Anti-bacteria effect of active ingredients of siraitia grosvenorii on the spoilage bacteria isolated from sauced pork head meat

X Li¹,²,³, L Y Xu¹,²,³, Y Q Cui¹,²,³, M X Pang¹,²,³, F Wang¹,²,³ and J H Qi¹,²,³,⁵

¹ College of Food Science and Engineering, Beijing Key Laboratory of Detection and Control of Spoilage Organisms and Pesticide Residues in Agricultural Products, Beijing 102206, China
² Food Chemistry teaching team, Beijing University of Agriculture, Beijing 102206, China
³ Innovation team, Modern agricultural industry technology system Beijing innovation team, Beijing 100102, China
⁴ High Level Talents of Beijing Universities Cross Training “Real Training Plan” Project, Beijing 102206, China
⁵ Email: abc960718@sina.com

Abstract. Extraction and anti-bacteria effect of active ingredients of Siraitia grosvenorii 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 optimum extraction condition of active ingredients extracted from Siraitia grosvenorii were described as follows: ethanol concentrations of sixty-five percent and twenty minutes with ultrasonic assisted extraction; the active ingredients of Siraitia grosvenorii had anti-bacteria effect on Staphylococcus epidermidis, Proteus vulgaris, Bacillus sp, Serratia sp and MIC was 0.125g/mL, 0.0625g/mL, 0.125g/mL and 0.125g/mL. The active constituent of Siraitia grosvenorii has obvious anti-bacteria effect on the spoilage bacteria isolated from Sauced pork head meat and can be used as a new natural food preservation to prolong the shelf-life of Low-temperature meat products.

1. Introduction

Low-temperature meat products are processed with the sterilizing condition of 68-72°C, which can retain mostly nutrition and flavour with short shelf life [1, 2]. Therefore, the preservatives have high lights on prolonging the shelf life of Low-temperature meat production recent years [3]. In this paper, Sauced pork head meat is the object of study whose main spoilage microorganisms include Staphylococcus epidermidis, Proteus vulgaris, Bacillus sp, and Serratia sp. as is shown in the research [4-6].

Up to now, there are two kinds of food preservations, including natural and chemical additives. Chemical additives were used widely in the past, but with the promotion of life standard, consumers prefer the natural, safe, efficient preservatives to chemical preservatives which is harmful [7]. At present, spices [8, 9] and Chinese herbal medicine [10, 11] were mostly used as natural bacteriostatic agent. In this paper, the anti-bacteria effect of Siraitia grosvenorii was investigated, which was a kind of medicinal and edible plant in China and obtained widespread application on Natural sweetener [12,
13] and Chinese herbal medicine [14, 15]. The previous studies showed that flavonoid [16, 17], mogroside, triterpene benzoate [18], saponins [19] were the main chemical substances of Siraitia grosvenorii and a new antibacterial compound named siraitiflavandiol was found by Zheng Y et al. [20]. Kaempferol was a kind of flavonoid [21, 22] which would be used to determine the extraction condition. In the paper, antibacterial effects of active ingredients of Siraitia grosvenorii on the spoilage bacteria isolated from Sauced pork head meat was studied aiming to prolong the shelf-life of Sauced pork head meat.

2. Material and methods

2.1. Sample
Siraitia grosvenorii 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 epidermidis(X12), Proteus vulgaris (T4), Bacillus sp. (X10) and Serratia sp.(N11) were isolated from Sauced pork head meat, and identified by Henan Academy of Sciences Institute of Biology Limited Liability Company, China.

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 aftercentrifuged 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 [23].

2.3.2. Concentration of ethanol. Five grams of sample were extracted with 125mL ethanol [24] 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 125mL ethanol of optimum concentration, 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 0.00125g/mL was suspended with 70% ethanol whose yield of kaempferol was determined by standard curve.

2.4. Anti-bacteria experiments
All the strains preserved at -80°C were thawed at room temperature and mixed with sterile nutrient broth of 50mL which was prepared by dissolving of 19g/L in water, next the sterile nutrient broth(Beijing land bridge technology CO., Ltd, China) inoculated with strains was incubated for 12h 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 33g/L in water was inoculated with bacterial suspension of 1×10⁸ CFU/mL diluted by plate counts method [25] (1mL bacterial suspension in 100mL agar medium) and poured into sterile petri dishes. Two sterile Oxford cups were placed on the surface of agar medium and marked with L and CK (L: antibacterial solution; CK: blank control), then 100μL sterile water and antibacterial
solution which was prepared by dissolving the paste of 1g/mL in sterile water were added into Oxford cups respectively. Finally all the plates were incubated for 16~18h at 37°C. Thereafter the antibacteria effect of active ingredients 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 [26]. The sterile nutrient agar prepared by dissolving of 33g/L in water was inoculated with bacterial suspension (1mL bacterial suspension in 100mL 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 100μL 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**
Figure 1 demonstrated that the concentration of ethanol had an obvious impact on the yield of kaempferol ($P<0.01$), with the increase of concentration, the kaempferol yield increased first and then decreased. The highest yield of kaempferol was 1.32mg/g with 70% ethanol.

![Figure 1. Effect of concentration of ethanol on Kaempferol yield.](image1)

![Figure 2. Effect of ultrasonic extraction time on Kaempferol yield.](image2)
Kaempferol is a kind of flavonols and slightly dissolved in water, easily dissolved in hot ethanol, existed in Siraitia grosvenorii with free form mainly and glycosides including kaempferol-3-c and kaempferol-3,7-double-glucoside[27]. As is showed in figure 1, the reason that yield of kaempferol decreased when the concentration of ethanol over 70% was that higher ethanol concentration restrained the hydrolysis of glycosides. Finally, the concentration of ethanol used in extraction was determined with 70%.

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 with kaempferol of 2.31mg/g. Compared to ethanol extraction, ultrasonic assisted extraction obtained higher yield. As is showed in figure 1 and figure 2, the extraction condition of active constituents of Siraitia grosvenorii was determined as follows: solid-liquid ratio of 1:25, 70% ethanol, temperature of 50℃, ultrasonic time of 20 minutes, power of 480W.

As is showed in figure 3, the active ingredients of Siraitia grosvenorii had different anti-bacteria effects on allstrains, especially the best anti-bacteria effect on X10 with inhibition zone of 18.6 mm, while the anti-bacteria effect on T4 was weakest whose diameter of inhibition zone was only 11.23mm.

The active ingredients of Siraitia grosvenorii showed distinct anti-bacteria effect on the strains which were isolated from Sauced pork head meat. What’s more, the results indicated that there were flavonols and flavonoids in extractive of Siraitia grosvenorii according to the chromogenic reactions with sodium hydrate and magnesium acetate. Asif Ahmad et al. studied that the molecular of flavonoids had included benzene ring and hydroxy, which can enter the cells of bacteria to destroy the plasma membrane and restrain the synthesis of nucleic acid [28]. Theryby the effective components of Siraitia grosvenorii can be used as a new natural food preservation and to delay the shelf-life of Sauced pork head meat through inhibiting microorganism in the future.
As was shown in figure 4, ultraviolet absorption wavelength of kaempferol was 369 nm. Figure 5 demonstrated linear regression equation: \( y = 69.92872 x + 0.0007, R^2=0.9996 \).

### Table 1. Determination of minimum inhibitory concentration of active substances of S. grosvenorii.

| 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 \leq 20 \text{mm} = "++"; 10 \text{mm} \leq d < 15 \text{mm} = "+"; d < 10 \text{mm} = "+"; "; -" means no inhibition zone.*

Table 1 showed that MIC of effective component extracted from Siraitia grosvenorii was 0.125g/mL, 0.0625g/mL, 0.125g/mL and 0.125g/mL respectively on X10, T4, X12 and N11. Effective components showed higher anti-bacteria effect on T4 than others. While with the concentration of effective component decreased, the anti-bacteria effects on X10 and X12 changed significantly.

### 4. Conclusion

The results showed that the extraction condition of Siraitia grosvenorii was determined as follows: solid-liquid ratio 1:25, 70% ethanol, ultrasonic temperature of 50°C, ultrasonic time of 20 minutes, ultrasonic power of 480W. The active ingredients of Siraitia grosvenorii showed bacteriostatic effects on Staphylococcus epidermidis, Proteus vulgaris, Bacillus sp, Serratia sp. and MIC was 0.125g/mL, 0.0625g/mL, 0.125g/mL and 0.125g/mL respectively.

The study results provide reliable data for the application of natural plant bacteriostatic agent on low-temperature meat products. But active components of Siraitia grosvenorii and anti-bacteria mechanisms need further research.

### References

[1] Yuan X Q 2012 Research on quality changes of stewed meat in seasoning Chongqing: Southwest University
[2] SB/T 10481-2008 Requirements of quality and safety on Pasteurized meat products China
[3] Zhang D Q, He Z 2008 Research progress of the preservation technology of processed meat products J.Meat Research 5 3-7
[4] Li Y L 2009 Isolation and identification of spoilage bacteria in low-temperature meat products J.Inner Mongolia Agricultural Science and Technology 3 62-63
[5] Xiao X 2013 Bacterial diversity and the inhibitory mechanism of antibacterial plant extract against specific spoilage organism in Yao meat
[6] Lei M, Jiang R R, Ju R H and Xiao Y 2013 Microbiology detection of pasteurized meat products anddominant microflom analysis and identification China Brewing 32(3) 83-86
[7] Fan J S 1993 Functional food additive Tianjin Science and Technology Press 318
[8] Tian F, et al. 2017 Study on antibacterial activity of 7 spices on Pseudomonas, Staphylococcus epidermidis and Brochothrix Journal of Beijing University of Agriculture 32(2) 10-14
[9] Liu L, Kong B H, et al. 2008 The inhibition effect of spices extraction on Listeria monocytogenes in culture medium and in chilled pork J.Science and Technology of Food Industry 29(9) 87-90
[10] Liu F, Cao X Z, et al. 2015 Study on bacteriostatic activity of total flavonol from angelica sinensis J.China Food Additives 1 60-63
[11] Zhou J X 2006 Reviews on research progresses, actual problem and prospects on natural food preservatives from plant materials J.Food Science 27(1) 263-268
[12] Chen Y 2005 Chemical and functional studies of Siraitia grosvenorii Shanghai:Shanghai Jiao
Tong University

[13] Qi Y P and Tang M Y 2001 The chemical component in Siraitia grosvenorii (Swingle) C. Jeffrey fruit and utility Fujian Medical Journal 23(5) 158-160

[14] Song F, et al. 2006 A natural sweetener, Momordica grosvenori attenuates the imbalance of cellular immune functions in alloxan-induced diabetic mice J.Phytotheray Research 20 552-560

[15] Wei R C, et al. 2013 Advances on Medicinal Plant Siraitia grosvenorii Hubei Agricultural Sciences 52(23) 5669-5672

[16] He K X, et al. 2013 Changes of total flavonoids in Siraitia grosvenorii and its antibacterial activity Amino Acids and Biological Resources 35(4) 16-20

[17] Chen Q B, et al. 2004 Study on tea value of Siraitia grosvenorii leaf Tea In Fujian 3 12-15

[18] Motohiko U, et al. 2002 Inhibitory effects of cucurbitane glycosides and other triterpenoids from the fruit of Momordica grosvenori on Epstein-Barr Virus early antigen induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate Agric Food Chem 50 6 710-6 715

[19] Shi D F 2016 Studies on the extraction, separation, structural identification and biological activities of the chemical compounds from Dictamus dasycarpus Turcz. and Siraitia grosvenorii swingle Changchun:Northeast Normal University

[20] Y Zheng, et al. 2009 A new antibacterial compound from Luo Han Kuo fruit extract (Siraitia grosvenorii) J Asian Nat Prod Res 11(8) 761-5. doi: 10.1080/10286020903048983.

[21] Huang X S 2007 Chemical constituents of Siraitia grosvenorii (Swingle) C. Jeffrey Guangxi:Guangxi Normal University

[22] Zhang H, et al. 2011 Research progress on chemical compositions of fruits Momopdicae Journal of Anhui Agri 39(8) 4555-4556,4559

[23] Chen Z G, et al. 2016 Determination of quercetin content in Raw drug by ultraviolet spectrophotometry J.Progress in Veterinary Medicine 37(1) 122-124

[24] Cui B 2012 Study on the extraction of purification technology of flavone of Mangosteen

[25] GB 4789.2-2010 National food safety standard Food microbiological examination: Aerobic plate count

[26] Li Y, Liu J, et al. 2015 Antioxidation and antibacterial property of flavonoids compounds extracted from Nitraria leaves J.Food Industry 36(3) 94-98

[27] Ma M, Wang L, et al. 2014 Optimization of extraction technology of kaemperol from Sophora japonica by orthogonal test J.China Pharmacy 25(43) 4069-71

[28] Asif Ahmad, Muhammad Kaleem, et al. 2015 Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections-A review J.Food Research International 77 221-235