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Development of antibrowning and antimicrobial formulations to minimize *Listeria monocytogenes* contamination and inhibit browning of fresh-cut "Granny Smith" apples☆

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ABSTRACT

In recent years, there have been a number of *Listeria monocytogenes* recalls involving fresh-cut apples treated with antibrowning solutions. In the present study, we used response surface methodology to develop and optimize formulations for reducing *L. monocytogenes* populations in the solutions and for maintaining cutting surface color of apple slices. The following two sets of three chemicals at various levels were combined: citric acid + calcium ascorbate + *N*-acetyl-L-cysteine (NAC), and citric acid + ascorbic acid + NAC. The survival of a 4-strain cocktail of *L. monocytogenes* cells (F2365, serotype: 4b; NRRL 33857, serotype: 1/2a; NRRL 33723, serotype: 1/2a; NRRL 33230, serotype: 4c) in the solutions prepared from the combinations was evaluated, and the changes in cutting surface color parameters (L*, a* and b*) and skin edge browning of "Granny Smith" apple slices dipped in the solutions for 3 min were assessed during 21 d of storage at 4 °C. Results showed that combinations of citric acid, calcium ascorbate and NAC were ineffective in achieving 5 log reduction of *L. monocytogenes*. However, formulations comprised of 4.0–4.5 % citric acid, 3–4% ascorbic acid and 1.5–2.0% NAC achieved more than 5 log reduction of *L. monocytogenes* in the solutions, and the cutting surface of apple slices treated with these formulations maintained L* values of > 70, and a* values of < -1.8 during 21 d of storage. Our results suggest that the combinations of citric acid, ascorbate and NAC may be used to enhance microbial safety of fresh-cut apples without compromising product quality.

1. Introduction

Fresh-cut fruit has become an important sector of fresh-cut produce in recent years due to convenience and fresh-like quality. Fresh-cut fruits, particularly apple slices, are offered in schools, supermarkets and fast-food restaurants. It is well known that, when being cut, apples are susceptible to enzymatic browning and have a relatively shorter shelf-life compared to whole fruit. The enzymatic browning of apple slices is catalyzed by polyphenol oxidase (PPO) which converts polyphenol compounds into quinines in the presence of oxygen. The quinines condense and react with amino acids and proteins, leading to formation of brown melanin pigments (Dawley and Flurkey, 1993). To control the browning, several types of antibrowning agents have been investigated. The most common antibrowning agents are ascorbic acid and their derivatives (Sapers and Miller, 1998; Chen et al., 1999; Sapers et al., 2002; Karaibrahimoglu et al., 2004; Fan et al., 2005a). Ascorbic acid prevents enzymatic browning by reducing the quinone products to their original polyphenol compounds. Calcium ascorbate at concentrations up to 7%, commonly used by the fresh-cut fruit industry, not only inhibits browning but also maintains firmness as a result of calcium effects.

Sulfur-containing amino acids are known for their antibrowning activities on apples (Buta et al., 1999) and potatoes (Gurbuz and Lee, 1997). The sulfur-containing amino acids have been evaluated as substitutes for sulfite to prevent enzymatic browning (ALi et al., 2015; Gomes et al., 2014; Kuijpers et al., 2012; Molnar-Perl and Friedman 1990; Oms-Oliu et al., 2010). Son et al. (2001) found that among the compounds tested, *N*-acetyl-L-cysteine (NAC), cysteine, and glutathione showed the highest inhibitory activity on apple browning. These sulfur-containing amino acids did not inhibit the oxidation of phenolics by PPO; rather, they prevented the subsequent polymerization of phenolics (Kajiwara et al., 2006) by forming colorless products with *o*-quinones (Kuijpers et al., 2012). NAC is also considered to be a powerful antioxidant, reacting with free radicals (De Vries and De Flora et al., 1993;

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Aruoma et al., 1989).

It is also known that many carboxylic acids have inhibitory effects on enzymatic browning. Son et al. (2001) showed that citric acid, pyruvic acid, malic acid and lactic acid at 1% moderately inhibited the browning of apple slices followed by the weaker inhibitory compounds (acetic acid, succinic acid, and fumaric acid). Citric acid has been reported to inhibit PPO by lowering the pH (below those for optimal PPO activity) and chelating copper (Altunkaya and Gökmen, 2008).

In recent years, there have been a number of recalls of cut apples and whole apples due to contamination with *Listeria monocytogenes* (FDA, 2012a, b, 2013, 2014, 2015, 2016; Angelo et al., 2017). The common antibrowning agents used by the industry, such as ascorbic acid and calcium ascorbate, are antioxidants and do not possess any antimicrobial properties (Fan et al., 2005b; Karaibrahimoglu et al., 2004). *L. monocytogenes*, being a psychrotropic bacterium, can survive and proliferate in the antibrowning solutions at 4 °C and spread to apple slices, resulting in contamination of antibrowning solutions and apple slices with *L. monocytogenes*. Therefore, there is an urgent need for new antibrowning formulations that possess antimicrobial properties.

It is well known that organic acids are antimicrobials. The antimicrobial ability of organic acids is due to penetration of the undissociated states of acids into microorganisms through the plasma membranes. Once inside, the molecules dissociate and release charged anions and protons that are toxic, damaging proteins and DNA structure and interrupting enzymatic activities and microbial metabolic reactions (Mani-Lopez et al., 2012; Nazer et al., 2005). However, the antibrowning properties of organic acids are not strong as compared with ascorbic acid or calcium ascorbic acid (Ali et al., 2015; Son et al., 2001), and can not ensure the long shelf-life as demanded by industry and consumers.

There have been a number of studies on the development of antibrowning formulations (Capotorto et al., 2018; Chen et al., 2016; Oms-Oliu et al., 2010; Wills and Li, 2016). However, there has been few reports dealing with the optimization of formulations addressing both tissue browning and inactivation of *L. monocytogenes*. An earlier study (Raybaudi-Massilia et al., 2009) showed that combination of 1% NAC, 1% glutathione, 1% calcium lactate and 2.5% malic acid resulted in more than 5 log reductions of *L. monocytogenes*, *S.* Enteritidis and *E. coli* O157:H7 on "Fuji" apple slices. However, apple slices treated with the combination had a short shelf life due to changes in physicochemical parameters such as firmness and color. Yan et al. (2017) showed that combination of ethanol (20–40 %) and ascorbic acid (1–3%) reduced browning of fresh-cut apple slices and reduced populations of pathogenic bacteria. However, the high concentrations of ethanol likely impact the taste of apple slices at least during earlier storage period. Because the *Listeria* contamination of a large amount of fresh-cut apples likely occurs during the dipping treatment with contaminated antibrowning solutions, elimination of *L. monocytogenes* in the antibrowning solutions would prevent cross contamination of apple slices. Therefore, the objectives of the present study were to study the combinations of citric acid, ascorbate (ascorbic acid and calcium ascorbate) and NAC to inactivate *L. monocytogenes* in the antibrowning solutions and maintain quality of apple slices.

2. Materials and methods

2.1. Materials

"Granny Smith" apples were harvested at commercial maturity from an orchard in Central Pennsylvania. Fruit were stored at 4 °C for up to 3 months before being used. Calcium ascorbate (98–100.5 %) was purchased from Spectrum Chemicals & Laboratory Products (New Brunswick, NJ, U.S.A.). NAC (≥ 99%) and L-ascorbic acid (≥ 99%) were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.) and citric acid (100%) was from Mallinckrodt Pharmaceuticals (St. Louis, Mo., U.S.A.).

Table 1
Uncoded levels for the concentrations of antibrowning compounds (independent factors) corresponding to coded levels. Alpha (α) = 1.682.

	Coded level				
Compounds	-α	−1	0	+1	+α
Calcium ascorbate	0	1.42	3.50	5.58	7
Ascorbic acid	0	1.42	3.50	5.58	7
Citric acid	0	1.01	2.50	3.99	5
N-acetyl-L-cysteine	0	0.41	1.00	1.59	2

2.2. Experimental design

A block central composite design (CCD) for three chemicals at five levels (−1.682, −1, 0, +1, +1.682) was used to study the effects of antibrowning/antimicrobial combinations on cutting surface and skin browning of apple slices and populations of *L. monocytogenes* in the solutions (Table 1). A total of 20 runs (14 combinations plus 6 center points) for each combination were used to guarantee minor departures from the rotatability and orthogonality of the experimental design. The center points were repeated six times to improve the precision of the experiment. The following combinations of chemicals were tested: citric acid + calcium ascorbate + NAC, and citric acid + ascorbic acid + NAC. Tested levels for ascorbic acids/calcium ascorbate were 0–7 % with 3.5% as center points; and for NAC, the levels tested were 1–2%. The concentration ranges were chosen based on levels commonly used by the industry (for calcium ascorbate/ascorbic acid) and our preliminary experiments. After the findings of formulations that achieved more than 5 log reductions of *L. monocytogenes* while maintaining desirable cutting surface color, separate experiments using apples from a second season were conducted to verify the effectiveness of these formulations.

2.3. Processing of apple slices

Medium size fruit were used to prepare apple slices by slicing apples into 8 equal unpeeled pieces without the core. The sliced apples were dipped into antibrowning solutions for 3 min. The pH values of the antibrowning solutions were measured using a pH meter (Orion 420A+, Thermo Electron Corp., Waltham, MA, U.S.A.). The experiments were conducted in ambient temperature (22 °C). The slices were drained and placed into zipper sealed bags (15.2 × 12.7 cm) with 4 holes (0.6 cm diameter) and stored for 21 d at 4 °C. There were 8 apple slices in each bag with each slice coming from a different apple, and 4 replicated bags for each experiment. Each replicated bag was from a different batch of apples and treated with different batches of solution. Cutting surface and skin edge browning were measured at d 1, 7, 14 and 21 of storage. Cutting surface (CIE L^*, a^*, b^*) was measured with a UltraScan Vis colorimetric spectrophotometer (Hunter Associates Lab, Reston, Va., U.S.A.) using a 9.5 mm (diameter) area view, and operated in the RSEX reflectance mode. The spectrophotometer was calibrated using the standard light trap and a white tile (L^* 93.50, a^* –0.89, and b^* 1.01). The illuminant/viewing geometry were D65/10°. Two readings were taken on each apple slice (one on each cutting surface). Measurements were made at the middle point of the cutting surface of apple slices. The green skin of the apple slices may turn brown, starting from the edge, after some treatments (Fan et al., 2009). The width of the browned skin of the apple slices was measured using a ruler at 1, 7, 14, and 21 d during storage at 4 °C. Eight apple slices were measured. Even though the cutting surface and skin edge browning were measured every week during storage, only data on d 21 are shown. A minimum shelf-life of 3 weeks is commonly sought by producers of apple slices.

2.4. Reduction of L. monocytogenes population

Four strains of *L. monocytogenes* were used: F2365 (Serotype: 4b;