

The Antimicrobial Efficacy of Chitosan Fiber from Mushrooms for Stabilization and Its Beneficial Impact on Brewing Sustainability for Low and Nonalcoholic Beers

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ABSTRACT

While conventional alcoholic beers are considered immune to spoilage from contamination with bacterial pathogens, their low-alcohol (LA) and nonalcoholic (NA) counterparts are not. Recent reports, including a major study published in the *Journal of Food Protection*, show that the pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella* survived in LA and NA beer for over 2 months after brewing. Another report indicated that LA and NA beer with a pH higher than 4.20 are at high risk for pathogen growth and should have their processes reviewed by an Association of Food and Drug Officials (AFDO)-accredited process authority. At the same time, LA and NA beers are also at risk for spoilage microorganisms such as yeasts, molds, and nonpathogenic bacteria, which can cause exploding cans and alcohol production, as well as off-flavors and a bad appearance. With the surge in popularity of LA and NA beer, brewers are responding by rapidly increasing their produc-

tion to meet demand, but there is little scientific evidence showing the safety and microbial stability of these beers over time. A new natural antimicrobial ingredient, a cationic biopolymer fiber, chitosan, extracted from white button mushrooms, has been studied for its ability to protect LA and NA beers from both pathogenic bacteria and spoilage microorganisms in a series of microbial challenge tests. Natural antimicrobial ingredients present an opportunity to reduce the risk of microbial spoilage of LA and NA beers, while eliminating the risk of contamination with pathogenic bacteria and improving the sustainability of producing these beverages.

Keywords: Chiber™ mushroom extract, low-alcohol beer, natural antimicrobial, NA beer antimicrobial, NA beer stabilizer, natural stabilizer, nonalcoholic beer

Introduction

Background

The brewing industry has witnessed a surge in the popularity of low-alcohol (LA) and nonalcoholic (NA) beers driven by increasing consumer demand for healthier beverage options. Unlike their alcoholic counterparts, LA and NA beers lack the intrinsic antimicrobial properties provided by ethanol, rendering them more susceptible to microbial contamination. Traditional alcoholic beers, with an alcohol content typically above 3% al-

cohol by volume (ABV), along with the use of hops, and a pH below 4.6, create an inhospitable environment for most pathogens and spoilage organisms. However, LA and NA beers, which contain 0.5% ABV or less, do not have the protective mechanism of ethanol on which to rely. Using process techniques such as tunnel pasteurization has proven to be a successful microbial kill step; however, tunnel pasteurization uses large amounts of energy and water and has a negative impact on the flavor of the beer, making it a necessary but undesirable step in LA and NA beer production.

Pathogen Survival in LA and NA Beers

Recent studies have revealed that pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* can survive in LA and NA beers for over 2 months postbrewing (2). These findings underscore the need for stringent microbial safety protocols in the production of these beverages, particularly for those with a pH above 4.20 (1), which further exacerbates the risk of hazardous pathogenic microbial growth.

Current Industry Practices and Challenges

Brewers are looking for rapid production techniques to meet the growing demand for LA and NA beers. However, there is a significant gap in scientific evidence supporting the long-term safety and microbial stability of LA and NA beers, and brewers are looking to the wider NA beverage industry (sodas, juices, etc.) for answers. The use of synthetic preservatives is limited due to consumer preference for natural and clean-label ingredients and

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unfamiliarity with these types of functional ingredients for beer. Consequently, there is a pressing need for effective natural antimicrobial agents that can ensure the microbial safety of LA and NA beers without compromising their appeal. Tunnel pasteurization is a reliable method for achieving microbial stability, but it's known to degrade volatile flavor compounds and introduce new undesirable flavors, along with being energy and water intensive. Tunnel pasteurization causes increased oxidation, caramelization, alteration of hop compounds, and negative chemical reactions due to the high temperature. In a review of the available literature, Escarpment Laboratories (5) found that compared to conventional alcohol-containing beers NA beer needed four to six times the number of pasteurization units (PUs) depending on the number of bittering units (IBUs) to achieve the necessary microbial reduction from tunnel pasteurization. This high amount of required PUs has a major impact on the degradation of flavor and the production of off-flavors in LA and NA beers. At the same time, tunnel pasteurization requires a significant amount of energy to generate the PUs required.

The solution is to avoid high-heat exposure and instead use natural antimicrobials like Chiber™ mushroom chitosan extract. This research investigates the use of low doses (2,000 ppm) of natural mushroom chitosan to maintain the original flavor of beer while inhibiting the growth of six major spoilage-causing microorganisms in NA beer stored at ambient temperature. Chitosan is a natural biopolymer typically commercially extracted from crustaceans, but it also is found in mushrooms and other fungi. The extraction of chitosan from button mushrooms (*Agaricus bisporus*) is an eco-friendly alternative to crustacean-based extract, as it avoids the use of aggressive acid treatment and uses much milder conditions (3,4). Chitosan's cationic charge under acidic conditions, such as those of a beverage, enables an electrostatic bond to form with anionic components of the cell wall of yeast, mold, and bacteria cells, such as peptidoglycan and other proteins. The electrostatic interaction causes a physical sheering of the cell wall, thereby causing cell leakage and eventual cell death. By leveraging natural antimicrobials, brewers can avoid the flavor impact of heat pasteurization associated with NA beer while safeguarding its quality and stability.

The results of a study by Singh et al. (6) indicated that the extrapolated energy consumption of tunnel pasteurization for a 12-oz can of a beverage was calculated to be 38 Wh compared to the estimated 0.05 Wh consumed with the use of a white button mushroom extract chitosan ingredient. A summary of the comparison of tunnel pasteurization with the white button mushroom chitosan ingredient made by Chinova Bioworks (Chiber™) is shown in Figure 1.

	Tunnel Pasteurization	Mushroom Chitosan
Energy Consumption per can	38 watt-hour	0.05 watt-hour
Carbonation Limit	max 2.8 - 3.0 (depending on temperature of TP)	Open (limited to rating of packaging used)
Sensory Impact	Degradation of flavor, nutritional content	No impact on flavor or nutritional content

Figure 1. Comparison of energy consumption for tunnel pasteurization versus a white button mushroom chitosan ingredient (Chiber™).

Objective

This study aimed to validate the suitability of chitosan (Chiber™) extracted from white button mushrooms (*A. bisporus*) for reducing spoilage microorganisms and pathogenic bacteria in LA and NA beers, while also reducing energy consumption and maintaining the original flavor and sensory attributes of the beverage.

Methods

Antimicrobial Challenge Testing

The antimicrobial properties of the white button mushroom fiber were assessed using standardized antimicrobial challenge tests in accordance with U.S. Pharmacopeia (USP) method 51 (7). Pathogenic bacteria (*E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*) were inoculated into NA beer (0.5% ABV) at an initial concentration of log 6 CFU/mL. Spoilage microorganisms (*Saccharomyces cerevisiae*, *Paenibacillus*, and *Pseudomonas*) were introduced at a concentration of log 3 CFU/mL. These populations for inoculation into the beer samples challenged the sample with a high enough population that it is an unlikely scenario for harmful pathogenic bacteria and a more likely scenario for spoilage yeast and bacteria. The chitosan mushroom fiber (Chiber™) was dosed into the beer samples at 2,000 ppm. Prior to inoculation into the NA beer samples, the yeast and bacteria were cultivated in potato dextrose (PBD) and MRS/TSB broth, respectively, at 30–35°C for 2 days. Stationary phase cells were harvested by centrifugation within 20 h of inoculation and washed in buffer water to remove nutrients or metabolites in the spent medium that could affect growth in the test product. The pH of the spent medium was recorded, followed by filtration to remove residual cells to assure that acidic conditions occurred.

The cell density of each of the cultures was estimated by measuring optical density using a spectrophotometer. The cultures were diluted in buffered peptone water (BPW) or multi-peptone water at pH 7.4 to produce a suspension containing approximately 5×10^4 cells/mL. These suspensions were used as inoculum in the challenge tests.

All NA beer samples (three replicates) were prepared in a biosafety cabinet under sterile conditions. Samples of the NA beer were supplemented with the 2,000 ppm dose of Chiber™ and mixed well, and 10–100 mL of the sample was transferred to sterile tubes, jars, or bags, inoculated with the target microorganisms, mixed well by vortexing, and stored at 20–22°C for 21 days. The sampling point was every 7 days (e.g., day 1, day 7, day 14, day 21). During the storage period, samples were taken to determine the density of viable cells by plate count method.

Microbial Enumeration

Microbial populations were quantified at specified intervals by standard plating and dilution techniques on selective agar media. The populations of *E. coli*, *Salmonella*, and *Listeria*, along with the spoilage microorganisms, were monitored over a 21-day period. To perform the viable count analysis, a serial 10-fold dilution range of the sample was prepared in BPW or peptone water. For analysis of the viable yeast, bacteria, or mold count, 0.1 mL of sample was spread on PDB, MRS, or TSB using the agar plating method (sterile beads). The plates were incubated at 30–35°C for 2–3 days. Viable cell counts were expressed as colony forming units per milliliter and then graphed in log format.

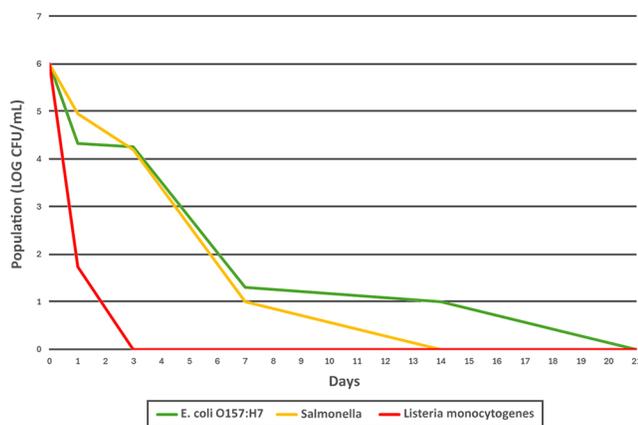


Figure 2. Reduction of pathogenic bacteria in nonalcoholic beer with inclusion of white button mushroom chitosan (Chiber™).

Results

Reduction of Pathogenic Bacteria

The introduction of white button mushroom fiber at 2,000 ppm demonstrated a significant antimicrobial effect on pathogenic bacteria in NA beer. The initial inoculated populations of these pathogens were 6 log CFU/mL, or 1,000,000 CFU/mL. *L. monocytogenes* was completely eradicated by day 3, while *Salmonella* and *E. coli* O157:H7 showed significant reductions, reaching undetectable levels by day 21. The results are shown in Figure 2.

The fiber also exhibited robust antimicrobial activity against spoilage microorganisms. It is evident that spoilage microorganisms, including *S. cerevisiae*, *S. cerevisiae diastaticus*, *Brettanomyces bruxellensis*, *Paenibacillus*, and *Pseudomonas*, were effectively reduced over a 21-day period. The populations of these microorganisms decreased from initial levels of approximately 3 log CFU/mL to undetectable levels (0 log CFU/mL) by day 14 for most species, indicating strong antimicrobial activity. The results are shown in Figure 3.

Overall, the Chiber™ mushroom extract at 2,000 ppm demonstrated robust antimicrobial properties, effectively reducing both spoilage and pathogenic microorganisms in NA beer to undetectable levels within a 21-day period.

Discussion

Implications for the Brewing Industry

The results of this study demonstrated the potential of white button mushroom fiber as a natural antimicrobial agent for LA and NA beers. Its efficacy in significantly reducing the population of both pathogenic and spoilage microorganisms offers a promising solution for brewers seeking to ensure the safety and microbial stability of their products. The use of natural antimicrobials aligns with consumer preferences for clean-label ingredients. In addition, the flavor of the LA or NA beer was not impacted by the use of white button mushroom fiber, whereas tunnel pasteurization of these beers is known to have a serious negative impact. Simultaneously, the use of Chiber™ could significantly reduce the cost of LA and NA beer production due to its low energy use.

Recommendations for Process Optimization

Brewers should carefully consider and test the options for ensuring the microbial stability and safety of their LA and NA

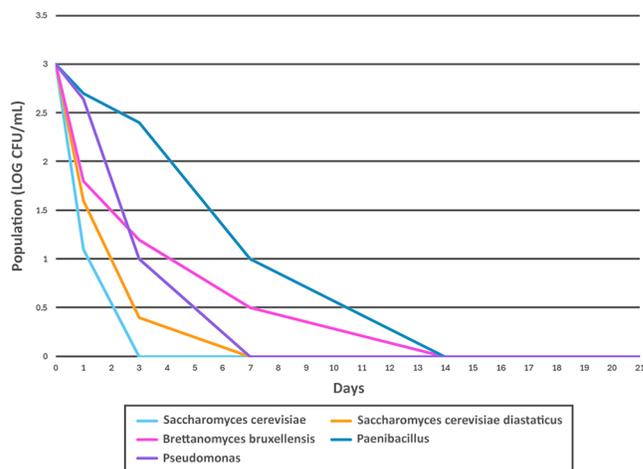


Figure 3. Reduction of spoilage microorganisms in nonalcoholic beer with inclusion of white button mushroom chitosan (Chiber™).

beers. The option of incorporating white button mushroom chitosan fiber into their production processes to mitigate the risk of microbial contamination is validated by the results of this study, but many factors can change its effectiveness. Microbiology testing is recommended to optimize dosing levels between 500 and 2,000 ppm and to evaluate the long-term stability and sensory impact of the fiber in various beer types and formulations. Collaboration with an Association of Food and Drug Officials (AFDO)-accredited process authority can help validate these processes and ensure compliance with safety standards.

Conclusions

The results of this study indicate that white button mushroom fiber (Chiber™) is an effective natural antimicrobial agent for LA and NA beers. Its ability to significantly reduce populations of pathogenic and spoilage microorganisms provides a viable solution for enhancing the microbial safety and stability of these beverages. As the demand for LA and NA beers continues to grow, the adoption of antimicrobial strategies will play an important role in meeting consumers' expectations for safe, high-quality products.

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