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Control of *Salmonella enterica* and *Listeria monocytogenes* in hummus using allyl isothiocyanate

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ABSTRACT

Hummus (chickpea dip) is a ready-to-eat product which has been implicated in several foodborne outbreaks and food recalls. This study aimed to screen the antimicrobial activity of allyl isothiocyanate (AITC) against 5 strains of each of *Salmonella enterica* and *Listeria monocytogenes* using a disc diffusion method. Additionally, the antimicrobial activity of 0.1–1.5% (v/w) AITC against both pathogens and aerobic bacteria in hummus was also investigated. The inhibition zones of AITC were 8.5–15 and 7.0–8.5 mm against the *S. enterica* and *L. monocytogenes* strains, respectively, at 37 °C. *S. enterica* numbers were reduced by > 6 $\log_{10}$ CFU/g in hummus containing ≥0.5% AITC by 3 days at both 4 and 10 °C. While 0.1–0.25% AITC reduced *S. enterica* by 2.5–5.1 $\log_{10}$ CFU/g at 4 °C or by 4.7–6.0 $\log_{10}$ CFU/g at 10 °C by 10 days. Similarly, *L. monocytogenes* numbers decreased by > 6 $\log_{10}$ CFU/g in hummus with ≥0.5% or ≥1.0% AITC at 4 or 10 °C, respectively, by 3 days. Further, 0.25% AITC significantly reduced *L. monocytogenes* in hummus by 2.7 and 4.3 $\log_{10}$ CFU/g at 4 and 10 °C, respectively. Moreover, 0.1% AITC reduced *L. monocytogenes* by 1.8 $\log_{10}$ CFU/g in hummus at 10 °C and inhibited the growth at 4 °C for up to 10 days. The aerobic bacterial count also significantly decreased in uninoculated hummus treated with 1.0–1.5% AITC at both 4 and 10 °C, while a concentration of 0.25–0.5% AITC inhibited their growth at 4 °C. AITC can be used to reduce the risk of salmonellosis or listeriosis in hummus and extend its shelf-life.

1. Introduction

Chickpeas are an important pulse crop, also known as the species *Cicer arietinum* L. and are a member of the Leguminosae family (Wallace et al., 2016). Chickpea consumption in the world is represented mainly by the consumption of hummus (chickpea dip) which is made from cooked, mashed chickpeas blended with tahini, lemon juice, olive oil and spices (Yamani and Mehyar, 2011). Hummus is one of the most popular traditional foods in the Middle East countries and it contains a high amount of moisture (71.0%), 14.3% carbohydrates, 7.9% protein, 9.6% fat, 1.7% ash and 6% fiber (Amr and Yaseen, 1994; Wallace et al., 2016). Hummus also provides the consumer with significant amounts of B vitamins and minerals including sodium, potassium, phosphorus, magnesium, calcium, selenium, iron and manganese. Therefore, it is considered that hummus can be a component in a healthy Mediterranean-style diet as well as being a desirable item in the 2015–2020 Dietary Guidelines for Americans. These features explain the significant increase in consumption of hummus in western countries in the last decade (Wallace et al., 2016).

Hummus is a ready-to-eat product consumed directly without further processing or additive treatments to achieve safety (Olaimat et al., 2017). However, foodborne pathogens such as *Salmonella* spp., *Listeria monocytogenes*, *Shigella* spp. and *Escherichia coli* have been isolated from hummus (Almualla et al., 2010; Khiyami et al., 2011; Varma et al., 2007). In the US in 2015, 30,000 containers of hummus were recalled due to contamination with *L. monocytogenes* (FDA, 2015). Further, the largest reported outbreak in the US, which involved 802 illnesses in 2007 was caused by hummus contaminated with *Salmonella* (CDC, 2010). A subsequent *Salmonella* outbreak in the US involving 16 cases was also associated with hummus (CDC, 2013).

Recently, the consumer demand for natural antimicrobials to replace artificial preservatives has increased. Few studies have investigated the effect of natural antimicrobials against foodborne pathogens in hummus (Al-Holy et al., 2006; Alali et al., 2012; Olaimat

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et al., 2017). Sinigrin is a glucosinolate present in Brussels sprouts, broccoli, Oriental mustard, and other cruciferous plants. When these plants are exposed to damage, the enzyme myrosinase (thioglucohydrolase, (EC3.2.1.147) is released to hydrolyze sinigrin and form allyl isothiocyanate (AITC) which is a natural, colorless and volatile compound with anticancer activity (Fahey et al., 2001). AITC is currently used as a food preservative in Japan, and as a GRAS flavoring agent in the US (Kim et al., 2002). In other work, AITC has been shown to have antimicrobial activity against spoilage bacteria and foodborne pathogens in a variety of food products (Olaimat et al., 2014a; Olaimat and Holley, 2015; Olaimat and Holley, 2016). However, no information is available on the inhibitory effect of AITC against *S. enterica* and *L. monocytogenes* in hummus. The objectives of this study were to screen the antimicrobial activity of AITC against 5 strains of each of *S. enterica* and *L. monocytogenes* using a disc diffusion method and different concentrations of AITC to control *S. enterica*, *L. monocytogenes* and aerobic bacteria in hummus.

2. Materials and methods

2.1. Preparation of hummus

Fresh hummus was prepared before the beginning of each trial. The preparation was done under aseptic conditions according to a recipe provided by a popular restaurant in Amman, Jordan as described by Olaimat et al. (2017). Briefly, one kg of sorted and cleaned dry chickpeas was soaked overnight in 2 l of potable tap water. The soaked chickpeas were strained and boiled for about 2 h in tap water containing 0.5% w/v sodium bicarbonate to soften the beans. The boiled chickpeas were strained and cooled in a refrigerator for approximately 1 h. Then boiled chickpeas were whipped in a high speed blender. Thereafter, 500 g of tahini was added to chickpea paste along with 250 ml of cooled water. All ingredients were mixed and blended again.

2.2. Bacterial strains

Five different strains of each pathogen used in the current study were obtained from the culture collection of the Food and Human Nutritional Sciences Department, University of Manitoba. The *L. monocytogenes* strains were 2–138, 2–243, GLM-1, GLM-3, and GLM-5, while the *S. enterica* serovars were *S.* Heidelberg 271, *S.* Typhimurium 02:8423, *S.* Copenhagen PT 99, *S.* Enteritidis CRIFS 1016, and *S.* Kentucky 64701. Bacteria were either food or human clinical isolates that have been used in previous work (Olaimat and Holley, 2013; Olaimat et al., 2014a, 2014b).

2.3. Culture preparation

All bacterial strains were stored individually at −80 °C in Brain Heart Infusion (BHI) broth (Oxoid Ltd., Basingstoke, England) with 25% glycerol. A loopful of each strain was transferred into 10 ml BHI broth which was incubated at 37 °C for 24 h. Thereafter, a loopful from each strain of *L. monocytogenes* or *S. enterica* was streaked, respectively, on *Listeria* selective agar base with *Listeria* selective supplement (LSA, Oxoid Ltd.) or on *Salmonella Shigella* (SS) agar (Oxoid Ltd.), and incubated at 37 °C for 24 h. A single colony of *L. monocytogenes* or *S. enterica* from the selective agars was transferred to BHI broth and incubated overnight at 37 °C. Then 0.1 % (v/v) of the culture was transferred individually to fresh BHI broth and incubated overnight at 37 °C. A cocktail culture of each pathogen was prepared by adding 2 ml of each strain to a sterile test tube.

2.4. Screening of antimicrobial activity of AITC

The inhibitory effect of AITC against *S. enterica* and *L. monocytogenes* strains was determined using a disc diffusion method (Klančnik et al., 2010). Briefly, aliquots of 20 µl of AITC (Combi-Blocks, San Diego, USA) were dispensed onto 6 mm-diameter sterile discs (Oxoid) with air drying at room temperature for 10 min. Each disc was placed on tryptic soy agar (TSA, Oxoid) plates that had been previously inoculated with 100 µl of approximately 6.0 $\log_{10}$ CFU/ml of each of the bacterial strains and incubated at 37 °C for 24 h. The inhibition zone was measured in mm using a caliper.

Table 1
Antimicrobial activity of AITC (20 µl/disc) against 5 serovars of *Salmonella* and 5 strains of *L. monocytogenes* using disc diffusion at 37 °C for 24 h.

Pathogen	Inhibition zone (mm)
S. Copenhagen PT 99	15.0 ± 2.2[a]
S. Typhimurium 02:8423	9.0 ± 1.4[bc]
S. Enteritidis CRIFS 1016	9.8 ± 1.0[bc]
S. Kentucky 64,701	10.3 ± 1.3[b]
S. Heidelberg 271	8.5 ± 1.9[bc]
L. monocytogenes GLM-1	8.3 ± 0.5[bc]
L. monocytogenes GLM-3	7.2 ± 0.5[c]
L. monocytogenes GLM-5	7.8 ± 0.5[bc]
L. monocytogenes 2–138	8.5 ± 1.0[bc]
L. monocytogenes 2–243	7.0 ± 0.0[c]

Means in the column with the same lowercase letters are not significantly different ($p > 0.05$).

2.5. The antimicrobial activity of AITC against S. enterica and L. monocytogenes in hummus

AITC at different concentrations was added directly and mixed gently to tahini (tahini: dry chickpea: 1:2) and then the mixture was added to the hummus as described in Section 2.1. The hummus was inoculated with a cocktail mixture of *S. enterica* or *L. monocytogenes* strains to obtain approximately 7 $\log_{10}$ CFU/ml. Then hummus containing 0.0 (control), 0.1, 0.25, 0.5, 1.0 or 1.5% (v/w) AITC was taken and subdivided into 50 g portions in 100 ml bottles, which were incubated at 10 or 4 °C for 10 days.

2.6. Bacterial enumeration

About 5.0 g of inoculated hummus was taken from each treatment using a sterile spoon and diluted with 45 ml of 0.1% peptone water for bacterial enumeration. Hummus was sampled at 0, 1, 4, 7, and 10 days of storage and the samples were analyzed for the presence of *S. enterica*, *L. monocytogenes* and aerobic bacterial survivors. The samples were homogenized in sterile stomacher bags for 1 min by means of a stomacher (Stomacher, Easy Mix, AES Laboratories, Combourg, France). The pummeled samples were 10-fold serially diluted and then 100 µl of each sample was spread-plated in duplicate onto the surface of plate count agar (PCA, Oxoid) for aerobic bacterial count, SS agar for *S. enterica* or LSA agar for *L. monocytogenes*. Plates were incubated aerobically for 24 to 48 h at 37 °C. Colonies typical of *S. enterica* and *L. monocytogenes* were enumerated. When bacterial cells were not detectable by spread plating onto selective media, an additional enrichment step was undertaken. Basically, 50 ml of double strength BHI broth was added to the homogenized hummus samples, incubated at 37 °C for 16–24 h, and plated on a selective agar and examined for the presence of *L. monocytogenes* or *S. enterica*.

2.7. Sensory evaluation

Sensory evaluation was conducted for the AITC treated (0.0 (control), 0.1, 0.25, 0.5, and 1.0%) hummus. Ten sensory judges (five males and five females) ages between 22 and 50 years were trained on descriptive analysis of hummus properties (Meilgaard et al., 2006). The panelists were consumers of hummus and were trained for 2 h (1-h