The antimicrobial activities of the cinnamaldehyde adducts with amino acids

Qing-Yi Wei a, Jia-Jun Xiong b, Hong Jiang c,*, Chao Zhang c, Wen Ye c

a College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510641, China
b College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
c Department of Chemistry, College of Science, Huazhong Agricultural University, Wuhan, 430070, China

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A B S T R A C T
Cinnamaldehyde is a well-established natural antimicrobial compound. It is probable for cinnamaldehyde to react with amino acid forming Schiff base adduct in real food system. In this paper, 9 such kind of adducts were prepared by the direct reaction of amino acids with cinnamaldehyde at room temperature. Their antimicrobial activities against Bacillus subtilis, Escherichia coli and Saccharomyces cerevisiae were evaluated with benzoic acid as a reference. The adducts showed a dose-dependent activities against the three microbial strains. Both cinnamaldehyde and their adducts were more active against B. subtilis than on E. coli, and their antimicrobial activities were higher at lower pH. Both cinnamaldehyde and its adducts were more active than benzoic acid at the same conditions. The adduct compound A was non-toxic by primary oral acute toxicity study in mice. However, in situ effect of the adduct compound A against E. coli was a little lower than cinnamaldehyde in fish meat. This paper for the first time showed that the cinnamaldehyde adducts with amino acids had similar strong antimicrobial activities as cinnamaldehyde, which may provide alternatives to cinnamaldehyde in food to avoid the strong unacceptable odor of cinnamaldehyde.

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1. Introduction
Enhancement of the safety of foods is one of the interests of food industry, and the usage of synthethic chemical food preservatives is one of the ways to control microbial growth and to reduce the incidence of food poisoning and spoilage (Eklund, 1980). Recently, however, consumers have grown concerned about the negative effects and expressed a desire for the reduction of chemical preservatives like benzoate, sorbate and propionate for preventing and controlling pathogenic microorganisms in foods (Salih, 2006), which have led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity (Marino et al., 2001). In this context extensive efforts are currently being made in research for alternative antimicrobial compounds based on plant extracts. One option is the use of essential oils as antibacterials which have GRAS (generally regarded as safe) status and a wide acceptance from consumers (Burt, 2004). Essential oils (EOs) are volatile aromatic oily liquids, obtained from plants that have been traditionally used to preserve foods as well as enhance food flavor that has been used in food preparation since antiquity. The antimicrobial properties of carvacrol, thymol, eugenol and cinnamaldehyde from EOs have been described extensively (Liolios et al., 2009; Si et al., 2006).

Cinnamaldehyde is an aromatic aldehyde and main component of bark extract of cinnamon (Cinnamomum verum) (approximately 65%) (Holley and Patel, 2005; Lens-Lisbonne et al., 1987). The main advantage of cinnamaldehyde is that direct contact is not required for being active as antimicrobial. Cinnamaldehyde has been shown to be active against a range of foodborne pathogens bacteria including Escherichia coli O157:H7 and Salmonella Typhimurium, fungi (Amalaradjou et al., 2010; Becerril et al., 2007; Guillier et al., 2007; Gutierrez et al., 2010; Goñi et al., 2009; Lee et al., 2005; Lopez et al., 2007; Montero et al., 2011; Rodriguez, et al., 2007, 2008) and viruses (Hayashi et al., 2007; Liu et al., 2009). Cinnamaldehyde is classified as a GRAS molecule by the United States Food and Drug Administration and is approved for use in foods (21 CFR 182.60) (Sivakumar et al., 2002).

However it has generally been found that higher concentrations of EOs were necessary to obtain the same antimicrobial effects in foods than in vitro (Carraminana et al., 2008; Burt, 2004). The ratio has been recorded to be approximately twofold in semi-skimmed milk (Karatzas et al., 2001), 10-fold in pork liver sausage (Sundan and Shlef, 1994), 50-fold in soup (Ullée and Smid, 2001) and 25 to 100-fold in soft cheese (Mendoza-Yepes et al., 1997). If high concentrations are required to achieve useful EO antimicrobial activity, unacceptable levels of inappropriate flavors and odors may result. Therefore, to successfully decrease the concentration of EOs in food systems, primary studies to determine potential interactions between main component in EOs and food components should be carried out (Burt, 2004).

Cinnamaldehyde, main component of cinnamon EO, can react with the amino group of amino acids or protein in food system forming new compounds. Cinnamaldehyde has free carbonyl group and the amino acids have free amino group, so the two groups can react...
readily forming Schiff base adducts (Fehn et al., 2001). Other essential oils have no main components containing free aldehyde groups, so we only choose cinnamaldehyde as the testing compound. Whether the new formed compounds have any impact on antimicrobial efficacy is unknown. Towards this end, we wish to report our observations on the antimicrobial activities of the adducts of cinnamaldehyde with amino acids. The main advantage of the adducts is their direct contact to some food systems to be active because of their solubility in water.

2. Material and methods

2.1. Chemicals and instruments

Methanol, cinnamaldehyde, L-amino acids, potassium hydroxide, sodium phosphate and benzoic acid were obtained from Shanghai Reagent Co. IR spectra were recorded on an Avatar 330 infrared spectrophotometer (KBr pellet); only the most significant absorption bands are reported (νmax, cm−1). 1H NMR data were acquired at room temperature on a Bruker AV 400-MHz operating at 400 MHz. CDCl3 or DMSO-d6 was used as solvent; chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (TMS) as internal reference. A Spectronic Genesys 8 UV/VIS spectrophotometer was used to record the UV of prepared compounds.

The preparation was exemplified by the adduct between glycine and cinnamaldehyde as follows:

2.2. The preparation of potassium glycinate

A mixture of glycine (6.7 mmol, 0.50 g) and potassium hydroxide (6.7 mmol, 0.38 g) in methanol (25 mL) was added into a 100 mL round-bottomed flask equipped with a condenser. Let them reacted at 50 °C for 2 h with stirring. The mixture was cooled followed by filtration. The filtrate was potassium glycinate solution.

2.3. The preparation of the adduct of cinnamaldehyde with potassium glycinate

To the above stirred solution of potassium glycinate was added dropwise cinnamaldehyde solution in 10 mL methanol (6.7 mmol, 0.84 mL) at room temperature under nitrogen in 1 h. The stirring was continued for 2.5 h. The precipitate was collected after filtration, then washed with ether and dried under vacuum, the Schiff base adduct of potassium glycinate and cinnamaldehyde was produced. Similarly other Schiff base adducts between different amino acids and cinnamaldehyde were also prepared by the same way.

The preparation reaction is shown in Scheme 1 and the structure of the prepared compounds is presented in Scheme 2.

2.4. Antimicrobial activities

For antibacterial assays gram positive (Bacillus subtilis MTCC 441) and gram negative (E.coli ATCC 25922) bacterial strains were employed. Antifungal activity was assessed on the yeast Saccharomyces cerevisiae ATCC 2365. These microorganisms were supplied by College of Biology, Huazhong Agricultural University, Wuhan, China.

All the following antimicrobial activities of prepared compounds were compared with commercial cinnamaldehyde and benzoic acid in order to have references reputed for their antimicrobial properties. All the data collected for each assay are the averages of three determinations of three independent experiments. Cultural media for yeast used in this study was Malt Agar, for the bacteria Nutrient Agar (Shanghai reagent co. China).

The biological activity against these classes of microorganisms was determined by employing the standard disks diffusion technique (Bauer et al., 1966; Shan et al., 2007; Rattanachaikunsopon and Phumkhachorn, 2010). Mother cultures of each microorganism were set up 24 h before the assays, in order to reach the stationary phase of growth. The tests were assessed by inoculating, from the mother cultures, Petri dishes with proper sterile media with the aim of obtaining a microorganism concentration of 10⁶ cfu (colony forming units)/mL and 10⁵ cfu/mL for bacteria and yeast, respectively. A measured amount of the test samples was dissolved in definite volumes of methanol to give solutions of known concentrations (200, 400, and 600 mg/L). Then, sterile Whatmann filter paper disks (10 mm diameter) were impregnated with known amounts of the tested compounds and dried at room temperature to remove any residual solvent. These disks were placed on plates containing agar media seeded with the test organisms. The Petri dishes were then incubated at 37 °C for bacterial and 28 °C for yeast for 24 h respectively. Methanol was used as negative controls, and cinnamaldehyde and benzoic acid were used as positive controls. The antimicrobial activities were measured by the inhibition zone diameter (IZD) expressed in mm (subtract the diameter of the filter paper disk). All experiments were carried out in triplicate.

2.5. Effect of pH on the antimicrobial activity

Test compounds (compound A, B, CINMAL (cinnamaldehyde), BENZ (benzoic acid)) at concentration of 200 mg/L were used. The growth of microbial was estimated by determining the absorbance at 560 nm in a Spectronic Genesys 8 UV/VIS spectrophotometer quantitatively (Bevilacqua et al., 2008; Michael et al., 2002; Giordani et al., 1997). Microorganisms were cultivated in liquid media (bacterial — Nutrient broth; yeast — Malt extract broth) supplemented with test compounds at various pH. The pH of cultures was adjusted to c. 4.0, 5.0, 6.0, 7.0, and 7.5 by addition of different pH buffer solutions at a concentration of 1% (w/v). Microbial was diluted to achieve 10⁴–10⁵ cfu/mL. 0.2 mL of the diluted microbial was added to the conical flask with 100 mL liquid media at different pH with tested compounds. Sterile medium without any addition of test compound but with microbial inoculums was used as a control. Liquid media with tested compound but without microbial inoculums served as a blank. After subtraction its absorbance from the absorbance of the samples, where the inoculum was added, absorbance of pure microorganisms was obtained. After shaking the plates for 15 s, the absorbance was determined after 24 h of incubation. All the analyses
were performed in triplicate and the experimental data represent the average of them. The inhibitory index was calculated as following:

\[ \text{In} X = 100 - (\Delta A_t / \Delta A_c) \times 100 \]

where:
\[ \Delta A_t \] — absorbance of treated samples subtract blank sample
\[ \Delta A_c \] — absorbance of the control sample subtract blank sample

2.6. In situ effect of compound A

The in situ efficacy was evaluated against *E. coli* ATCC 25922, in a food model (fresh fish). This assay was performed according to the method reported by Al-Zoreky (Al-Zoreky, 2009) with some modification. *E. coli* are recognized as the food-borne pathogen of serious gastrointestinal diseases in humans, and have been responsible for several food-borne outbreaks (Newell et al., 2010). Fishery products are usually distributed with minimal treatment before sale to the consumer, and they were one of the main sources of such diseases. In addition fish meats are one of the main protein sources for south China people. Therefore, it was appropriate to conduct in situ experiments for evaluating the efficacy of A against *E. coli* in a food model during typical storage conditions of the test food (chilled fish).

Grass carp is a commonly consumed fish in south China, so fresh Grass carp was used to evaluate the antibacterial activity of A and cinnamaldehyde towards *E. coli*. The fresh fish samples were rinsed with running tap water, drained and cut to 5 g per piece (5 × 2 × 0.5 cm) with a sterile knife under aseptic conditions. The pieces were then put inside plastic bags (3 pieces per bag) and stored at 4 °C for approximately 3 h. *E. coli* was pre-cultured at 37 °C for 18–20 h in beef extract-peptone broth. The freshly grown pathogen was serially diluted with sterile normal saline water to 10^8 cfu/mL, as determined by spread plating on selective agar medium for *E. coli* and cinnamaldehyde towards *E. coli*. The fresh food samples were dipped for 15 min in pre-sterilized containers containing 30 mL of A and cinnamaldehyde of different concentrations (25 mM and 50 mM), compound A and cinnamaldehyde were dissolved with phosphate-buffered saline (PBS) (consisting of 137 mM NaCl, 2.7 mM KCl, 8.1 mM NaHPO4 and 1.5 mM KH2PO4, pH 6.6) with 1% Tween-20. Control experiments containing equivalent PBS pH 6.6 with 1% Tween-20 were always conducted under the same condition. Removing excess solution was done by gently pressing each piece against the inside walls of containers. Pieces were vacuum-sealed in plastic bags and immediately stored at 4 °C. After predetermined intervals, each fish piece was aseptically removed and blended with 45 mL normal saline water. Aliquots (0.1 mL) were spread onto surfaces of the selective agar medium for *E. coli* followed by incubation at 37 °C for 24 h. The experiment was performed in triplicate.

2.7. Oral acute toxicity study in mice

Kunming strain male and female mice (a closed strain coming from Kunming, Yunnan Province, China) were obtained from the Experiment Animal Center (Wuhan, China) with a body weight range from 18 to 22 g. The animals were housed in cages in a temperature-controlled animal room (20–25 °C) with a relative humidity of 40–60%. They were fasted overnight but given water ad libitum prior to dosage. The animals were divided into four groups of five males and five females, and the adduct product A was administered by oral gavage at doses of 2500, 1250, 625, and 312 mg/kg body weight. After a single administration, signs of possible toxicity were observed every hour for the first six hours and every day for 14 days. The observations of general status, toxic symptom and mortality in mice were continued for two weeks after treatment. Finally, acute toxic classification was determined.

2.8. Statistical analysis

In each test, three experiments were carried out and each experiment was done in duplicate. Student’s t test was used for comparison of the results.

3. Results and discussion

3.1. Antimicrobial activities of the tested compounds

The paper disk assay results are presented in Table 1. All of the prepared compounds in this study, which were the adducts of cinnamaldehyde and amino acids, exhibited antimicrobial activity against *B. subtilis*, *E. coli*, and *S. cerevisiae*, but showed different degrees of inhibition. Based on inhibition zone diameter (IZD), cinnamaldehyde produced the largest inhibition zone, whereas benzoic acid produced the smallest at the same mass concentration. Moreover, the stronger flavor of the cinnamaldehyde disappeared because of the non-volatility of the adducts formed, which was confirmed by a panel of experienced people in food sensory test. The IZD, as expected, increased with increasing concentration in the tested concentration range. For cinnamaldehyde, at the concentration of 200 mg/L, 400 mg/L, 600 mg/L, the IZD to *E. coli* and *B. subtilis* were 5.5 mm, 10.3 mm, 16.2 mm; 6.5 mm,11.0 mm,17.9 mm, respectively, so cinnamaldehyde was more active against Gram-positive bacteria (*B. subtilis*) than Gram-negative bacteria (*E. coli*). This is likely due to the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Grampositive bacteria (Nikaido, 1996; Duffy and Power, 2001). The resistance of Gram-negative bacteria toward antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules, and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Nikaido, 1994; Gao et al., 1999).

### Table 1

The antimicrobials (IZD, mm) of compounds A to I.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Escherichia coli</th>
<th>Bacillus subtilis</th>
<th>Saccharomyces cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mg/L</td>
<td>400 mg/L</td>
<td>600 mg/L</td>
</tr>
<tr>
<td>A</td>
<td>4.2 ± 0.3A</td>
<td>7.3 ± 0.4A</td>
<td>11.8 ± 0.6B</td>
</tr>
<tr>
<td>B</td>
<td>4.3 ± 0.2B</td>
<td>7.6 ± 0.3B</td>
<td>12.4 ± 0.5B</td>
</tr>
<tr>
<td>C</td>
<td>3.1 ± 0.4A</td>
<td>5.8 ± 0.5A</td>
<td>10.3 ± 0.6B</td>
</tr>
<tr>
<td>D</td>
<td>4.1 ± 0.3A</td>
<td>7.1 ± 0.5B</td>
<td>11.3 ± 0.6B</td>
</tr>
<tr>
<td>E</td>
<td>4.3 ± 0.4A</td>
<td>6.4 ± 0.3B</td>
<td>11.6 ± 0.5B</td>
</tr>
<tr>
<td>F</td>
<td>3.4 ± 0.2A</td>
<td>5.6 ± 0.5B</td>
<td>10.8 ± 0.6B</td>
</tr>
<tr>
<td>G</td>
<td>3.1 ± 0.4B</td>
<td>5.4 ± 0.2B</td>
<td>10.6 ± 0.4B</td>
</tr>
<tr>
<td>H</td>
<td>3.3 ± 0.4B</td>
<td>7.2 ± 0.3B</td>
<td>11.5 ± 0.5B</td>
</tr>
<tr>
<td>I</td>
<td>3.5 ± 0.5A</td>
<td>7.1 ± 0.3B</td>
<td>11.9 ± 0.5B</td>
</tr>
<tr>
<td>CINMAL</td>
<td>5.5 ± 0.2A</td>
<td>10.3 ± 0.2B</td>
<td>16.2 ± 0.3B</td>
</tr>
<tr>
<td>BENZ</td>
<td>3.5 ± 0.2A</td>
<td>6.7 ± 0.1B</td>
<td>9.2 ± 0.3B</td>
</tr>
</tbody>
</table>

Antimicrobial activities were evaluated by measuring the inhibition zone diameter (IZD) of the tested microorganisms *Escherichia coli*, *Bacillus subtilis*, and *Saccharomyces cerevisiae*. The concentrations were 200 mg/L, 400 mg/L, 600 mg/L for tested compounds, for clarity, the data for 200 mg/L was not provided. IZD value = mean ± SD. Means with the same letter in the same column were not significantly different (p < 0.05). CINMAL is cinnamaldehyde, BENZ is benzoic acid.
Gram-positive bacteria do not have such an outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Kalemba and Kunicka, 2003; Lin et al., 2000). From the point of mass concentration, at the same concentration cinnamaldehyde was more active than its adduct with amino acids, but when it is convert into molar concentration, the antimicrobial activity of the adducts were very near or higher than the cinnamaldehyde. For example, the molecular weight for compound A is 227, while for cinnamaldehyde is 132, so 400 mg/L cinnamaldehyde is equal to 3.0 mmol/L, and 600 mg/L compound A is equal to 2.64 mmol/L, that is, the number of molecules of 400 mg/L cinnamaldehyde is near that of 600 mg/L compound A. The IZD of compound A for Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae at 600 mg/L is 11.8 mm, 12.6 mm, 12.3 mm, repectively, while IZD of cinnamaldehyde for E. coli, B. subtilis, S. cerevisiae at 400 mg/L is 10.3 mm, 11.0 mm, 10.8 mm, respectively. Therefore, it is obvious that at the same molar concentration, compound A is more active than cinnamaldehyde (Table 2). It means that when cinnamaldehyde react with the amino acids of the food system to form adducts, the antimicrobial activity will not decrease, because the number of molecules of the adduct formed is equal to that of the original cinnamaldehyde added. Table 1 also demonstrates that all the prepared adducts had better antimicrobial activities than benzoic acid, however, presently no activity-structure can be found from them.

### 3.2. Effect of pH on the antimicrobial activity

The results of Fig. 1, Fig. 2, and Fig. 3 showed the antimicrobial activities were higher at lower pH. On one hand, at lower pH, the viability of the microorganisms is not good enough, on the other hand, the proportion of undissociated molecules at different pH values modify the antimicrobial activity. It is established that the antimicrobial activity of weak acids is pH dependent due to the influence of this parameter on the proportion of undissociated acid molecules in solution (Wen et al., 2003). The proportion of undissociated acid molecules is higher at lower pH and reduced at higher pH. The undissociated acid is less hydrophilic than the dissociated form, so the undissociated acid is more active. At pH 4.0, the inhibitory rates of A and B for E. coli, B. subtilis, S. cerevisiae are 60.8%, 82.0%, 57.8%; 82.4%, 84.6%, 74.4%, respectively, so the antimicrobial activities for both compound A and B decreased in the following order B. subtilis > E. coli > S. cerevisiae.

### 3.3. In situ effect of compound A

The results in food system revealed that cinnamaldehyde and compound A dipping (25 and 50 mM) possessed an obvious inhibition against E. coli in fish meat (Fig. 4). However, E. coli was gradually increasing at 4 °C, for control slices, reaching more than 7.18 log cfu/g

### Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mg/L)</th>
<th>Molecular weight</th>
<th>Molecular concentration (mmol/L)</th>
<th>E. coli (mmol/L)</th>
<th>B. subtilis (mmol/L)</th>
<th>S. cerevisiae (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>400</td>
<td>227</td>
<td>1.76</td>
<td>4.1±0.2</td>
<td>4.8±0.3</td>
<td>4.3±0.2</td>
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<tr>
<td>B</td>
<td>400</td>
<td>241</td>
<td>1.66</td>
<td>4.5±0.2</td>
<td>5.2±0.2</td>
<td>4.9±0.4</td>
</tr>
<tr>
<td>C</td>
<td>400</td>
<td>269</td>
<td>1.49</td>
<td>3.9±0.3</td>
<td>4.8±0.3</td>
<td>4.6±0.4</td>
</tr>
<tr>
<td>D</td>
<td>400</td>
<td>317</td>
<td>1.26</td>
<td>5.6±0.4</td>
<td>6.5±0.3</td>
<td>6.0±0.4</td>
</tr>
<tr>
<td>E</td>
<td>400</td>
<td>283</td>
<td>1.41</td>
<td>4.5±0.2</td>
<td>5.3±0.3</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>F</td>
<td>400</td>
<td>338</td>
<td>1.18</td>
<td>4.7±0.4</td>
<td>6.3±0.5</td>
<td>6.2±0.2</td>
</tr>
<tr>
<td>G</td>
<td>400</td>
<td>324</td>
<td>1.23</td>
<td>4.4±0.1</td>
<td>5.9±0.2</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>H</td>
<td>400</td>
<td>297</td>
<td>1.35</td>
<td>5.3±0.2</td>
<td>6.0±0.3</td>
<td>5.4±0.3</td>
</tr>
<tr>
<td>I</td>
<td>400</td>
<td>287</td>
<td>1.39</td>
<td>5.1±0.2</td>
<td>5.7±0.4</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>CINMAL</td>
<td>400</td>
<td>132</td>
<td>3.03</td>
<td>3.4±0.1</td>
<td>3.6±0.1</td>
<td>3.6±0.1</td>
</tr>
<tr>
<td>BENZ</td>
<td>400</td>
<td>122</td>
<td>3.28</td>
<td>2.0±0.1</td>
<td>1.8±0.1</td>
<td>2.1±0.1</td>
</tr>
</tbody>
</table>

Antimicrobial activities were evaluated by measuring the inhibition zone diameter (IZD) of the tested microorganisms Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae. The data were from Table 1 of 400 mg/L for tested compounds in the form of ratio IZD/molecular concentration. CINMAL is cinnamaldehyde, BENZ is benzoic acid.
after a 6-day storage, while for 50 mM cinnamaldehyde and compound A, the corresponding values were 4.76 log cfu/g and 5.80 log cfu/g, respectively. So both cinnamaldehyde and compound A significantly (P<0.05) prevented the proliferation of E. coli in fish pieces. Cinnamaldehyde was more effective than compound A at the same concentration, which might be attributed to its poor solubility of compound A in the water phase of fatty fish meat. When fatty fish meat was dipped with compound A, which is water-soluble, so it is hard for compound A to adhere to fatty tissues of fish meat.

3.4. Oral acute toxicity study in mice

To establish the safety of the adducts, compound A at 2500, 1250, 625, and 312 mg/kg body weight was administered to both male and female mice. We observed no death and no apparent toxic during the 14 day observation period. None of the mice showed clinical toxic signs such as anorexia, depression, lethargy, jaundice, dermatitis, except that at dose of 2500 mg/kg body weight, the mice drink more water. Because of the limitation of the solubility in water, larger dose than 2500 mg/kg body weight was not tested. The oral acute toxicity study was more than 2,500 mg/kg body weight mice. Therefore, compound A was practically no toxicity according to the criteria of acute toxic classification (Ministry of Health PR China, 2003).

4. Conclusion

The adducts between cinnamaldehyde and amino acids showed good antimicrobial effects against the studied microorganisms (E. coli, B. subtilis, S. cerevisiae), and for each strain the effects were dependent on the medium pH, which were higher at lower pH. After addition with amino acid, cinnamaldehyde was attached with a carboxylic acid group making them water-soluble, so the volatile property of cinnamaldehyde disappeared, hence the unacceptable levels of inappropriate strong flavors and odors from cinnamaldehyde can be prevented. Moreover, the prepared adducts are non-toxic by primary oral acute toxicity study in mice. However, in situ effect of compound A against E. coli was a little lower than cinnamaldehyde in fish meat.

Considering the olfactory factor, replacing cinnamaldehyde with cinnamaldehyde adducts is worth further investigation in the future.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.ijfoodmicro.2011.07.034.

References


