Figure 3. The anti-bacteria effect of active ingredients of Cacumen Platycladi. X10=Bacillus, T4=Proteus, X12=Staphylococcus, N11=Serratia](image3)

As is showed in figure 3, the active ingredients of *Cacumen Platycladi* had different anti-bacteria effects on all the strains, especially the highest anti-bacteria effect on X10 with inhibition zone of 27.4 mm. While the anti-bacteria effect on X12 was weakest whose diameter of inhibition zone was only 11.37 mm.

![Figure 4. spectral scanning curve of Kaempfero; Figure 5. The standard curve of Kaempferol](image4)

As was shown in figure 4, ultraviolet absorption wavelength of Kaempferol was 369 nm. Figure 5 demonstrated linear regression equation: \( y = 69.92872 x + 0.0007 \)  \( R^2=0.9996 \)

Study demonstrated that kaempferol was the main active substance in the extractive [25,26] whose molecular structure included much hydroxy, which can enter the cells of bacteria and fungus to destroy the plasma membrane and restrain the synthesis of nucleic acid [13], thereby the active ingredients of *Cacumen Platycladi* can prolong the shelf-life of Sauced pork head meat through inhibiting microorganism.
Table 1. Determination of minimum inhibitory concentration of active substances of *Cacumen Platycladi*.

| Strains | The concentration of antibacterial solution (g/mL) |
|---------|-----------------------------------------------|
|         | 1.0   | 0.5   | 0.25  | 0.125 | 0.0625 | 0.0313 | 0.0157 |
| X10     | +++   | ++    | ++    | ++    | ++     | ++     | -      |
| T4      | +     | +     | -     | -     | -      | -      | -      |
| X12     | +     | +     | -     | -     | -      | -      | -      |
| N11     | +     | +     | +     | +     | -      | -      | -      |

“d” means diameter of inhibition zone, $d \geq 20\text{mm} = "+++"$; $15\text{mm} \leq d < 20\text{mm} = "++"$; $10\text{mm} \leq d < 15\text{mm} = "+"$; $d < 10\text{mm} = "-"$; “-” means no inhibition zone.

Table 1 demonstrated that MIC of active ingredients of *Cacumen Platycladi* was 0.0313 g/mL, 0.5 g/mL, 0.5 g/mL and 0.0625 g/mL respectively about X10, T4, X12 and N11. active ingredients showed higher anti-bacteria effect on X10 than others. While with the concentration of effective component decreased, the anti-bacteria effect on T4 and X12 changed significantly.

4. Conclusion

The results showed that the extraction condition of *Cacumen Platycladi* was determined as follows: solid-liquid ratio 1:25, 65% ethanol, ultrasonic temperature of 50 °C, ultrasonic time of 20 minutes and power of 480 W. The active ingredients of *Cacumen Platycladi* showed anti-bacteria effect on *Proteus, Staphylococcus, Serratia, Bacillus* and their MIC was 0.0313 g/mL, 0.5 g/mL, 0.0625 g/mL and 0.5 g/mL respectively.

The study results provide reliable data for the use of natural plant bacteriostatic agent on low-temperature meat products. But effect components and bacteriostatic mechanisms need further research.

5. References

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