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Inhibitory disc diffusion assays of antioxidants on foodborne pathogenic bacteria

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ABSTRACT

Aims: To find the minimum inhibitory concentration (MIC) in an in vitro assay of the antioxidants ascorbic acid, naringenin and quercetin against the foodborne pathogens Escherichia coli serotype O157:H7 and Staphylococcus aureus and the opportunistic pathogen Pseudomonas aeruginosa.

Results: Inhibition zones were recorded using a broad range of concentrations from 10,000 to 1 microgram/ml on Escherichia coli serotype O157:H7, Staphylococcus aureus and Pseudomonas aeruginosa. The MIC for the antioxidants ascorbic acid and naringenin were 1 microgram/ml for P. aeruginosa and E. coli serotype O157:H7, respectively. The MIC for quercetin was 1 microgram/ml on E. coli serotype O157:H7 while high concentrations of the antioxidant were required to inhibit S. aureus (MIC of 5,000 micrograms/ml) and P. aeruginosa (MIC of 7,500 micrograms/ml).

Conclusions: Low concentrations (1 microgram/ml) of the antioxidants ascorbic acid and naringenin were effective on some of the evaluated bacteria (P. aeruginosa and E. coli serotype O157:H7, respectively) and therefore could be incorporated into foods. The antioxidant quercetin was effective against the pathogenic bacteria Escherichia coli serotype O157:H7 at low concentration (1 microgram/ml) while inhibition against S. aureus and P. aeruginosa required concentrations that if used as food additives would have a negative effect on the organoleptic properties of the foods. The assessment of the effective antioxidant concentration to eliminate these bacteria can be useful in developing value-added and safe food products.

Key words: Antioxidants, Minimum inhibitory concentration, Foodborne pathogenic bacteria

RESUMEN

Objetivos: Determinar la concentración mínima de inhibición (CMI) de los antioxidantes ácido ascórbico, naringenin y quercetin utilizando pruebas de difusión en agar contra los patógenos relacionados a alimentos Escherichia coli serotipo O157:H7 y Staphylococcus aureus y el patógeno oportunist Pseudomonas aeruginosa.

Resultados: Zonas de inhibición fueron determinadas utilizando un rango variado de concentraciones que incluyeron un máximo de 10,000 microgramos/ml a un mínimo de 1 microgramo/ml en Escherichia coli serotipo O157:H7, Staphylococcus aureus y Pseudomonas aeruginosa. La CMI de los antioxidantes ácido ascórbico y naringenin fueron 1 microgramo/ml para P. aeruginosa y E. coli serotipo O157:H7, respectivamente. La CMI para el antioxidante quercetin fue de 1 microgramo/ml para E. coli serotipo O157:H7, mientras que las altas concentraciones del antioxidante

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fueron requeridas para encontrar inhibición de S. aureus (CMI de 5,000 microgramos/ml) y de E. coli serotipo O157:H7 (CMI de 7,500 microgramos/ml).

Conclusiones: Bajas concentraciones (1 microgramo/ml) de los antioxidantes ácido ascórbico y naringenin fueron efectivos en P. aeruginosa y E. coli serotipo O157:H7, respectivamente. Consecuentemente éstos podrían ser incorporados en alimentos. El antioxidante quercetin demostró un efecto antimicrobiano contra el patógeno E. coli serotipo O157:H7 a concentraciones tan bajas como 1 microgramo/ml, mientras que inhibición contra los patógenos S. aureus y P. aeruginosa requirieron altas concentraciones que de ser incorporadas en alimentos tendrían un efecto negativo en las propiedades organolépticas. El estudio de las concentraciones efectivas de estos antioxidantes para eliminar bacterias patogénicas en alimentos es de gran importancia en el desarrollo de alimentos inocuos y de valor añadido.

**Palabras clave:** Antioxidantes, Concentración mínima de inhibición, Patógenos de alimentos

**INTRODUCTION**

Foodborne pathogenic and spoilage bacteria represent a mayor concern to the food industry. Current health issues such as foodborne illness appear to be increasing and are becoming a challenge for the food industry. Therefore, the control of these microorganisms in our food supply is of great importance. Several reports have shown the potential use of antioxidants, especially phenolic flavonoids compounds such as quercetin and naringenin as antimicrobial agents (Cushnie and Lamb, 2005; Martini et al., 2004; Veluri et al., 2004). Based on the literature, the minimum inhibitory concentration (MIC) of some of these aromatic antioxidants against Gram-positive bacteria appears to be only 2 to 8 micrograms/ml (Park et al., 2004). Other studies have shown a range of 25 to 50 micrograms/ml to be inhibitory on both Gram-positive and Gram-negative bacteria (Dastidar et al., 2001; Martini et al., 2004).

Our preliminary studies on quercetin and naringenin (data not published) have previously evaluated a range on 100 to 500 micrograms/ml of these antioxidants and shown an MIC of 250 to 500 micrograms of quercetin/ml for most Gram-positive and Gram-negative pathogens using disc inhibitory assays. On the other hand, an MIC of 100 micrograms/ml of the antioxidant naringenin was observed for Gram negative pathogens (data not published).

The study presented herein included a wider range of concentrations from 1-10,000 micrograms/ml including 0 (DMSO or sterile water control), 1, 5, 10, 15, 30, 45, 60, 75, 100, 250, 500, 5,000 and 10,000 micrograms of the antioxidants/ml on three microorganisms of importance to the food industry. These included the Gram negative pathogen Escherichia coli serotype O157:H7, the Gram positive pathogen Staphylococcus aureus and the opportunistic Gram negative pathogen Pseudomonas aeruginosa. Therefore, the main aim of this study was to find the minimum inhibitory concentration (MIC) of the antioxidants mentioned above on these bacteria using a disc diffusion inhibitory assay.
MATERIALS AND METHODS

**Antioxidants.** Ascorbic acid (vitamin C), quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) and naringenin (4', 5, 7-trihydroxy flavanone) were evaluated on this study. Sterile water was used as solvent for ascorbic acid and dimethyl sulfoxide (DMSO) was used as organic solvent for the antioxidants quercetin and naringenin.

**Bacterial Strains.** The foodborne pathogenic bacteria included *Escherichia coli* serotype O157:H7 and *Staphylococcus aureus*, as representatives of Gram negative and positive, respectively and the opportunistic pathogen *Pseudomonas aeruginosa* were kindly provided by the Biology Department, Microbiology Laboratories at our Institution.

**Growth media and conditions.** Bacteria were grown in tryptic soy broth (TSB) for 18-24 h at 35 ± 2° C until mid-log phase was reached. Tryptic soy agar (TSA) plates were streaked with the metabolically active culture to verify purity and phenotypical characteristics for each bacteria including Gram staining reaction, cell morphology, colony morphology and catalase reaction. The *Escherichia coli* serotype O157:H7 isolate was further confirmed using the RIM E. coli O157:H7 serological agglutination test (Remel Co., Lenexa, KS) prior to the assays.

**Determination of the Minimum Inhibitory Concentration (MIC).** The MIC was determined using a disc diffusion assay. All bacteria were grown in tryptic soy broth (TSB) for 18 to 24 h until mid-log phase was reached. Tryptic soy agar (TSA) plates were streaked with the metabolically active culture using a sterile swab to create an even lawn of bacterial growth. Sterile paper filter discs were aseptically dipped into the different ascorbic acid, quercetin and naringenin solutions and placed onto the inoculated plate. Plates were incubated for 18 to 24 h at 35 ± 2° C. After incubation, the zones of inhibitions (diameters) were recorded in mm. Controls including a disc dipped into the pure solvents (DMSO and sterile water) and one with a dry sterile disc were included. The sterility of the swabs and the agar media was also verified on all assays. All assays were performed in duplicate.

RESULTS AND DISCUSSION

Inhibition zones were recorded using a broad range of concentrations from 10,000 to 1 micrograms/ml of the antioxidants ascorbic acid, quercetin and naringenin on *Escherichia coli* serotype O157:H7, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC for the antioxidants ascorbic acid and naringenin were observed at low concentrations of 1 micrograms/ml for *P. aeruginosa* and *E. coli* serotype O157:H7, respectively while the pathogen *S. aureus* was not inhibited by neither ascorbic acid nor naringenin at any of the evaluated concentrations (data not shown). Furthermore, naringenin was also ineffective at the evaluated concentration range on the bacteria *P. aeruginosa*.

The assessment of the minimum inhibitory effect of antioxidant quercetin (Figure 1) on the other hand, was effective against the pathogenic bacteria *Escherichia coli* serotype O157:H7 at all concentration including a concentration as low as 1 microgram/ml while inhibition against *S. aureus* (MIC of 5,000 micrograms/ml) and *P. aeruginosa* (MIC of 7,500 micrograms/ml) required higher concentrations that if used as
food additives would have a negative effect on the organoleptic properties of the foods. The assessment of the effective antioxidant concentration to eliminate and control these bacteria in our foods can be useful in developing value-added and safe food products.

Figure 1. Inhibitory disc diffusion assay of quercetin on *Escherichia coli* serotype O157:H7, *Staphylococcus aureus* and *Pseudomonas aeruginosa* grown on Tryptic Soy Agar (TSA) for 18-24 h at 35 ± 2 °C

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LITERATURE CITED


