FRESHLY CUT ONIONS PACKAGED WITH CYCLODEXTRIN-ENCAPSULATED ALLYL ISOTHIOCYANATE SHOW ANTIMICROBIAL ACTION IN A MODEL SYSTEM

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ABSTRACT

This study aimed to investigate the antibacterial activity of inclusion complexes of allyl isothiocyanate (AIT), beta- and alpha-cyclodextrin. In order to carry out these fictitious experiments, the target pathogen was plated onto the agar surface of a Petri dish, and the AIT formulations were spotted onto filter paper discs that were then adhered inside the dish's lid. The concentrations of AIT formulations in the static headspace were measured using solid phase microextraction and gas chromatography. By storing freshly cut onions at 5 °C for 20 days in sealed containers, beta IC's antibacterial effectiveness was evaluated. AIT that had been beta IC-encapsulated had better antimicrobial effectiveness than AIT that had not been encapsulated (p 0.05). In the static system, unentrapped AIT had the highest vapor concentrations, followed by beta IC and then alpha IC. significant reduction in L. When fresh-cut onions were exposed to beta IC (200 l/l) before being packaged, monocytogenes was found, but a smaller decrease in the number of L was seen. monocytogenes found in the raw, cut onion. The total aerobic count in onions was about 4 log CFU/g lower than in controls after 10 days of treatment with beta IC. Based on these results, beta IC might be used as an antibacterial treatment in pre-packaged fresh-cut vegetables.

1. INTRODUCTION

Due to its potent, vaporous smell, allyl isothiocyanate, which is derived from mustard seeds, is widely used as a flavoring ingredient and antibacterial agent. Even though it is largely insoluble in aqueous systems, it quickly interacts with peptides and proteins. In glass jars that have been tightly sealed, bactericidal effects of air concentrations of 2e8 ml/l are visible, while inhibitory effects of air concentrations of 0.1 ml/l are visible. Only at doses of 100 ml/l was AIT vapour effective in Petri dishes against tomato pathogens.

AIT's reaction time, odor, volatility, and flavor must all be considered in order to decide if it is a good choice for food preservation. Additionally, it's possible that over time, AIT vapor will pass

through the polymer materials used in food packaging. Entrapping AIT in CD could help achieve the same objectives as CD-entrapped volatile flavor compounds, which were found to reduce odor, get rid of unwanted reactivity, and control release. Five (alpha), six (beta), or seven (gamma) glucose monomers are joined by alpha 1-4 bonds to form the cyclic structure of CDs. The hollow core of CDs is frequently apolar, and this characteristic makes it easier to form stable associations with hydrophobic "guest" molecules like antimicrobials, flavors, or medications. The inclusion complex is made up of a host and a guest. In more detail, both temperature and relative humidity affect how quickly AIT bound in alpha or beta CD becomes unbound and evaporates when it comes into contact with water. It has been suggested by a number of academics that ICs used in packaged food products release volatile antibacterial compounds to help preserve food. It has been demonstrated that the production of their volatile antimicrobials correlates with the relative humidity, and that beta ICs from thyme and, more recently, garlic oil, can inhibit fungal growth on tomatoes and in vitro. As previously mentioned, AIT can be trapped in CDs and can prevent Penicillium spp. growth when combined with an alpha IC preparation. But AIT's antibacterial properties in food products or in vitro have not yet been given a name. This study compared the effectiveness of beta IC and unentrapped AIT against Escherichia coli, Listeria monocytogenes, and Penicillium expansum on a product of fresh-cut onions packaged aerobically before and after being inoculated with Listeria monocytogenes.

2. MATERIALS AND METHODS

Synthesis & characterizing - AIT inclusion complexes

0 point 1 g of the compound was dissolved in a mixture of hexane and distilled water, and the solution was then subjected to a 20-minute sonication process to ascertain the AIT content of three batches of each IC. The collected and injected hexane fractions were examined using gas chromatography, flame ionization detection, and helium as the carrier gas. Both the measuring temperature and the inlet temperature were set to 250 C. The temperature was raised from 50 degrees Celsius to 96 degrees Celsius in five minutes by adding 11 points 5 degrees every minute. The software VarianStar was used to examine the generated peak areas. Using a standard curve, we measured the areas under the peaks to calculate the concentration of AIT in the ICs. We were able to determine the moisture percentage and CD mass of ICs by drying them to a constant weight in the oven. The molecular weights of CD mass per IC weight unit and the AIT were used to convert in order to figure out the inclusion efficiency.

Evaluation of AIT and ICs for their antibacterial activity using in vitro vapour tests

To develop P. We used Potato Dextrose Agar and 26 C for four days as the expansum temperature. By gently stirring mature growth plates in 6 ml of sterile distilled water, conidia were obtained. Then, at a rate of about 3 log CFU/plate, we infected brand-new PDA plates with conidia. As calculated by dividing the AIT concentrations by the Petri dish headspace volume, i. e. Initial concentrations ranging from 0 point 2 to 6 ml AIT/l air were pipetted into sterile filter paper discs and put inside the Petri dish covers, with the dish volume subtracted 30 ml to account for the volume of the agar. The AIT-infused paper disc was placed facing down on top of the inoculated agar surface, which was then sealed with Parafilm and the Petri dish cover. The maturation of the plates at 6 and 11 C for 11 days or at 24 C for 4 days, followed by another incubation at the same time and temperature, was done to see if the treatments had fungistatic or fungicidal effects. The experiment was performed three times, and duplicate samples of each treatment were created. In addition to being tested as controls, cyclodextrins, water, and ICs made without AIT were also used.

Gram-positive L monocytogene and Gram-negative E coli were both diluted in peptone saline and distributed at an intensity of 3 log CFU/plate on tryptic soy agar plates following an overnight incubation in tryptic soy broth. Bacteriostatic concentrations were calculated by incubating plates with initial AIT loads ranging from 6 to 60 ml/l for 4 days at 30 C, followed by observing growth. After removing the filter paper discs, the plates were reincubated to determine the bactericidal concentration. AnaeroIndicator tablets, used in various controls, were used to demonstrate that the environment in the Petri dish remained aerobic throughout the incubation process. Each control was examined several times. Following incubation, fungistatic or bacteriostatic effects were quantified as a 150-fold decrease in CFU/plate compared to the control. We observed no regrowth of the 150-fold reduction in CFU/plate after AIT was removed from the system and the plates were re-incubated, indicating bactericidal or fungicidal effects. Damaged cells may still be viable, but they cannot be cultured.

Beta IC's antimicrobial activity on packaged fresh-cut onions

2020's May and August will see Nova Agri Inc. a fresh batch of yellow onions that were chopped. The effects of AIT encapsulated in beta CD on the native onion microbiota and the fate of inoculated L. Monitoring was done for monocytogenes 568. To sum it up, L. Monocytogenes 570 was grown overnight in PS, diluted to 6 log CFU/ml, and then resuspended.

An initial concentration of 3 log CFU/g was obtained by adding 1 ml of PS or listerial suspension to the onion. Onion samples weighing 25 grams each were sealed in 6-8 cm polyethylene bags with sterile filter paper discs containing water or beta IC to create 0, 100, and 200 ml AIT/l bag headspace. Following heat sealing, the bags were put in an incubator set to 6 C. On the sampling day, onions were removed from their bags and homogenized for 1 minute in a sterile environment after being diluted with 220 ml PS. Following 49 hours of incubation at 36 degrees Celsius, the enumeration was performed by spot plating the appropriate serial dilutions on Oxford Agar for Listeria counts and Plate Count Agar for total aerobic counts. The fate of the inoculated bacteria was ascertained using a 3-tube MPN method, in which aliquots of 0 point 1, 1 point 0, and 10 ml from the 100 dilution were enriched in Fraser broth before being detected on Palcam agar, when the amount of Listeria in a sample was approaching the detection limit using the spot plate method. Each treatment was applied twice during each of the three runs of the entire experiment. The Holm-Sidak test was used to compare the treatment effect statistically, with a significance level of 4%.

3. RESULTS

AIT and moisture levels in beta IC are significantly higher than in alpha IC. Inclusion efficiencies for the wet IC preparations were 87 percent for alpha ICs and 127 percent for beta ICs, indicating that some AIT was linked to the beta IC's surface. Both inclusion complexes had AIT odors after being filtered and washed, but they were less potent than in the unentrapped AIT. The IC preparations were kept in a hydrated form suitable for direct application onto filter paper discs to make it easier for them to be transported into the vapour phase of the petri dish or packages of fresh-cut onions. Minimum initial concentrations with fungistatic action of 0.6e1 ml AIT/l air and bacteriostatic concentrations of 24 and 52 ml AIT/l for E. coi and L. AIT encapsulated in beta CD was found to have the greatest antibacterial effect when monocytogenes, respectively, were observed. In a similar vein, P. At an initial concentration of 2 ml/l, expansum was successfully inhibited by both AIT and alpha IC, whereas at a concentration of 6 ml/l, growth was only slowed by air. Both alpha IC and unentrapped AIT failed to inhibit E when given at a dose of 48 ml/l. Colitis or L. monocytogenes. Beta IC and alpha IC were fungicidal at initial concentrations of 6 ml/l air, but AIT was not. Only

beta IC killed the target bacteria, even at high concentrations; alpha IC and AIT had no impact. At 4 C compared to 12 C and 26 C, AIT was generally more inhibitive against the fungus. Growth wasn't in any way slowed down in the untreated controls. Concentration in the static headspace was 12 times higher over suspensions of unentrapped AIT than it was over beta IC, which was 10 times higher than it was over alpha IC. The system reached equilibrium after 2 days and remained steady for 6 days. When compared to the control, adding beta ICs with initial concentrations of 120 ml AIT/l air significantly decreased aerobic plate counts from days 4 through 14 and adding beta ICs with initial concentrations of 220 ml AIT/l air significantly decreased microflora levels from days 4 through 16, with neither treatment's levels ever exceeding 4 log CFU/g. Without AIT, aerobic plate counts increased over the course of 8 days from 4 point 8 log CFU/g to 6 log CFU/g before steadily declining to 3 log CFU/g at the end of the aerobic storage period. L. was vaccinated after 15 days. progressively decreased from 2.5 to 4 log CFU/g in onion samples treated with either no AIT or 120 ml AIT/l, indicating that L. monocytogenes was present. On raw, thinly sliced onions, monocytogenes cannot survive. L. After 6 days, the monocytogenes count on onions treated with 220 ml AIT/l dropped to 2.4 log CFU/g, with notable drops on days 6, 8, 10, and 12 compared to the control. By day 22, all samples had almost surpassed the MPN detection limit. samples free of Listeria spp. were applied as a check.

4. DISCUSSIONS

In conclusion, the findings are in line with the fact that Alpha IC contained the expected quantity of AIT. Beta IC showed a higher than expected concentration of AIT. This could be explained by the hydrated beta IC preparation having surface-associated, unentrapped AIT. We used washed, hydrated ICs because they were easy to hydrate, dilute with water to the desired AIT concentration, and then apply to the filter paper disc-based delivery system, which then released the AIT into the gas phase of the food package or Petri dish model.

An in vitro antimicrobial vapour assay, which involves spraying an antimicrobial agent onto a disc of filter paper and then sealing it inside a package with agar containing water, protein, and carbohydrates, can be thought of as a conceptual representation of an aerobic model food package. Higher concentrations of free AIT were needed here in order to achieve the same effect as that observed in a prior experiment using sealed glass jars. This might be because AIT vapour quickly forms from unentrapped AIT and escapes the Petri dish, delaying the detection of the antibacterial effect. It was discovered that beta IC had much stronger inhibitory or lethal effects than alpha IC. Their different antibacterial effects could be explained by how beta and alpha ICs release in the vapour system. The static headspace experiment showed that alpha IC from a hermetically sealed glass container produced ten times less AIT than beta IC. On the other hand, it's possible that the beta IC preparation's inclusion of some unencapsulated AIT helped the AIT release. Previous studies discovered that beta IC sped up the release of AIT compared to alpha IC, likely due to the wider beta CD. In a Petri dish environment with low headspace concentrations, this effect would cause the antibacterial efficacy of alpha ICs to decline. Beta IC would release more AIT, which would result in a stronger antibacterial action, in contrast, as demonstrated in the antimicrobial vapour model system. These results imply that a sweet spot must be found between the too rapid and too slow release of AIT from an IC, as well as between the too slow and too fast release of AIT from an unentrapped IC. The beta IC equilibrium was the best, as demonstrated by our open aerobic model system.

This supports what has previously been noticed, but it remains unclear why bacteria have higher AIT resistance. Stringent aerobes are particularly vulnerable to the effects of AIT because it acts as an uncoupler of oxidative phosphorylation, which is one potential mechanism for its effectiveness against microorganisms. Although this may simply be a result of the fungus growing more slowly, lower temperatures appear to increase AIT activity when applied to it to the untrained eye. The release of AIT from beta IC, which prevented the growth of microorganisms, was advantageous for onions kept at 6 C for a long time in a plastic with high oxygen permeability. According to recent studies, beta ICs can effectively suppress tomato microflora when combined with other antimicrobials. These studies focused on the effectiveness of beta IC formulations containing garlic oil. The headspace levels are less than they would be if AIT were not trapped because beta IC is released more slowly than it would otherwise. This ought to lessen the unpleasant AIT smell that customers complain about when opening products containing beta IC. It is typical to need to use more antimicrobial agent when going from in vitro testing to a food product because antimicrobials can be lost during packing, suffer chemical interactions with food components, and have target cells become more resistant due to stress response. L. was immunized. The results also demonstrated that endogenous onion antimicrobials suppressed growth, which is supported by a recent publication by Santas et al (2010). Monocytogenes were effectively inhibited or killed after being treated with beta IC. To treat a mixture of ground vegetables, the authors of this study vaporized AIT. An alternative application could involve combining AIT ICs. right in the center of the food chain. Similar methods were used for the delivery of minced meat and sausages as well as acacia-gum-encased AIT, and it was shown to be effective. E. coli inhibitor Strain E. coli O157:H7 at concentrations that do not affect the flavors and odors of the food.

5. CONCLUSION

In conclusion, P was more effectively inhibited by the beta IC formulation, in which AIT is trapped in beta CD. spread, E. and L. coli. monocytogenes than the untrapped AIT and alpha IC. The three examined microorganisms were all vulnerable to beta IC, but the fungus was the most so. Beta IC also reduced L's viability and prevented the growth of aerobic microflora. fresh-cut onions stored with monocytogenes. Antimicrobial food technology has advanced as a result of concerns about cost, effectiveness against infections, extension of shelf life, and consumer acceptance. If further research is done, AIT might be able to take the place of beta IC as a food preservative.

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