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Inhibition effects of carvacrol on biogenic amines formation by common food-borne pathogens in histidine decarboxylase broth

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ABSTRACT

The effect of carvacrol at different doses (0.1, 0.5 and 1 ml/100 ml) on ammonia (AMN) and biogenic amines (BAs) production by 8 food-borne pathogens (FBP), which are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Aeromonas hydrophila* and *Salmonella enterica* serovar Paratyphi A was investigated in histidine decarboxylase broth (HDB) using HPLC method. Significance differences were found in histamine (HIS), AMN and other BAs production ($P < 0.05$). The highest HIS production was obtained by *K. pneumoniae* (2.5 mg L^{-1}) and the lowest by *E. faecalis* (1.6 mg L^{-1}). Almost all other BAs such as PUT, CAD, SPD and PHEN were formed by pathogens. The highest concentration of dopamine (DOP) was given by *K. pneumoniae* which produced 1004.8 mg L^{-1} while agmatine (AGM) was 72.5 mg L^{-1} for *L. monocytogenes*. The most significant amounts of AMN were noticed for *E. coli* and *E. faecalis* with a value of 908.19 mg L^{-1} and 685.46 mg L^{-1} respectively. Consequently, the results of this current study shows that all FBPs tested are capable of decarboxylating more than one amino acid and the effect of carvacrol on AMN and BAs formation by FBPs depends on bacterial strains as well as on carvacrol doses.

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1. Introduction

Biogenic amines (BAs) are low-molecular weight organic bases which are found in a wide range of food and beverages such as dairy products, fish, meat, wine, beer, and fermented vegetables (Landete, de Las Rivas, Marcobal, & Munoz, 2007). The decarboxylation of free amino acids is the most common way of synthesis of BAs. Decarboxylases are enzymes (substrate-specific) produced by microorganisms present in the food and in most cases they are strain-specific rather than species-specific (Buňková et al., 2010; Landete et al., 2007). Amino acid decarboxylases are found in certain group of bacteria including *Enterobacteriaceae*, *Clostridium*, *Lactobacillus*, *Streptococcus*, *Micrococcus*, and *Pseudomonas* (Shalaby, 1996).

Amines along with ammonium (AMN) detection have been used as food quality indicators since they are generated by microbial activity. Thus, their presence is a sign of spoiled foodstuff (Gram & Dalgaard, 2002; Gram et al., 2002; Özogul, Kuley, & Kenar, 2011). The main BAs encountered in foods and drinks include histamine, tyramine, putrescine, cadaverine, tryptamine, agmatine, spermine and spermidine (Silla Santos, 1996; Visciano, Schirone, Tofalo, & Suzzi, 2012). There are variable factors that have limiting impacts on the BAs formation such as availability of free amino acids (substrate), pH, and temperature. Due to their psychoactive and vasoactive properties, consumption of foods containing high amounts of BAs (especially tyramine and histamine) can cause food poisoning and various pharmacological reactions (Moret, Smela, Populin, & Conte, 2005).

Once BAs are formed, it is difficult to eliminate them by high temperature treatment since they are reported to be heat stable compounds (Cardozo, de Souza, Lima, & Lima, 2011). BAs formation should be prevented by strict use of good hygiene to avoid the microbial contamination in both raw and processed food (Valsamaki, Michaelidou, & Polychroniadou, 2000). Therefore, different antimicrobial components have been used in food products to reduce or eliminate health hazard of BAs and ammonia formation by foodborne pathogens.

Many people in the world suffer from food-borne diseases due to food spoilage by microorganisms. Therefore, there is an increasing interest in antimicrobial compounds especially plant-

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derived (Burt, 2004). In this context, essential oils from aromatic plants have been used as favoring as well as natural food preservatives. The antimicrobial activities of several essential oils have been linked to the presence of phenolic compounds such as thymol, eugenol and carvacrol (Ben Arfa, Combes, Preziosi-Belloy, Gontard, & Chalier, 2006). Carvacrol, one of the major components of oregano (12%–70%) and thyme oils (9%–60%) has been studied extensively *in vitro* and *in vivo* (De Vincenzi, Stammati, De Vincenzi, & Silano, 2004; Ultee, Bennik, & Moezelaar, 2002). Its antimicrobial effectiveness against most of Gram-positive and Gram-negative bacteria (Veldhuizen, Tjeerdsma-van Bokhoven, Zweijtzer, Burt, & Haagsman, 2006) including *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Vibrio vulnificus*, *Staphylocoocus aureus,* and *Bacillus cereus* has been proved (Cosentino et al. 1999; Kim, Marshall, & Wei, 1995; Lambert, Skandamis, Coote, & Nychas, 2001). Besides its wide spectrum antibacterial activity, carvacrol possesses other important biological properties namely antifungal, anti-inflammatory, antioxidant, insecticidal, antiparasitic, hepatoprotective and antitumoral activities (Kordali et al., 2008; Manohar et al., 2001; Nostro et al., 2007). The antibacterial mechanism of carvacrol against bacteria consists in targeting the cytoplasmic membrane (Xu, Zhou, Ji, Pei, & Xu, 2008). Due to its hydrophobic character, it is able to partition lipids and disrupt structure of microorganisms' membranes. Thereby, this phenolic compound alters the membranes permeability leading to leakage of cell contents and ions (phosphate and potassium). Furthermore, a decrease in the pH gradient across the cytoplasmic membranes and a collapse of the membranes potential are noticed followed finally by the death of the bacterial cells in case of massive loss of critical molecules (Burt, 2004; Ultee et al., 2002).

Until now, most of the studies have focused on the antimicrobial activity of carvacrol on Gram-positive and Gram-negative bacteria *in vitro* and *in vivo*. However, to our best knowledge there are no informations about the effect of carvacrol on histamine, AMN and BAs production by FBPs in *vitro*. Therefore, the purpose of this study was to investigate the antibacterial efficacy of different concentrations of carvacrol (0.1, 0.5 and 1 ml/100 ml) on HIS, AMN and other BAs production by eight common foodborne pathogens in histidine decarboxylase broth (HDB). The obtained results may be used for the development of novel natural substances in food preservation in order to reduce formation of BAs by FBPs.

2. Material and methods

2.1. Bacterial strains

The selected 8 FBPs were *S. aureus* (ATCC29213), *E. coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Enterococcus faecalis* (ATCC29212), *Pseudomonas aeruginosa* (ATCC27853), *L. monocytogenes* (ATCC7677) which were purchased from American Type Culture Collection (Rockville, MD, USA). *Aeromonas hydrophila* (NCIMB1135) and *Salmonella* Parathypi A (NCTC13) were obtained from National Collections of Industrial Food and Marine Bacteria (Aberdeen, UK) and National Collection of Type Cultures (London, UK), respectively.

2.2. Culture media and bacterial extraction

The production of ammonia and BAs by all FBPs strains used in this work were monitored using histidine decarboxylase broth (HDB) proposed by Klausen and Huss (1987). Nutrient broth was used for propagation of FBPs cultures and growing temperature was 37 °C for all FBP strains. They were incubated according to this growth temperature for 2 or 3 days after which 0.5 ml of these bacterial cultures was removed and put into the HDB to allow them to decarboxylate histidine for 24 h. After that carvacrol, at doses of 0.1 ml/100 ml, 0.5 ml/100 ml and 1 ml/100 ml were also added into the HDB for treatment groups. Actual concentrations of carvacrol per doses were calculated as 97.6 mg/100 ml, 448 mg/100 ml and 976 mg/100 ml, respectively. For the extraction of FBPs, 5 ml of the HDB containing FBP strains were removed to separate bottles and then 2 ml trichloroacetic acid was added. They were centrifuged at 3000 × g for 10 min and then filtered through a filter paper (Milipore). After that, 4 ml of bacterial supernatant were taken for derivatisation from each FBP strains in order to analyze BAs using HPLC.

2.3. Chemical reagents

Carvacrol and all biogenic amine standards were purchased from Sigma–Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and HPLC grade water for amine analyses.

2.4. Biogenic amine analysis

Preparation of standard amine solution and derivatisation of biogenic amines were made according to method of Özogul (2004) and measured in mg amines per liter broth. The confirmation of BAs production was accomplished using a rapid HPLC method with a reversed phase column by using a gradient elution program. Ammonia and TMA separation were also achieved using same injection of BAs analysis.

The detected compounds were ammonia (AMN), putrescine (PUT), cadaverine (CAD), histamine (HIS), spermidine (SPD), tryptamine (TRPT), 2-Phenyl-ethylamine (PHEN), spermine (SPN), serotonin (SER), tyramine (TYR), trimethylamine (TMA), dopamine (DOP), agmatine (AGM).

2.5. HPLC apparatus and method

For the BAs and AMN analyses a Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with an SPD-M20A diode array detector and two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC), a communication bus module (CBM-20A) with valve unit FCV-11AL and an ODS Hypersil column, 5 μ, 250 × 4.6 mm (Phenomenex, Macclesfield, Cheshire, U.K.) was used.

Chromatographic separation was performed by continuous gradient elution, with acetonitrile (eluant A) and HPLC grade water (eluant B), during 25 min to permit total separation. The gradient passed gradually from 40% to 60% acetonitrile in 20 min. A volume of 10 ml was injected and detection was monitored at 254 nm. The gradient elution program used in this study was satisfactory since a linear relationship between amine concentration and detector response was observed.

2.6. Statistical analysis

The mean value and standard deviation were calculated using the data obtained from the four samples for each treatment. One way ANOVA was used to determine the significance of differences at $P < 0.05$. All statistics were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL. USA). The results were labeled using a, b, c, d letters, where results with different letter differ significantly from each other ($\alpha < 0.05$).

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