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The impact of natural clinoptilolite on ammonia, cadaverine and other polyamine formation by food-borne pathogen in lysine decarboxylase broth

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ABSTRACT

The influence of natural clinoptilolite (CLIN) on ammonia (AMN), cadaverine (CAD) and other polyamines (PAs) production by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Aeromonas hydrophila* and *Salmonella* Paratyphi A was investigated in lysine decarboxylase broth (LDB). Significant differences were found in AMN and PAs production ($P < 0.05$) among the groups. *E. coli* yielded the highest amounts of AMN (240.24 mg/L), CAD (278.24 mg/L) and dopamine (DOP) (143.52 mg/L). *S. aureus* also produced substantial amounts of AMN (222, 99 mg/L), CAD (124.75 mg/L) and DOP (207.29 mg/L). The addition of different doses of zeolite (1% and 5%) was very effective on CAD and AMN amounts produced by *E. coli*. However in some cases the presence of CLIN increased AMN and PAs concentrations. For instance *L. monocytogenes* that produced 111.31 mg/L of AMN for the control group donated 192.40 mg/L at 1% zeolite. Thus, it can be concluded that the results of the present research show that all food-borne pathogens (FBP) tested are capable of decarboxylating more than one amino acid and the inhibition effect of zeolite on AMN and PAs production by pathogens depends on the zeolite doses and the bacterial strains. The obtained results could be used in the food sectors to prevent undesirable compounds production (AMN and amines) by pathogenic bacteria which constitute a risk to the consumers' health and can cause several food-borne diseases.

1. Introduction

Biogenic amines (BAs) are low molecular weight organic basis. They exist in a large variety of food and beverages (dairy products, meat, fish, wine, and fermented vegetables) (Landete, de Las Rivas, Marcobal, & Munoz, 2007; Spano et al., 2010). The common way of BAs synthesis is the decarboxylation of free amino acids by decarboxylases. These components are enzymes (substrate-specific) produced by microorganisms that are strain-specific rather than bacterial species (Buňková et al., 2010; Landete et al., 2007). Many Gram positive and Gram negative bacteria such as *Enterobacteriaceae*, *Clostridium*, *Lactobacillus*, *Streptococcus*, *Micrococcus*, and *Pseudomonas* are able to produce BAs through amino acid decarboxylation activity (Linares et al., 2012; Shalaby, 1996).

The detection of amines and ammonium (AMN) in foodstuff has been used as food quality indicator since their presence is a sign of spoilage (Gram & Dalgaard, 2002; Gram et al., 2002; Özogul, Kuley, & Kenar, 2011). Histamine (HIS), tyramine (TYR), putrescine (PUT), cadaverine (CAD), tryptamine (TRPT), agmatine (AGM), spermine (SPM) and spermidine (SPMD) are the principle BAs found in foods and beverages (Silla Santos, 1996, Visciano, Schirone, Tofalo, & Suzzi, 2012). Different factors are found to have limiting effects on the BAs formation such as availability of free amino acids (substrate), pH, and temperature. Consumption of food containing high amounts of BAs especially tyramine and histamine can cause various toxicological reactions (Moret, Smela, Populin, & Conte, 2005; Wunderlichovà, Bunkovà, Koutny, Jancovà, & Bunka, 2014) which lead to different types of foodborne diseases including histamine poisoning (scombroid poisoning) and tyramine toxicity (cheese crisis) (Shalaby, 1996). Histamine is known to cause headaches, low blood pressure, heart palpitations, edema,

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vomiting, and diarrhea (Maintz & Novak, 2007; Hungerford, 2010). While tyramine can engender hypertension, dilate the pupils and the palpebral tissue, cause lacrimation and salivation, increase respiration and escalates the blood sugar (Abreu Gloria, 2005). The diamines like putrescine and cadaverine, although not toxic themselves, (Landete et al., 2007) aggravate the adverse effects of histamine and tyramine. Diamines compete for some of the mechanisms involved in the detoxification of BAs by inhibiting their metabolizing enzymes (Košmerl, Šućur, & Prosen, 2013; Straub, Kicherer, Schilcher, & Hammes, 1995).

BAs are heat-stable, after their formation it is difficult to remove them by treatment of high temperature. Therefore, it is better to avoid BAs formation by strict use of good hygiene in raw and processed food (Valsamaki, Michaelidou, & Polychroniadou, 2000). Nowadays different antimicrobial compounds have been used in food products to reduce foodborne pathogens that yield BAs and ammonia which constitute a health hazard.

There is an increasing interest in natural antimicrobial compounds since each year many people in the world suffer from foodborne diseases related to microbial deterioration of foodstuff (Burt, 2004). Zeolites are natural (CLIN, erionite, chabazite, heulandite, mordenite, stilbit, and philipsite) or synthetic minerals (zeolites A, X, Y, and ZMS-5). They are hydrated microporous crystals with well-defined structures (Pavelić et al., 2001). There are more than 50 zeolites found in nature and more than 150 that have been synthesized (Ming & Allen, 1999). The Greek words of "Zeolite" means "boiling stones since it seems that they have the ability to froth under fast heating conditions" (Inglezakis & Loizidou, 2012; Polat, Karaca, Demir, & Naci-Onus, 2004). CLIN ((Na_4K_4) (Al_8Si_{40}) $O_{96}.24H_2O$) is an abundant natural zeolite that exist in igneous, sedimentary, and metamorphic deposits (Kithome, Paul, Lavkulich, & Bomke, 1998).

Zeolites are flavourless, odourless, and harmless minerals. They possess unique chemicals and physical characteristics such as cation exchange properties, easiness to hydration and dehydration and stability of their crystal framework (Ulmanu & Anger, 2012). Besides they have antimicrobial effects against several bacteria including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, and *Pseudomonas aeruginosa* (Demirci, Ustaoğlu, Yılmazer, Sahin, & Baç, 2014; Kawahara, Tsuruda, Morishita, & Uchida, 2000).

Due to their large geographic distribution, their discovery in wide deposits and their low cost, natural zeolites gained a significant role over the synthetic ones (Perić, Trgo, & Vukojević Medvidović, 2004). CLIN has been used in a variety of industrial applications ranging from soil amendment (Kithome et al., 1998), through environmental improvements especially in wastewater treatments (Barakat, 2011; Kesraoui-Ouki, Cheeseman, & Perry, 1994) and dyes removal (Meshko, Markovska, Mincheva, & Rodrigues, 2001; Wang & Peng, 2010) to food sector in which it has been used as odour control and as food supplements for the body detoxification (Tzia & Zorpas, 2012). Moreover this natural mineral has been investigated for medical utilization where antidiarrheic drugs have been developed and its anticancer effects have been proved (Kahler, 2014; Pavelić et al., 2001; Rodriguez-Fuentes, Barrios, Iraizoz, Perdomo, & Cedre, 1997; Tomečková et al., 2012).

Until now, most of the studies have been related to the effect of lactic acid bacteria and phenolic compounds such as carvacrol on BAs and AMN production by FBP in decarboxylase broth. However, to our best knowledge there are little information about the action of the natural zeolite (CLIN) on BAs and AMN production by bacteria *in vitro*. Only one research paper has looked into this matter using the decarboxylation of amino acids by FBP in histidine decarboxylase broth (Gokdogan et al., 2012). The objective of this study was to test the influence of different concentrations of zeolites (1% and 5%) on CAD, AMN and other PAs production by eight common foodborne pathogens in lysine decarboxylase broth (LDB). The effectiveness of CLIN as natural antimicrobial in reducing the spoilage compounds (AMN and PAs) production by pathogens was investigated.

2. Materials and methods

2.1. Bacterial strains

The selected 8 FBP were *S. aureus* (ATCC29213), *E. coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Enterococcus faecalis* (ATCC29212), *P. aeruginosa* (ATCC27853), *Listeria monocytogenes* (ATCC7677) which were purchased from American Type Culture Collection (Rockville, MD, USA) and *Aeromonas hydrophila* (NCIMB1135) and *Salmonella paratyphi* A (NCTC13) which 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 cadaverine by all FBP strains used in this work was monitored using LDB proposed by Klausen and Huss (1987). 1 g peptone, 0.5 g Lab-Lemco powder, 2.5 NaCl, 4.01 g L-histidine HCl and 2.5 mg pyridoxal HCl in 500 ml distilled water and the pH were adjusted according to their optimum growth pH with 1 M NaOH or 0.1 M HCl. The LDB was pipetted in 10 ml bottles and then autoclaved at 121 °C in 15 min prior to use.

Nutrient broth was used for propagation of FBP cultures and growing temperature was 37 °C for all FBP strains. FBP strains were incubated according to their optimum growth temperature for 2 or 3 days after that 0.5 ml of these bacterial cultures was removed and put into the LDB to allow them to decarboxylate lysine after 24 h. Then 1% and 5% zeolite (clinoptilolite) doses were also added into the LDB for treatment groups.

For the extraction of the FBP strains, 5 ml of the LDB 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). Then, 4 ml of bacterial supernatant were taken for derivatisation from each FBP strains in order to identify AMN and BAs concentrations by HPLC analysis.

2.3. Chemical reagents

L-lysine monohydrochloride (H8125) and all BAs standards were purchased from Sigma–Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and HPLC grade water for amine analyses.

2.4. Preparation of standard amine solution

Histamine dihydrochloride (165.7 mg), tyramine hydrochloride (126.7 mg), typtamine hydrochloride (122.8 mg), putrescine dihydrochloride (182.9 mg), 2-phenylethylamine hydrochloride (130.1 mg), cadaverine dihydrochloride (171.4 mg), spermidine trihydrochloride (175.3 mg), spermine tetrahydrochloride (172.0 mg), 5-hydroxytryptamine (serotonin) (133.9 mg), 3-hydroxytyramine hydrochloride (dopamine) (123.8 mg), agmatine sulphate (175.4 mg), trimethylamine hydrochloride (161.7 mg) and ammonium chloride (296.9 mg) were dissolved in 10 ml HPLC grade water. The final concentration of free base for each amine was 10 mg/ml solution.