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Impact of volatile allyl isothiocyanate on fresh produce

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ABSTRACT

The applicability of allyl isothiocyanate (AITC) for the preservation of mung bean sprouts as well as fresh-cut iceberg lettuce was investigated. For this purpose, produce samples were stored in high gas barrier plastic bags containing a filter paper soaked with AITC (10 µl). Microbial loads as well as color changes and respiration of both products were monitored over 9 days of storage at 5 °C. The results showed that AITC (0.81–1.41 µg/ml) exhibits strong antimicrobial properties via the gas phase against the native microflora of iceberg lettuce and mung bean sprouts. However, the quality of both tested products was significantly affected, which limits the applicability of AITC for such sensitive produce commodities. The respiration activity of mung bean sprouts and fresh-cut salad was decreased by AITC while discolorations as well as changes to the texture were evident even at low concentrations (0.11–0.16 µg/ml), which did not provide antimicrobial effects.

1. Introduction

Within the last years, significant research has been focused on seeking new strategies to preserve fresh produce, especially industrially processed and packed fruits and vegetables (Meireles, Giaouris, & Simões, 2016). This commodity represents particularly perishable foods since cutting and peeling remove the protecting shell, increase cross contaminations and expose the tissue, which usually causes accelerated deterioration due to microbial growth, tissue softening or water loss. Especially sprouts are known to be very vulnerable to microbial spoilage since conditions during germination (high humidity and temperatures) usually lead to high microbial loads (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; BfR, 2011). Allyl isothiocyanate (AITC) is a naturally occurring compound with a strong typical flavor, which is well-known from mustard, horseradish or wasabi. Isothiocyanates occur in plants belonging to the family of cruciferae where they derive from glucosinolates, a group of glucosides stored in the cell vacuoles (Delaquis & Mazza, 1995; Sekiyama, Mizukami, Takada, & Numata, 1994). Their antimicrobial activity has been known for long, which is why they have been used as biocides in agriculture and forestry or also as natural antimicrobials in food applications in Japan (Lin, Kim, Du, & Wei, 2000; Sekiyama et al., 1994). However, the broad application of AITC for food preservation is still limited although many studies have proven its efficiency to inactivate food pathogens or spoilage microorganisms (Dufour, Stahl, & Baysse, 2015), especially yeast and molds but also bacteria (Pires et al., 2009). AITC has been generally recognized as safe by the Food and Drug Administration (Delaquis & Mazza, 1995; Otoni, Soares, Silva, Medeiros, & Baffa Junior, 2014) and it has been shown not to be carcinogenic in mice (EFSA, 2010). AITC has been tested as volatile preservative for a bunch of different foods like cottage cheese (Gonçalves, Junqueira, Dos Santos Pires, Soares, & Araújo, 2009), mozzarella cheese (Pires et al., 2009), spinach leaves (Seo et al., 2012), peanuts (Otoni et al., 2014) or minimally processed shredded cabbage (Banerjee, Penna, & Variyar, 2015) with very promising results. However, reports about the impact of AITC on quality attributes of food are rare and the effect of AITC on very sensitive products like fresh cut salad or mung bean sprouts has not been elucidated so far. The purpose of this study was therefore to assess the suitability of volatile AITC regarding the inhibition or inactivation of naturally occurring microorganisms on fresh cut iceberg lettuce and mung bean sprouts as well as its impact on food quality.

2. Material and methods

2.1. Product preparation and setup of storage trials

Whole Iceberg lettuce heads as well as fresh mung bean sprouts were obtained from local retail and used immediately for storage trials. Salad heads were trimmed, cut and washed in a box with 3 l of sterile potable water in order to homogeneously distribute the microorganisms on the whole product surface. Sprouts were washed similarly. About 20 ± 1 g of each product were placed in bags (10 × 15 cm) made of a sealable multilayer film (PET, 12 µm thickness; PA/EVOH/PP, 50 µm thickness) with an oxygen permeability of < 2 $cm^3/(m^2$ d bar) and a

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carbon dioxide permeability of < 5 $cm^3/(m^2\ d\ bar)$ (Südpack). This film with high gas barrier was chosen in order to prevent permeation of AITC through the packaging. Cellulose filter papers (diameter: 6 mm) soaked with 10 µl of undiluted or 1:10 diluted (Ethanol) AITC (Sigma Aldrich) were added inside the bags which were finally sealed manually with a hand sealing device. Reference samples without AITC were included for both products. Three independent replicate samples for each sampling day were stored at 5 °C in the dark. After 1, 3, 6 and 9 days, samples were taken and the following parameters were assessed.

2.2. Color measurements

L*, a*, and b* values were recorded with a DigiEye digital color imaging system (VerVide). The product color was determined inside the bags from both sides respectively and mean values as well as standard deviations were calculated.

2.3. Quantification of oxygen and carbon dioxide in the packaging

The respiration activity of iceberg salad and mung bean sprouts was monitored by periodically analyzing the headspace gas concentration of O_2 and CO_2 inside the bags. O_2 and CO_2 percentages in the headspace were determined with a gas analyzer CheckMate3 (Dansensor). Mean values as well as standard deviations were calculated.

2.4. Quantification of microbial populations

The colony count on the surface of iceberg salad and mung bean sprouts was determined by the plate count method whereas different microbial populations were discriminated. About 10 g of the samples were transferred into sterile stomacher bags (Seward) before 90 ml of sterile ringer solution (Oxoid) supplemented with 0.1% Tween 80 was added. The samples were subsequently homogenized in a Stomacher 400 circulator (Seward) for 1 min at 260 rpm to detach microorganisms from the product surface. Serial dilutions of the sample suspensions were made with sterile ringer solution and 1 ml of each dilution was plated by the pour-plating method in triplicate. For the determination of the total mesophilic aerobic cell counts, tryptic soy agar (TSA) (Oxoid) was used and the plates were incubated at 30 °C for 72 h. *Pseudomonas* spp. was quantified by use of CFC Pseudomonas Selective Agar (Merck) and plates were incubated for 48 h at 30 °C. Enterobacteria were determined with VRBD Agar (Merck) and plates were incubated at 37 °C for 24 h. An agar overlay was used in this case. Lactic acid bacteria were determined with MRS Agar (Merck) and plates were incubated at 30 °C for 5 days under anaerobic conditions. Yeasts and moulds were quantified by use of Rose Bengal Chloramphenicol Agar (Oxoid) and plates were incubated at 25 °C for 5 days. After the respective incubation period, the colony count was determined manually and the number of colony forming units (cfu) per g of product was calculated as mean values with standard deviations.

2.5. Determination of AITC in the headspace of the packaging

The concentration of AITC in the headspace of the bags was determined during storage at days 3, 6 and 9 by headspace gas chromatography-mass spectrometry (HS/GC–MS). Calibration curves (five concentrations) were included in every measurement by adding 5 µl of AITC dilution series in water to headspace vials of 22 ml. Samples of 1 ml were taken from the packaging headspace with a syringe and injected into 22 ml headspace vials respectively. Tempering of standards and samples was done in a TurboMatrix HS-40 Autosampler (Perkin Elmer) for 30 min at 60 °C before quantification on a QP 2010 Ultra (Shimadzu) using an Optima-WAX separation column (J&W Scientific). Concentrations are given as µg AITC per ml of headspace volume (mean values from three samples).

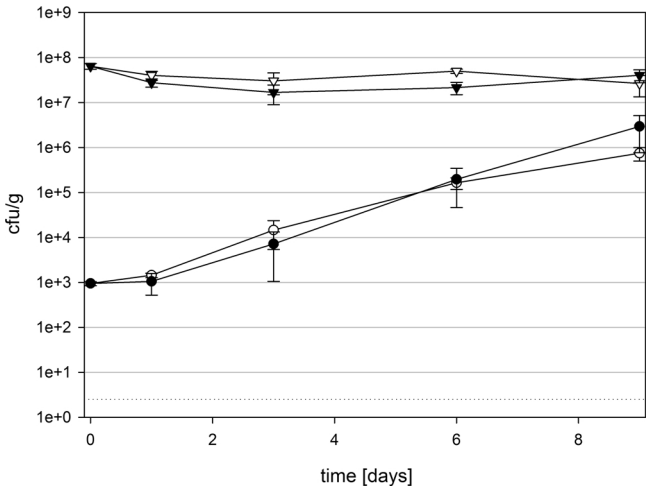

Fig. 1. Impact of AITC on the total aerobic mesophilic count of mung bean sprouts (triangles) and fresh-cut iceberg lettuce (circles); White symbols refer to reference samples without AITC, black symbols indicate samples stored with 10 µl of 1:10 diluted AITC.

2.6. Statistical analysis

Data is presented as mean values ± standard deviation (n = 3). Where indicated, differences between mean values were tested for significance by t-testing at a significance level of 0.05 using Sigmaplot 12.5 (Systat).

3. Results and discussion

3.1. Impact of AITC on native microflora of mung bean sprouts and iceberg lettuce

When 10 µl of AITC was applied 1:10 diluted, no distinct reduction of the total aerobic mesophilic counts on mung bean sprouts and fresh-cut iceberg lettuce was observed during storage (Fig. 1). No increase of the colony count was evident on mung bean sprouts with or without AITC during storage, while microbial growth by about 3 log was found on iceberg lettuce. Since the growth kinetics of microbial populations are similar for references without AITC and samples with 10 µl of 1:10 diluted AITC, it can be concluded that the measured headspace concentration of AITC in the range of about 0.11–0.16 µg/ml is too low to cause a significant effect on the natural microflora of fresh-cut iceberg lettuce or mung bean sprouts.

In a second trial, a 10-fold higher concentration of AITC was applied whereby a strong antimicrobial effect of gaseous AITC was found on both products (Fig. 2). When 10 µl of undiluted AITC were applied in the bags, the measured concentrations of AITC in the headspace of the packaging system ranged between 1.12 and 1.41 µg/ml for fresh-cut iceberg lettuce and between 0.81 and 1.16 µg/ml for mung bean sprouts during storage. All microbial populations on sprouts showed a decreasing viable count, except lactic acid bacteria, which remained on a constant level. Reductions by up to 3 log were found in case of Enterobacteria while *Pseudomonas* spp. and the total aerobic mesophilic count decreased by about 2–2.5 log within 9 days. Yeast and moulds were present at low levels and decreased to the detection limit after only 1 day of storage. The populations on the reference samples without AITC remained on a constant level except for lactic acid bacteria, which increased by about 2 log within 9 days (Fig. 2A).

For fresh-cut iceberg lettuce stored in the presence of AITC, a growth inhibition was found in case of the total aerobic mesophilic count, while *Pseudomonas* spp. decreased by approximately 1 log. Enterobacteria as well as yeast and moulds decreased down to the detection limit after 1 day of storage (Fig. 2B) while counts of lactic acid

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