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Improving water solubility of natural antibacterials to inhibit important bacteria in meat products

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Abstract

The use of natural antimicrobial compounds, such as cinnamic acid and its phenolic derivatives, may help prevent survival of *Escherichia coli* O157:H7 and other pathogenic bacteria in foods. The limitation of the widespread use of these acids is their low solubility in water systems. Therefore the objective of this work was to evaluate the impact of the concentration of soluble salts of hydroxycinnamic acids on the viability of *E. coli* O157:H7 and *S.* Gallinarum. The solubility enhancement of the compounds at pH 7 could bring important consequences, since, for instance, the majority of outbreaks involving *E. coli* O157:H7 have been caused by the ingestion of meat and lacteous derivatives that present nearly neutral pH. Also, experimental errors in the evaluation of inhibitory concentrations may be prevented, avoiding lack of solubility of phenolic acids and their tendency to precipitate during incubation of media. Morphological changes on ultrastructure of *E. coli* O157:H7 was analyzed using transmission electron microscopy. Sodium salts of ferulic and *p*-coumaric acids were prepared and tested against *E. coli* O157:H7 in concentrations of 0.2, 0.4, 0.6, 0.8 and 1% in trypticase soy medium at pH 7. A bacteriostatic and/or bactericidal effect was also concentration-dependent. A bactericidal effect was found at concentrations of 0.8 and 1%, a bacteriostatic effect at the intermediate concentration of 0.6%, and an almost normal growth at the lowest concentrations of 0.2 and 0.4% showing their potential to be used as pathogen inhibitors.

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1. Introduction

A variety of foods have been implicated in outbreaks of illness attributed to pathogenic bacteria, such as *E. coli* O157:H7. Food industry has re-focused attention on *E. coli* O157:H7 as a cause of significant morbidity and mortality in outbreaks of food-borne illness. Among foods implicated in outbreaks due to

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E. coli O157:H7 are: milk and milk derivatives such as cheeses, un-pasteurized cream; meat products such as hamburgers, undercooked beef patties, cooked meats, and canned salmon [1]. Foods with lower pH have also been implicated, such as apple juice. Indeed, inadequate thermal treatments of foods and lack of good manufacturing practices are associated with the outbreaks. However, the presence of antimicrobial compounds may constitute an additional barrier to the survival of pathogenic bacteria.

On this regard, the antimicrobial activity of phenolic compounds has been challenged against several bacteria, recognizing that Gram-positive are generally more sensitive to them. Among these compounds, the substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids present in foods from vegetable origin [2]. As an example, the antibacterial activity of Capsicum extracts against several pathogenic bacteria has been demonstrated by our working group [3]. In most cases, the extracts of Capsicum have been prepared using isopropanol and the active compounds using small quantities of ethanol, since most of the active compounds present poor solubility in water. Nevertheless, the presence of isopropanol in foods is not allowed, and an exhaustive evaporation of the solvent should be done following the extraction.

Considering the above the objective of this work was to improve the solubility of two natural antimicrobials, ferulic and coumaric acids, in water, and to test its antibacterial activity against two bacteria important in meat products.

2. Materials & Methods

The sodium salts of the phenolic acids were prepared mixing equimolecular amounts of the phenolic acid and sodium hydroxide. The solubility of ferulic and coumaric acid was successfully increased and a challenge test was performed with the salts. Two media were used for the challenge test: one was trypticase soy broth (TSB) and the other was a meat soup. This was prepared in such a way as to have 9% of meat solids, 1% of salt, and 90% of water. Several groups of beakers were prepared: the first froup (controls) contained 50 mL of soy broth; the second group besides the broth contained 0.2, 0.4, 0.6,0.8 and 1% ferulate; and the third group besides the broth contained 0.2,0.4,0.6, 0.8 and 1% coumarate. Each beaker was inoculated with 10^4 CFU/mL of *Escherichia coli* O157 and incubated at 37ºC for 24 and 48 hours. Afterwards, a bacterial count was performed using the technique of Miles and Mishra [4]. Additionally a challenge test was prepared with the meat soup using 1% ferulate and 1% coumarate and challenged as described above with the bacteria.

The ultra-structural analysis was made on *E. coli* O157:H7 treated with two different concentrations of coumarate, one with bactericidal effect (1%) and another with bacteriostatic effect (0.6%). Two millilitres of bacterial suspensions were grown overnight and added to 18 mL of TSB containing the corresponding concentration of coumarate, and then incubated at 37ºC for 24 h. Aliquots of 1.5 mL were taken every 2 h until a time of 10 h had elapsed, and then a final one after 24 h. Each sample was centrifuged at 6000 rpm for 5 min to be fixed with 2.5% glutaraldehyde (Electronic Microscopy Science-EMS; Washington, USA) in phosphate regulator solution (pH 7.3) for 1 h. It was washed three times with the regulator solution and then post fixed with 1% osmium tetraoxide (EMS; Washington, USA) in the same solution for 1 h. Afterwards, each sample was washed with ethanol-water solutions of increasing concentrations (40-90%) for 10 min and then 100% ethanol (three changes of 10 min each). The samples were mixed with propylene Epson-oxide resin (EMS; Washington, USA) 1:2, 1:1 and 3:1 for 2 h. Finally, they were included in 100% Epson resin with two changes of 2 h each, then identified and incubated at 60ºC for 24 h to achieve resin polymerization. The polymerized samples were cut with a Leica ultra- microtome model Ultracut UCT, contrasted with uranyl acetate and lead citrate, before they were examined with the electron transmission microscope Jeol model JEM 1010 at an acceleration voltage of 60KV, Akishima, Japan [5].

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