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An innovative design and application of natural antimicrobial gelatin based filling to control risk of listeriosis from caramel apples

Pelin Barış Kavur, Ahmet Yemenicioğlu *

Department of Food Engineering, Faculty of Engineering, Izmir Institute of Technology, 35430, Gülbahçe Köyü, Urla, Izmir, Turkey

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ABSTRACT

The aim of this study is to design and apply antimicrobial gelatin based filling with an innovative method in production of safe caramel apples that caused deadly listeriosis cases. For this purpose, water holding capacity and mechanical stability of gelatin (GEL) gels were improved by incorporation of soy proteins (SP) and inulin (IN), respectively. The water activity (a_w) of gels were also reduced < 0.9 by addition of sucrose (SUC). The application was conducted by coring of apples, filling void core with GEL-SP–IN–SUC blend gel solution containing synergetic antimicrobials, nisin and lysozyme, and antibrowning agent ascorbic acid (each at 1% in gel). Candy sticks were then placed into gel-filled void cores of apples, and apples were kept at 4 °C for 20 h for gelation of filling before they were dip-coated with caramel. The inoculated caramel apples with antimicrobial GEL-SP–IN–SUC filling showed 2.4–2.9 D lower Listeria count than traditional inoculated caramel apples during 7-day cold storage. The active blend gel-filling also successfully prevented the discoloration of apple core and accumulation of apple juice between apple surface-caramel coating interface. Mechanical tests showed strong binding of candy sticks by GEL-SP–IN–SUC gel-filling. Active blend gel-filling applied in an innovative way showed good potential to improve safety of candied apples.

1. Introduction

The prevention of food contamination by *Listeria monocytogenes*, and application of hurdles to prevent listerial growth in risky food are critically important since this bacterium may cause deadly infections in pregnant women, old people and the immunosuppressed people (Vázquez-Boland et al., 2001; Álvarez-Ordóñez, Leong, Hickey, Beaufort, & Jordan, 2015). The prepacked caramel apple was designated as a Listeria risk-food after it had caused dead of 7 people and hospitalization of 34 from listeriosis in 12 states of United States of America (Angelo et al., 2017; Centers for Disease Control and Prevention, 2015). This infection occurred unexpectedly since apples are too acidic, and the caramel coating applied at a high temperature (71–88 °C) is too dry (a_w < 0.80) to support Listeria growth (Bouvier, 2015; Salazar et al., 2016). However, the research by Glass, Golden, Wanless, Bedale, and Czuprynski (2015) clearly showed how Listeria developed in caramel apples during storage. These workers showed that stick insertion into apples at the stem-end that contains the potential Listeria contaminant causes leakage of apple juice from core region to the interface between the apple surface and caramel coating. Then, during cold storage the released apple juice neutralized by the basic caramel becomes a suitable medium for growth of *L. monocytogenes* contaminated at apple peel, calyx or stem regions. The findings of Salazar et al. (2016) also supported that Listeria contaminated at the stem-end of apples is highly protected, and it might survive and grow in caramel apples.

Although the mechanism of listerial development in caramel apples has been characterized sufficiently, efforts to develop technological solutions to this problem are very limited. For example, Gustafson and Ryser (2017) tried thermal inactivation of *L. monocytogenes* by increasing temperature of caramel dipping solutions for current industrial practice from 82 °C to 99 °C. However, it was reported that the high temperature caramel coating process cannot ensure Listeria elimination at the stem-end of apples, and it causes undesirable cooking of apple surface as well as formation of a too thin caramel coating (Gustafson & Ryser, 2017; Salazar et al., 2016). Murray, Moyer, Wu, Goyette, and Warriner (2018) achieved the inactivation of *L. monocytogenes* on surface and scar tissue of apples by applying the combination of UV-C light, ozone, and hydrogen peroxide via forced air circulation. Moreover, Carstens et al. (2018) evaluated the efficiency of impregnating different antimicrobials such as ascorbic acid, nisin, potassium sorbate and

* Corresponding author.
E-mail address: ahmetyemenicioglu@iyte.edu.tr (A. Yemenicioğlu).

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sodium benzoate into wooden sticks inserted into caramel apples. However, none of these methods prevent the leakage of apple juice at the interface between caramel coating and apple surface. Moreover, apples processed by the traditional method still contain risks associated with Listeria contaminated at the protected sites like stem-end and calyx.

Different edible antimicrobial materials could be applied as a fruit coating to suppress the respiration rate and inactivate spoilage and pathogenic bacteria in whole or minimally processed sliced fruits (Park, 1994; Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). In contrast, the application of edible materials capable to produce hydrogels as an antimicrobial and antioxidant gel filling for fruit preservation is scarce. However, different basic research studies have been conducted to develop gel-based delivery systems incorporated with natural active compounds suitable for biomedical, pharmaceutical and food applications. For example, Campia et al. (2017) showed that aerogels of galactomannan extracted from leguminous plant sources could be promising for bio-medicinal and food packaging purposes to deliver antibiotics, bacteriocins, and enzymes (such as polymyxin B, nisin, lysozyme, protease and lipase). Abhari, Madadlou, Dini, and Naveh (2017) developed starch hydrogels cross-linked by trisodium citrate for controlled release of caffeine. Moritaka and Naito (2002) investigated the flavor release properties of agar and gelatin gel. *Aloe vera* gel has also been tested as a fruit coating to prolong shelf-life and quality of grapes and nectarine during cold storage (Ahmed, Singh, & Khan, 2009; Serrano et al., 2006). A hydrogel could be employed not only as a high capacity reservoir to deliver active compounds (antimicrobials and antioxidants) onto food surface, but also it could be used as an absorbent pad to bind drip-loss fluids from food (Batista et al., 2019).

In the current study, a smart preservation system was formed by an innovative application of antimicrobial and antioxidant gelatin (GEL) based blend gel as a multi-functional filling material for caramel apples (see Fig. 1). The developed composite gels formed by mixing GEL with soy proteins (SP), inulin (IN) and sucrose (SUC) were incorporated with generally recognized as safe (GRAS) antimicrobials, nisin (an antimicrobial peptide) and lysozyme (an antimicrobial enzyme), that synergetic action against Listeria frequently exploited in different food systems (Gill & Holley, 2000a, 2000b; Monticello, 1990; Morsy, Elsabagh, & Trinetta, 2018; Proctor & Cunningham, 1992; Sozbilen & Yemenicioğlu, 2020; Takahashi et al., 2012). The gel pH was lowered and browning of cored apple was prevented by addition of the antioxidant ascorbic acid. This work is the first study in the literature that applied a tailor-made antimicrobial gel-filling in an innovative way to prevent risk of listeriosis from a minimally processed fruit product as caramel apple.

2. Materials and methods

2.1. Materials

Type B gelatin (from bovine skin) was kindly provided by Seljel (Sel Sanayi, Balıkesir, Turkey) (bloom strength: 220, protein content $\geq$ 70%, viscosity: 2.5 mPa s at the concentration of 6.67%). Commercial soy protein isolate (Dunasoy 90 at 90% w/w protein) obtained from non-GMO defatted and dehulled soybeans was kindly provided by Euroduna Food Ingredients GmbH (Barmstedt, Germany). Sucrose (MW 342.30 g/mol) was purchased from Panreac Quimica SLU (Barcelona, Spain). The commercial inulin (from chicory root) was obtained from Beneo-Orafti HSI (Oreye, Belgium) (minimum 90% inulin, DP $\geq$ 10). Lyophilized powder of lysozyme from chicken egg white (L6876) (Product information: $\geq$ 90%, activity: $\geq$ 40,000 U/mg) and nisin from *Lactococcus lactis* (N5764) (Product information: 2.5%, activity: $\geq 10^6$ IU/g) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-ascorbic acid was purchased from Merck (Darmstadt, Germany). *Listeria innocua* NRRL-B 33314 (ATCC 1915) was from culture collection of the microbiology laboratory of the Department of Food Engineering at Izmir Institute of Technology. Whole fresh Starking Delicious apples (average weight: 169.0 $\pm$ 3.7) and commercial caramel sauce (Kenton, Istanbul, Turkey) were purchased from a local retail supermarket.

2.2. Methods

2.2.1. Production of gelatin based blend gels

The gel solution was prepared by blending and dissolving gelatin

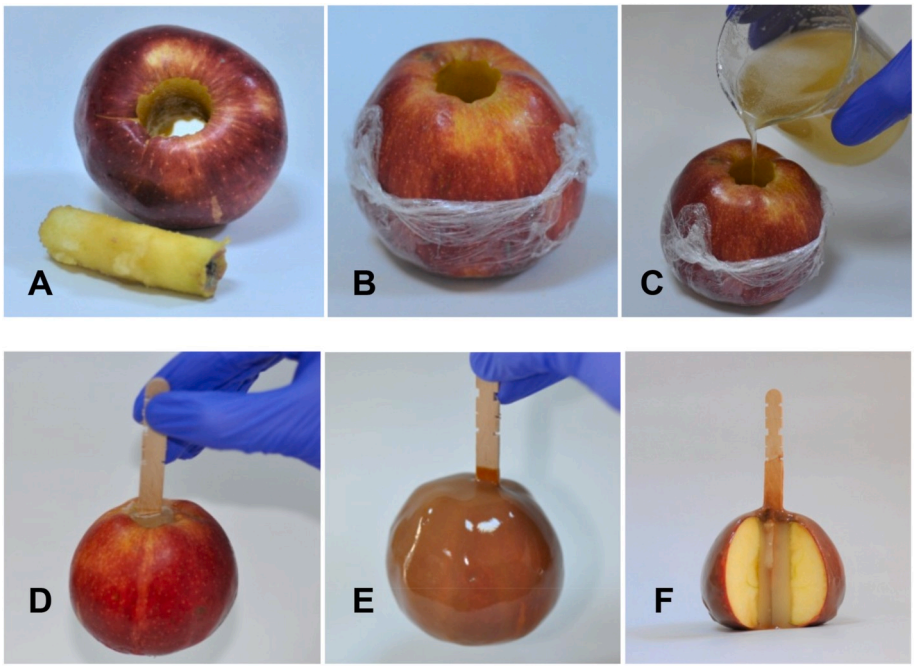

Fig. 1. Representative images of the novel method used in caramel apple production [A: coring of apple; B: wrapping of stem-end with a stretch film to prevent leakage of the gel-filling; C: pouring of gel-forming solution from blossom-end; D: solidification of gel-filling following stick immersion; E: caramel coating of apple; F: cross-sectional view of obtained caramel apple].

(GEL), inulin (IN) and sucrose (SUC) at concentrations of 14, 31 and 31% (w/v) in warm water (~50 °C) by stirring at 500 rpm, respectively. The soy protein isolate (SP) was then added into the blend at the concentration of 1.4% (w/v), and the blend was homogenized at 10,000 rpm for 2 min using a homogenizer (Heidolph Instruments, Silent Crusher M, Schwabach, Germany). In this formulation, SUC was used to control development of Listeria and other bacteria in the gel by reducing its water activity (a_w) < 0.9 while SP and IN were employed mainly to increase water holding capacity and mechanical stability of GEL based gel, respectively. The concentrations of each component were optimized with detailed preliminaries that targeted optimization of specified gel properties (data were not reported). The blend GEL-SP–IN–SUC gel solution was heated in a water bath at 85 °C for 45 min to induce gel formation of proteins. After cooling, nisin (NIS), lysozyme (LYS) and ascorbic acid (AA) were added and then dissolved (each at 1% (w/v) alone or in combination) in warm gel solutions by stirring. The heated gel solutions were then poured into molds (12-well cell culture plate having well depth of 17 mm and well diameter of 22 mm) and incubated for 20 h at 4 °C to achieve complete gelation. The cooled gels were removed gently from molds and used in different characterization studies. During application for caramel apples, the heated gels were directly used as a filling material (see section 2.2.5).

2.2.2. Mechanical and physicochemical properties of gel-forming solutions and gels

Texture profile analysis (TPA) of gels was characterized using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a compression plate (plate diameter: 75 mm; crosshead speed: 0.5 mm/s, cell load: 50 kg). Test conditions used by Fiszman and Damásio (2000) were applied with slight modifications based on our gel properties. Cylindrical gel samples (22 mm in diameter × 15 mm in height) at 4 °C were compressed up to 50% deformation. The hardness (N), springiness or elasticity (mm), resilience (dimensionless), cohesiveness (dimensionless) and gumminess (N) were determined according to Bourne (2002). Experiments of each gel were replicated twice with five repetitions.

Total soluble solid (TSS) content of gel-forming solutions was determined with a digital refractometer (Atago 3830, PAL-3, Tokyo, Japan). Measurements were replicated twice with three repetitions.

The turbidities of gel-forming solutions were determined by measuring their transmittance (% T) at 640 nm using a spectrophotometer (Shimadzu UV–Vis, Model 2450, Tokyo, Japan). Measurements were replicated twice with three repetitions.

Water activity (a_w) of gels was measured directly by placing 7.5 g gel in an a_w-meter (HygroLab, Rotronic AG, Bassersdorf, Switzerland). Measurements were replicated twice with three repetitions.

pH of gels was determined using a digital pH-meter (inoLab, Terminal, Level 3, WTW GmbH, Weilheim, Germany) as described in Brewer (2012). The samples were prepared by homogenizing 1 g of gel with 9 mL of distilled water. Measurements were replicated twice with three repetitions.

Water binding capacity (WBC) of gels was determined according to a gravimetric method of Witono, Noordergraaf, Heeres, and Janssen (2014) at 4 °C. Measurements were replicated twice with three repetitions.

2.2.3. Antimicrobial activity of gels against L. innocua in broth media

The antimicrobial tests were conducted by *L. innocua* growth and adapted at 4 °C. Discs of gels (~10 g, diameter: 6.6 cm, thickness: 0.5 cm) were placed into flasks containing 45 mL Nutrient Broth and 5 mL bacterial culture (10^7 cfu/mL). The flasks were sealed and placed into a shaker working at 80 rpm and 4 °C. The bacteria were counted at day 0, 1, 4, 7 and 14. For this purpose, 1 mL aliquot from each flask was serially diluted and spread-plated onto Oxford Listeria Selective Agar (Merck, Darmstadt, Germany) enriched with Oxford Listeria Selective Supplement (Merck, Darmstadt, Germany). The plates were incubated at 37 °C for 24 h, the colonies formed were counted and expressed as log cfu/mL. Experiments of each gel were replicated twice with two repetitions.

2.2.4. LYS and NIS release profiles of gels

To determine their soluble/bound LYS and NIS contents, gels were subjected to release tests in distilled water at 4 °C. Discs of gels (diameter: 22 mm, thickness: 5 mm) were placed into beakers containing distilled water ten times their weight (25 mL) and shaken at 80 rpm. The release tests continued until reaching equilibrium for release of LYS or NIS. The release curves were formed by plotting the calculated released activities (Unit (U) and International Unit (IU) per g of gel for LYS and NIS, respectively) versus time. The LYS activity was determined by the spectrophotometric method given by Arcan and Yemenicioğlu (2013) using *Micrococcus lysodeikticus* ATCC No. 4698 (Sigma-Aldrich, MO, USA) as a substrate. NIS activity (as IU) was determined using classical zone of inhibition assay against *Lactobacillus plantarum* as described by Teerakarn, Hirt, Acton, Rieck, and Dawson (2002). All calculations were corrected by considering the activity removed from the aqueous media during sampling. The recoveries (%) of LYS and NIS were calculated considering the ratio of total activities (U or IU) released and total activities added into gels. Experiments of each gel were replicated twice with three repetitions.

2.2.5. Application of developed antimicrobial gels to produce safe caramel apples

Briefly, apples washed extensively with tap water were cored using a stainless steel apple coring knife (Fig. 1A). This eliminates stem-ends and calyx that provide a protective site for internalization of pathogenic contaminants. The stem-end side of apples were then wrapped with a plastic stretch film to prevent leakage of gel-filling (Fig. 1B), and apples were then placed onto a flat surface keeping their stem-ends at the bottom. Gel solution was then poured inside the void cores from the blossom-end of each apple (Fig. 1C). After that, sticks were immersed into gel fillings along the vertical axis of apple core and apples were incubated at 4 °C for 20 h for solidification of antimicrobial gel filling (Fig. 1D). At the end of incubation period, the stretch film was removed and apples were dip-coated with commercial caramel solution at ~45 °C (Fig. 1E).

2.2.5.1. Effect of gel-filling on listeria counts of inoculated caramel apples.

L. innocua used in the inoculation tests was prepared by transferring one loop of frozen culture to Nutrient Broth and incubating culture at 37 °C for 24 h. One-mL aliquot from the active culture was then transferred into fresh Nutrient Broth, and it was incubated at 4 °C for 24 h to achieve adaptation of its growth at 4 °C. The initial number of the culture was ≈10^8 cfu/mL. The inner surfaces of cylindrical holes opened in apples by coring were inoculated with 0.5 mL of inoculum by spreading with a sterile plastic rod (this represents contamination of inner surface of core during processing). The inoculated apples were kept under aseptic conditions in safety cabinet for 15 min for absorption of the inoculum. The inoculated cored apples were then processed into caramel apple as described at section 2.2.5. Two different gels, GEL-SP–IN–SUC and GEL-SP–IN–SUC with LYS + NIS + AA, were filled into cored apples to obtain control gel-filled cored apples (GF_{apple}) and active gel-filled cored apples (AGF_{apple}), respectively. Some inoculated cored apples without gel-fillings and caramel coating were wrapped with stretch films to obtain control apples (Ctr_{apple}). Standard caramel apples (SC_{apple}) were also prepared by inserting sticks into apples through stem-end, pipetting 0.5 mL of inoculums between inserted stick and stem-end tissues of apples, and then coating apples with the caramel as described above. All apple samples were prepared in duplicate and stored at 4 °C for 7 days for microbiological analysis. Samples were enumerated for Listeria at day 0, 1, 3 and 7. For sampling, all caramel apples were first halved, and then the solidified gel at their center was removed and discarded. The inoculated inner surface of apple cores was then carefully excised with a

sterile knife, and a 20 g sample put into a sterile stomacher bag (BagFilter, Interscience, France) was homogenized for 210 s with 180 mL sterile 0.1% peptone water in a stomacher (BagMixer 400, Interscience, France). The serial decimal dilutions prepared from this homogenate were spread-plated onto Oxford Listeria Selective Agar (Merck, Darmstadt, Germany) enriched with Oxford Listeria Selective Supplement (Merck, Darmstadt, Germany). The plates were incubated at 37 °C for 24 h for enumeration and the colonies were counted. Listeria counts were expressed as log cfu/g apple. Experiments of each caramel apple were replicated twice with three repetitions.

2.2.5.2. Effect of gel-filling on browning of caramel apples. The color measurements were conducted using a digital colorimeter (Chromometer type, Konica Minolta, CR-400, Tokyo, Japan) standardized with a white plate (Y = 93.80, X = 0.3159, y = 0.3322) at the inner parts of halved caramel apples, after solidified gels inside their cores were removed. The measured CIE; L*, a* and b* values were used for calculation of browning index (BI) (see equation (1)) at day 0, 1, 3 and 7 days of cold storage according to Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, and Swanson (1999). Experiments of each sample were replicated twice with five repetitions.

$$BI = \frac{100(X - 0.31)}{0.172} \quad (1)$$

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (2)$$

2.2.5.3. Adhesion strength of gel-filling against sticks placed for handling of caramel apples. Tension test was performed to determine the adhesion strength of solidified gel-filling within cores of caramel apples against handling sticks placed within gel before complete solidification. This test reflected the work required to pull and liberate the stick from solidified gel in the core of caramel apple. The following texture parameters were determined by TA.XT plus texture analyzer equipped with tensile grips (crosshead speed: 0.5 mm/s, cell load: 50 kg) using stress-strain curve: tensile strength (TS) value is determined by finding maximum stress needed to liberate stick from gel-filling; elongation at break (E) value is determined from percent elongation before stick liberated from gel-filling, Young's modulus (YM) value is calculated from slope of the linear region of stress-strain curve. The area under the curve (AUC) value determined from the area of stress-strain curve gives the required energy before stick liberated from gel-filling. Peak force (PF) value is the maximum force to liberate stick from gel-filling. Measurements were done with three repetitions.

2.2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to process the data of gel samples while two-way ANOVA was performed to evaluate the storage period analysis of caramel apple samples using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., USA). Statistical differences among means were compared with Duncan's multiple range test at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Development of GEL based filling with desired mechanical properties

The stick used in candied apple products is a characteristic and essential component since it enables practical handling during consumption (Glass et al., 2015). Thus, the solidified form of developed blend GEL based filling should gain the desired mechanical properties (e.g., high firmness, gumminess, cohesiveness and resilience) essential to hold and to support the stick placed at the void apple core strongly (see Fig. 1F). A filling containing only GEL alone forms brittle gels with low adhesion to stick and it developed deep cracks and detachments during handling and storage. Thus, to improve the mechanical properties of developed gel, GEL matrix was supported by IN a prebiotic oligosaccharide that has been known for its ability to act synergetically with different hydrocolloids to improve their gel strength (BeMiller, 2018; Delgado & Bañón, 2018; Roberfroid, 2005). The texture profile analysis (TPA) results given at Table 1 clearly showed the significant improvements in hardness, cohesiveness, gumminess and resilience of GEL gels by addition of IN ($P < 0.05$). On the other hand, SP and SUC showed significant improvements only in cohesiveness and gumminess of GEL gels, respectively ($P < 0.05$). The combination of SUC with IN in GEL gels also gave significantly more cohesive and gummy gels than control GEL and GEL/IN gels. These results clearly showed that the improved mechanical characteristics of GEL gel originated from IN and SUC that improve gelation, networking and viscoelastic behavior of GEL gels.

3.2. Performance of developed GEL based filling with stick placed at cores of caramel apples

The results in Table 2 show the effects of different gel components on mechanical performance determined during pulling of sticks from cores of caramel apples containing solidified gels by using the texture analyzer. TS, E and YM values observed during pulling of sticks from solidified GEL, GEL/SP and GEL/SP/IN gel-fillings within caramel apples were not significantly different ($P > 0.05$). However, caramel apples with GEL/SP/IN gel-fillings showed 1.8 and 2.0 fold higher AUC and PF values than those of GEL filled control. Moreover, caramel apples with GEL/SP/IN/SUC gel-filling exhibited a very high adhesion to sticks and showed almost 1.9, 2.8, 1.7, 3.2, 2.2 fold higher TS, E, YM, AUC and PF values than control caramel apples filled with GEL filling. Furthermore, the gel-filling with SP and IN showed no cracking during handling and cold storage (results were not given). These results clearly showed the suitability of mechanical properties for GEL-SP–IN–SUC as a gel-filling to tightly hold the sticks placed at apple cores before gel solidification.

3.3. Physicochemical properties of GEL based gel

Some physicochemical properties of developed gels are presented in Table 3. The gel development studies were focused not only to obtain desired mechanical and textural properties, but also to minimize water activity (a_w) of gels and to increase their water binding capacity that is essential to prevent leakage of fruit juice from cored apples during storage. The addition of IN increased the total soluble solids (TSS) of GEL solutions to almost 40% while addition of IN with SUC further increased the TSS close to 60%. Thus, the a_w of GEL/SP/IN/SUC dropped below a_w of 0.92 that is a critical limit for growing of *L. monocytogenes* (Snyder, 2018; Wagner & McLauchlin, 2008). The SP did not cause an increase in TSS of gel solutions since it formed a colloidal dispersion. However, colloidal dispersion of SP tuned clear GEL solutions to highly turbid, thus, this caused dramatic drops of gel transmittance (T) values. IN alone caused less reduction in T than SP alone, but combination of IN with SUC caused a dramatic reduction in T similar to that caused by SP. It seemed that the presence of SUC turned soluble IN into colloidal form due to the increased competition of both carbohydrates for binding with water molecules (Cui, Wu, & Ding, 2013). Thus, GEL gels with SP, IN and SUC were highly turbid with T values lower than 1%. However, visual appearance is not a critical parameter in the current work since gel fillings developed will serve as a filling at the cores of apples.

The SP was added into GEL solutions to improve their water binding capacity (WBC). The results of WBC tests showed that the addition of SP increased the WBC of different gels (GEL/SP, GEL/SP/IN and GEL/SP/IN/SUC) 1.6–1.7 fold while IN and SUC had no considerable effect on WBC of gels. These results showed parallelism with those of Zhang and Zhao (2013) who reported that the combination of GEL with SP (cross-linked with enzyme transglutaminase) gave a hydrocolloid blend with higher water holding capacity. Finally, it is important to note that

Table 1
Development of mechanical properties of GEL gel to obtain a suitable caramel apple filling[b,c].

Gel samples[a]	Hardness (N)	Cohesiveness	Gumminess (N)	Springiness (mm)	Resilience
GEL	41.38 ± 6.06[b]	0.55 ± 0.13[d]	22.44 ± 5.82[d]	0.87 ± 0.07[c]	0.62 ± 0.06[b]
GEL/SP	43.70 ± 6.25[b]	0.62 ± 0.08[c]	26.80 ± 4.47[d]	0.91 ± 0.04[abc]	0.65 ± 0.05[b]
GEL/IN	51.93 ± 8.20[a]	0.77 ± 0.02[b]	39.92 ± 7.03[c]	0.91 ± 0.04[abc]	0.72 ± 0.04[a]
GEL/SP/IN	54.43 ± 5.14[a]	0.79 ± 0.02[ab]	42.89 ± 4.25[bc]	0.89 ± 0.06[bc]	0.70 ± 0.03[a]
GEL/IN/SUC	58.00 ± 5.51[a]	0.84 ± 0.01[a]	48.77 ± 5.03[a]	0.95 ± 0.02[a]	0.72 ± 0.02[a]
GEL/SP/IN/SUC	56.70 ± 6.14[a]	0.84 ± 0.01[a]	47.64 ± 5.30[ab]	0.94 ± 0.01[ab]	0.71 ± 0.03[a]

[a] GEL: gelatin, SP: soy protein, IN: inulin, SUC: sucrose.
[b] Different lower superscripts in the same column indicate significant difference ($P < 0.05$).
[c] Values are presented as mean value ± SD (n = 10).

Table 2
Adhesion strength of solidified gel-filling against sticks placed at apple cores (Note: sticks were placed at cores before solidification of gels)[b,c].

Gel samples[a]	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	AUC[d] (MPa)	Peak force (N)
GEL	1.72 ± 0.40[b]	2.91 ± 0.38[b]	0.69 ± 0.04[b]	6.67 ± 0.51[c]	29.81 ± 2.13[b]
GEL/SP	1.74 ± 0.16[b]	3.72 ± 1.02[b]	0.82 ± 0.04[b]	9.43 ± 1.09[bc]	34.84 ± 3.17[b]
GEL/SP/IN	2.53 ± 0.74[ab]	4.74 ± 2.05[b]	0.91 ± 0.03[ab]	12.15 ± 5.30[b]	59.24 ± 2.56[a]
GEL/SP/IN/SUC	3.25 ± 0.11[a]	8.08 ± 2.48[a]	1.15 ± 0.31[a]	21.21 ± 0.55[a]	64.99 ± 2.34[a]

[a] GEL: gelatin, SP: soy protein, IN: inulin, SUC: sucrose.
[b] Different lower superscripts in the same column indicate significant difference ($P < 0.05$).
[c] Values are presented as mean value ± SD (n = 3).
[d] AUC: Area under the curve.

Table 3
Total soluble solid content (%), water activity, pH, transmittance (%) and water binding capacity (g water/g gel) of GEL based gels[b,c].

Gel samples[a]	TSS (%)	a_w	pH	T (%)	WBC[d] (g water/g gel)
GEL	15.8 ± 0.40[c]	0.96 ± 0.005[a]	5.42 ± 0.20[b]	92.0 ± 1.75[a]	0.35 ± 0.07[b]
GEL/SP	16.2 ± 0.32[c]	0.97 ± 0.01[a]	5.64 ± 0.20[ab]	1.8 ± 0.12[cd]	0.60 ± 0.11[a]
GEL/IN	40.9 ± 1.01[b]	0.93 ± 0.005[b]	5.50 ± 0.16[ab]	31.0 ± 2.15[b]	0.38 ± 0.07[b]
GEL/SP/IN	40.2 ± 1.37[b]	0.94 ± 0.008[b]	5.77 ± 0.12[a]	2.20 ± 0.30[c]	0.61 ± 0.10[a]
GEL/IN/SUC	59.7 ± 2.15[a]	0.90 ± 0.01[c]	5.39 ± 0.20[b]	0.64 ± 0.05[d]	0.36 ± 0.08[b]
GEL/SP/IN/SUC	59.1 ± 0.90[a]	0.89 ± 0.006[c]	5.61 ± 0.09[ab]	0.60 ± 0.06[d]	0.55 ± 0.06[a]

[a] GEL: gelatin, SP: soy protein, IN: inulin, SUC: sucrose.
[b] Different lower superscripts in the same column indicate significant difference ($P < 0.05$).
[c] Values are presented as mean value ± SD (n = 6).
[d] Data referred to 72 h after water immersion of gels.

the pH of gels showed a limited variation between pH 5.4 and 5.8. The isoelectric points (pIs) of GEL type B and SP are ~4.8 and ~4.5, respectively (Hartel, von Elbe, & Hofberger, 2018; Lusas & Rhee, 1995). Thus, it is clear that both GEL and SP are mainly negatively charged within the gels.

3.4. LYS and NIS release profiles of GEL based gel

The release profiles of major antimicrobial compounds, LYS and NIS, from developed GEL/SP/IN/SUC (G) blend gels with or without AA are seen in Fig. 2. The release tests were conducted with G gels with LYS or NIS alone (not with combination of LYS + NIS) to prevent interaction of LYS with the NIS determination method that depends on a zone inhibition test (see section 2.2.4). The very low maximum recoveries of LYS (~2% at day 4) and NIS (~0.6% at day 1) from G/LYS and G/NIS gels clearly showed that the G gel binds the protein-based antimicrobial compounds effectively. This result was expected since LYS (pI: 11.4) and NIS (pI: ~9) (Losso, Nakai, & Charter, 2000; Silvetti, Morandi, Hintersteiner, & Brasca, 2017; Zhang, 2019) are positively charged while major proteins of gel matrix, GEL and SP, are negatively charged at the G gel pH of 5.6. On the other hand, the maximum recoveries of LYS and NIS from G/LYS + AA and G/NIS + AA gels were almost 76 and 4%, respectively. The addition of AA into G gel reduced the gel pH from 5.6 to 4.1, a pH below the pI values of GEL and SP. Thus, it appeared that the reduced negative charges, but increased positive charges of G gel matrix caused liberation of a significant portion of positively charged bound LYS from the gels. In contrast, only a slight portion of bound NIS was liberated from the G gels by the pH change caused by AA. This finding suggested that the binding of NIS by the G gel matrix occurs by some other bonds and/or interactions (e.g. hydrophobic interactions and/or H-bonds).

3.5. Antimicrobial activity of GEL based gel against L. innocua in broth media

The antimicrobial effects of GEL/SP/IN/SUC (G) blend gel with different active agents (LYS, NIS, AA) alone or in combination on *L. innocua* was studied in broth media incubated at 4 °C (Table 4). There were no significant differences among initial *L. innocua* counts of broth cultures containing different gels ($P > 0.05$). The control culture lacking G gel and culture with G gel showed no significant change in Listeria counts during 1-week incubation ($P > 0.05$). The G/AA is also not very effective on *L. innocua* and caused only a very limited drop (−0.3 log) in

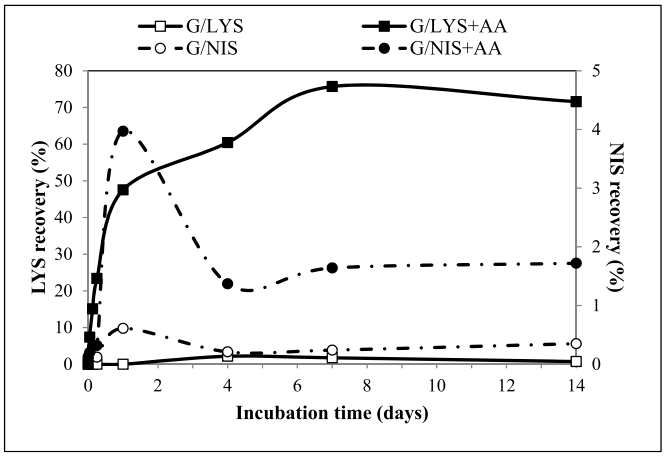

Fig. 2. LYS (primary y-axis) and NIS (secondary y-axis) release profiles of GEL-SP–IN–SUC gels (G) with or without ascorbic acid (AA) during incubation in distilled water at 4 °C.

Table 4
Antimicrobial activity of GEL based gels against *L. innocua* in broth media incubated at 4 °C.

Gel samples[a]	*L. innocua* (Log cfu/mL)[b,c]				
	Day 0	Day 1	Day 4	Day 7	Day 14
Control	7.6 ± 0.04^{a,A}	7.6 ± 0.12^{a,A}	7.6 ± 0.08^{a,A}	7.6 ± 0.04^{a,A}	7.5 ± 0.06^{a,A}
G	7.5 ± 0.13^{a,A}	7.4 ± 0.31^{a,A}	6.8 ± 0.73^{a,BC}	6.8 ± 0.64^{a,BC}	7.2 ± 0.90^{a,A}
G/LYS	7.6 ± 0.08^{a,A}	7.4 ± 0.16^{a,A}	6.6 ± 0.26^{b,C}	6.7 ± 0.36^{b,C}	5.8 ± 0.21^{c,B}
G/NIS	7.6 ± 0.05^{a,A}	6.0 ± 0.40^{b,B}	4.2 ± 0.50^{c,D}	4.2 ± 0.16^{c,D}	2.5 ± 0.58^{d,C}
G/AA	7.6 ± 0.05^{a,A}	7.6 ± 0.07^{a,A}	7.4 ± 0.04^{b,AB}	7.3 ± 0.08^{b,BA}	7.0 ± 0.14^{c,A}
G/LYS + NIS	7.6 ± 0.02^{a,A}	5.7 ± 0.11^{b,B}	3.3 ± 0.75^{c,E}	3.2 ± 0.15^{c,E}	1.3 ± 0.02^{d,D}
G/LYS + NIS + AA	7.6 ± 0.05^{a,A}	4.8 ± 0.33^{b,C}	3.7 ± 0.40^{c,DE}	2.4 ± 0.35^{d,F}	<1

[a] Control: control culture (no gel); G: GEL/SP/IN/SUC gel; G/LYS: GEL/SP/IN/SUC gel with lysozyme; G/NIS: GEL/SP/IN/SUC gel with nisin; G/AA: GEL/SP/IN/SUC gel with ascorbic acid; G/LYS + NIS: GEL/SP/IN/SUC gel with lysozyme and nisin; G/LYS + NIS + AA: GEL/SP/IN/SUC gel with lysozyme, nisin, and ascorbic acid.
[b] Different lower and capital letter superscripts in the same row and column indicate significant differences ($P < 0.05$), respectively..
[c] Values are presented as mean ± SD (n = 4).

initial bacterial counts within 1-week. However, dramatic drops were observed in *L. innocua* counts of cultures with G/NIS (−1.6 log), G/LYS + NIS (−1.9 log), or G/LYS + NIS + AA (−2.8 log) after 1 day incubation ($P < 0.05$). The *L. innocua* counts of cultures incubated 1 day with G/NIS and G/LYS + NIS were not significantly different from each other while culture incubated with G/LYS + NIS + AA for 1 day had significantly lower *L. innocua* count than cultures with other gels ($P < 0.05$). The G/LYS was less effective than these three potent gels and it took 4 days for this gel to cause a significant drop (−1 log) in initial *L. innocua* counts of culture. The respective log reductions in initial Listeria counts of cultures with different active gels after 1- and 2-weeks cold storage showed that the ranking of antimicrobial potency of gels from lowest to highest is as follows: G/LYS (−0.9 and −1.8 log) < G/NIS (−3.4 and −5.1 log) < G/LYS + NIS (−4.4 and −6.3 log) < G/LYS + NIS + AA (−5.2 and > −6.6 log). These results clearly proved the effectiveness of NIS alone on Listeria, but the most potent gels were obtained by combinational use of LYS + NIS or LYS + NIS + AA. The release tests conducted with G/LYS + AA and G/NIS + AA gels showed that the pH drop caused by AA increased the amounts of soluble LYS and NIS (see Fig. 2). Thus, this explained why G/LYS + NIS + AA was the most effective gel on Listeria. The synergy formed by combinational use of LYS + NIS against Listeria has also recently been demonstrated by our research group for both soluble and bound forms incorporated into chitosan films (Sozbilen & Yemenicioğlu, 2020). Moreover, synergy between LYS and NIS has also been demonstrated by different workers against *L. monocytogenes*, *Brochothrix thermosphacta, Staphylococcus aureus, Carnobacterium* sp. and food spoilage lactobacilli (Anderson, 1992; Chung & Hancock, 2000; Gill & Holley, 2000a, 2000b; Monticello, 1990; Nattress, Yost, & Baker, 2001; Proctor & Cunningham, 1992; Takahashi et al., 2012). It was reported that the decomposition of peptidoglycan layer of bacterial cell walls by LYS, enables easy access and then subsequent binding of NIS to the sensitive cell membranes which otherwise exist buried beneath the cell surface (Dawson et al., 1996; Monticello, 1990). Thus, combinational use of LYS and NIS has been suggested not only to increase effectiveness of bacterial inactivation, but also to prevent bacterial resistance problems (Pereda, Ansorena, & Marcovich, 2017; Sudagidan & Yemenicioğlu, 2012).

3.6. Effect of GEL based filling on listeria counts of inoculated caramel apples

The results showing antimicrobial effect of developed G (GEL-SP–IN–SUC) gel with LYS + NIS + AA when it was filled into cores of Listeria inoculated caramel apples during 1-week cold storage are presented in Table 5. There were no significant differences among the initial (day 0) Listeria counts of Ctr_{apple}, SC_{apple} and GF_{apple} while AGF_{apple} that contained the developed antimicrobial gel-filling had 2 to 2.5 lower log initial Listeria counts than the controls and other caramel apples. No significant changes occurred in Listeria count of Ctr_{apple} within 3 days, but extended cold storage for 7 days caused a significant increase in Listeria count ($P < 0.05$). The Listeria counts of SC_{apple} and GF_{apple} samples at the end of 3 days were significantly higher than those of their respective initial counts at day 0 ($P < 0.05$). The significant increase in Listeria load of SC_{apple} also continued between days 3 and 7 while GF_{apple} did not show further significant increase in its Listeria load within the same time period. In contrast, the Listeria counts of AGF_{apple} at days 3 and 7 were not significantly different than that at day 0. This result showed that the developed antimicrobial gel-filling prevented the development of Listeria in caramel apples during 7 days of cold storage. As a result, the AGF_{apple} showed 2.5 to 2.9 lower log Listeria counts than other samples at the end of day 7. The release tests conducted in distilled water suggested that addition of AA into gels solubilized majority of LYS. Thus, it appeared that the inoculated Listeria outside the active gel in AGF_{apple} (at apple core tissue around the gel and at apple-gel interface) interacted mainly with soluble LYS while Listeria contacted with or penetrated within gel interacted with soluble and bound forms of both LYS and NIS.

It was reported in the literature that Listeria growth in inoculated caramel apples produced by classical caramel dipping process applied at 95 °C initiated after first week of refrigerated storage (at 7 °C) and increased almost 1 log at the second week (Glass et al., 2015). However, listeria growth in caramel apples exposed to temperature abuse at room temperature initiated rapidly and showed a 3 log increase within 3 days (Glass et al., 2015). Thus, it appears that caramel apples produced by the current antimicrobial gel-filling procedure and stored for 1-week under cold storage (showed 2.9 lower log Listeria count than SC_{apple}) gained a significant protection against Listeria. However, further studies are needed to conduct inoculation tests directly with different *Listeria monocytogenes* strains and determine safety limits of novel caramel apple product at temperature abuse or extended storage conditions.

In the literature, the LYS + NIS combination has been tested against *Listeria* spp. in different risky food such as sausages (Monticello, 1990), hotdog (Proctor & Cunningham, 1992), ham or bologna (Gill & Holley, 2000a, 2000b; Mangalassary, Han, Rieck, Acton, & Dawson, 2008), minced meat (Morsy et al., 2018) and seafood products such as smoked salmon (Datta, Janes, Xue, Losso, & La Peyre, 2008), minced tuna and

Table 5
Effect of GEL based antimicrobial gel-filling on *L. innocua* counts of inoculated caramel apples stored at 4 °C.

Samples[a]	*L. innocua* count (Log cfu/g)[b,c]			
	Day 0[d]	Day 1	Day 3	Day 7
Ctr_{apple}	5.3 ± 0.98^{b,A}	5.8 ± 0.27^{b,A}	5.6 ± 0.28^{b,B}	6.8 ± 0.12^{a,A}
SC_{apple}	5.8 ± 0.07^{c,A}	5.7 ± 0.17^{c,A}	6.0 ± 0.34^{b,A}	6.9 ± 0.55^{a,A}
GF_{apple}	5.8 ± 0.24^{c,A}	6.1 ± 0.20^{bc,A}	6.4 ± 0.35^{ab,A}	6.5 ± 0.28^{a,A}
AGF_{apple}	3.3 ± 0.26^{ab,B}	2.9 ± 0.89^{b,B}	3.6 ± 0.44^{ab,C}	4.0 ± 0.69^{a,B}

[a] Ctr_{apple}: control cored inoculated apple; SC_{apple}: standard inoculated caramel apple; GF_{apple}: cored inoculated apple filled with GEL-SP–IN–SUC gel; AGF_{apple}: cored inoculated apple filled with GEL-SP–IN–SUC gel with LYS + NIS + AA (each at 1% concentration).
[b] Different lower and capital letter superscripts in the same row and column indicate significant differences ($P < 0.05$), respectively.
[c] Values are presented as mean ± SD (n = 4).
[d] Listeria load of apples immediately after caramel coating.

salmon roe (Takahashi et al., 2012). Moreover, recently synergetic mixture of LYS and NIS incorporated into chitosan films has been successfully used to inactivate Listeria on seeds destined for edible sprout production (Sozbilen & Yemenicioğlu, 2020). However, to our knowledge, the current work is the first report about anti-Listeria potential of LYS + NIS combination in minimally processed fruits.

3.7. Effect of GEL based filling on browning index and color of caramel apples

The polyphenoloxidase (PPO), an oxidoreductase group enzyme that catalyzes the oxidation of polyphenols, is responsible for the browning of apples. The control of discoloration in core regions of apples is a highly challenging issue since PPO locates mainly around these tissues (Bhowmik & Dris, 2004). The browning index (BI) of core regions for controls and caramel apples are presented at Table 6. There were no significant differences among BI of apples used for Ctr_{apple}, GF_{apple} and AGF_{apple} immediately after coring. The BI of all apples increased significantly during the time past between coring and caramel coating ($P < 0.05$), but the increases in BI of Ctr_{apple} and GF_{apple} (3.4 and 3 fold, respectively) at day 0 of cold storage were considerably higher than that of AGF_{apple} (1.9 fold) containing ascorbic acid (AA) within its gel-filling. As expected, the BI of Ctr_{apple} lacking any gel-filling within its core increased most rapidly and reached significantly higher levels than those of GF_{apple} and AGF_{apple} within 3 days ($P < 0.05$). The BI of GF_{apple} reached a peak point at day 1 and it declined slightly by further storage. It is clear that the control gel-filling lacking AA is also slightly beneficial to suppress browning, possibly by limiting contact of PPO with air oxygen. On the other hand, it is important to note that the BI of AGF_{apple} did not change significantly during cold storage ($P > 0.05$). The photos of Ctr_{apple}, GF_{apple} and AGF_{apple} samples after 1-week cold storage clearly showed the beneficial effect of using active gel-filling with AA (Fig. 3). The cores of Ctr_{apple} and GF_{apple} turned dark brown (Fig. 3A) and brown (Fig. 3B), respectively. In contrast, AGF_{apple} maintained its light yellowish color (Fig. 3C). These results clearly showed the effective inhibition of browning by AA that is not only a reducing agent that turns enzyme-oxidized quinones back to diphenols, but also inactivates enzyme PPO by showing competitive inhibition (Mishra, Gautam, & Sharma, 2012). The gel-fillings with AA have not been applied previously to prevent enzymatic browning of cored apples. However, different reports exist related to use of 1% AA in edible coatings (e.g., those from whey protein-beeswax composite, alginate, pectin and gellan gum) to prevent enzymatic browning in fresh-cut apples (Moreira, Cassani, Martín-Belloso, & Soliva-Fortuny, 2015; Perez-Gago, Serra, & del Río, 2006).

Table 6
Effect of active gel-filling on browning of caramel apples stored at 4 °C.

Samples[a]	Browning index[b,c]				
	After coring[d]	Day 0[e]	Day 1	Day 3	Day 7
Ctr_{apple}	41.75 ± 14.31^{d,A}	140.49 ± 18.48^{c,A}	174.35 ± 26.02^{b,A}	202.74 ± 22.65^{a,A}	181.35 ± 14.07^{b,A}
GF_{apple}	42.97 ± 13.62^{d,A}	129.01 ± 15.27^{c,A}	185.33 ± 43.22^{a,A}	165.91 ± 30.58^{ab,B}	154.85 ± 22.95^{b,B}
AGF_{apple}	42.11 ± 11.98^{b,A}	81.79 ± 15.38^{a,B}	76.02 ± 18.36^{a,B}	76.77 ± 9.58^{a,C}	71.16 ± 9.16^{a,C}

[a] Ctr_{apple}: control cored apple; GF_{apple}: cored apple filled with GEL-SP–IN–SUC gel; AGF_{apple}: cored apple filled with GEL-SP–IN–SUC gel with LYS + NIS + AA (each at 1% concentration).

[b] Different lower and capital letter superscripts in the same row and column indicate significant differences ($P < 0.05$), respectively.

[c] Values are presented as mean ± SD (n = 10).

[d] ,[e] Color of apples immediately after coring of apples and caramel coating, respectively.

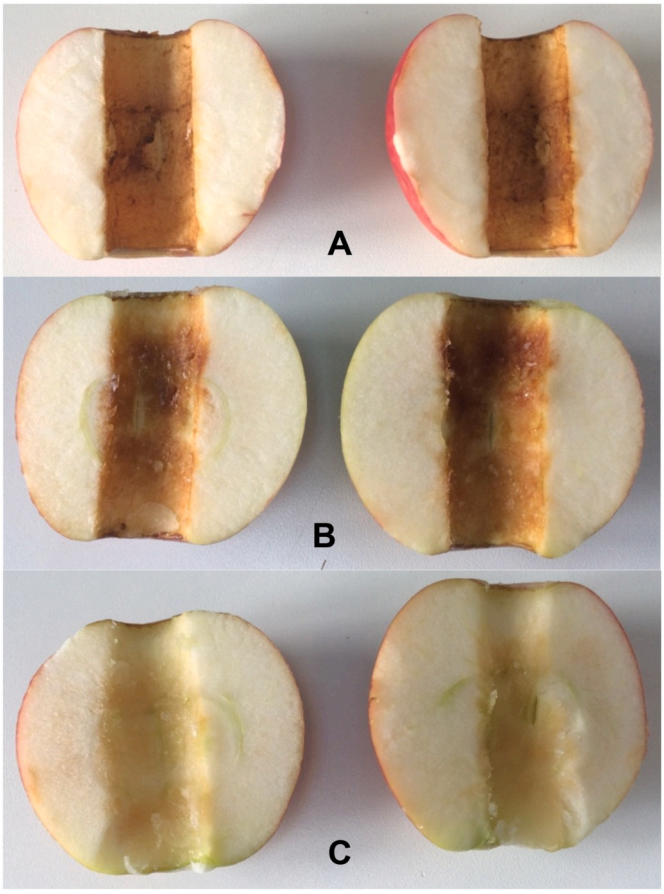

Fig. 3. Cross-section photos of different cored apples after 1-week cold storage at 4 °C [A: Ctr_{apple}: control cored apple; B: GF_{apple}: cored apple filled with GEL-SP–IN–SUC gel; C: AGF_{apple}: cored apple filled with GEL-SP–IN–SUC gel with LYS + NIS + AA (each at 1% concentration)] (Note: gels were removed from cores just before photographing).

4. Conclusions

The current work clearly showed possibility of changing classical candied apple processing method and adapting active gel-filling in their production with an innovative design. The GEL based gel-forming solution supported with SP, IN and SUC forms hard, mechanically stable, adhesive gel when it is filled within cored apples. The gel-filling strongly holds and supports the stick placed within the core of caramel apple before gel solidification. The active compounds (LYS, NIS and AA) added within the gel-filling successfully inhibits Listeria and enzymatic browning in caramel apples. This work is the first study that exploits both the physicochemical and active properties of gels to increase safety and quality of candied apples. Further studies are needed to improve in industrial applicability of developed safe caramel apple production technique against Listeria.

Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

CRediT authorship contribution statement

Pelin Barış Kavur: Formal analysis, Data curation, Writing - original draft. **Ahmet Yemenicioğlu:** Formal analysis, Writing - original draft.

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References

Abhari, N., Madadlou, A., Dini, A., & Naveh, O. H. (2017). Textural and cargo release attributes of trisodium citrate cross-linked starch hydrogel. *Food Chemistry, 214*, 16–24. https://doi.org/10.1016/j.foodchem.2016.07.042.

Ahmed, M. J., Singh, Z., & Khan, A. S. (2009). Postharvest *Aloe vera* gel-coating modulates fruit ripening and quality of 'Arctic Snow' nectarine kept in ambient and cold storage. *International Journal of Food Science and Technology, 44*(5), 1024–1033. https://doi.org/10.1111/j.1365-2621.2008.01873.x.

Álvarez-Ordóñez, A., Leong, D., Hickey, B., Beaufort, A., & Jordan, K. (2015). The challenge of challenge testing to monitor *Listeria monocytogenes* growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. *Food Research International, 75*, 233–243. https://doi.org/10.1016/j.foodres.2015.06.004.

Anderson, W. A. (1992). Compositions having antibacterial properties and use of such compositions in suppressing growth of microorganisms, eg. Listeria bacteria. *European Patent*, 0 466 244 A1 (pp. 6-11).

Angelo, K. M., Conrad, A. R., Saupe, A., Dragoo, H., West, N., Sorenson, A., et al. (2017). Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014-2015. *Epidemiology and Infection, 145*(5), 848–856. https://doi.org/10.1017/S0950268816003083.

Arcan, I., & Yemenicioğlu, A. (2013). Development of flexible zein-wax composite and zein-fatty acid blend films for controlled release of lysozyme. *Food Research International, 51*(1), 208–216. https://doi.org/10.1016/j.foodres.2012.12.011.

Batista, R. A., Espitia, P. J. P., Quintans, J. D. S. S., Freitas, M. M., Cerqueira, M.Â., Teixeira, J. A., et al. (2019). Hydrogel as an alternative structure for food packaging systems. *Carbohydrate Polymers, 205*, 106–116. https://doi.org/10.1016/j.carbpol.2018.10.006.

BeMiller, J. N. (2018). Inulin and konjac glucomannan. In J. N. BeMiller (Ed.), *Carbohydrate chemistry for food scientists*. Elsevier.

Bhowmik, P. K., & Dris, R. (2004). Enzymes and quality factors of fruits and vegetables. In R. Dris, & S. M. Jain (Eds.), *Production practices and quality assessment of food crops, volume 3: Quality handling and evaluation*. Kluwer Academic.

Bourne, M. C. (2002). Principles of objective texture measurement. In M. C. Bourne (Ed.), *Food texture and viscosity: Concept and measurement*. Elsevier.

Bouvier, N. M. (2015). The curious case of the caramel apples. *Science Translational Medicine, 7*(311), 311ec184. https://doi.org/10.1126/scitranslmed.aad5500.

Brewer, S. (2012). Quality measurements in beef. In L. M. L. Nollet (Ed.), *Handbook of meat, poultry and seafood quality*. Wiley-Blackwell.

Campia, P., Ponzini, E., Rossi, B., Farris, S., Silvetti, T., Merlini, L., et al. (2017). Aerogels of enzymatically oxidized galactomannans from leguminous plants: Versatile delivery systems of antimicrobial peptides and enzymes. *Carbohydrate Polymers, 158*, 102–111. https://doi.org/10.1016/j.carbpol.2016.11.089.

Carstens, C. K., Salazar, J. K., Bathija, V. M., Narula, S. S., Wang, P., & Tortorello, M. L. (2018). Control of *Listeria monocytogenes* in caramel apples by use of sticks pretreated with potassium sorbate. *Journal of Food Protection, 81*(12), 1921–1928. https://doi.org/10.4315/0362-028X.JFP-18-175.

Centers for Disease Control and Prevention. (2015). Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples (Final Update). https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html Accessed January 2020.

Chung, W., & Hancock, R. E. W. (2000). Action of lysozyme and nisin mixtures against lactic acid bacteria. *International Journal of Food Microbiology, 60*(1), 25–32. https://doi.org/10.1016/s0168-1605(00)00330-5.

Cui, S. W., Wu, Y., & Ding, H. (2013). The range of dietary fibre ingredients and a comparison of their technical functionality. In J. A. Delcour, & K. Poutanen (Eds.), *Fibre-rich and wholegrain foods*. Woodhead.

Datta, S., Janes, M. E., Xue, Q. G., Losso, J., & La Peyre, J. F. (2008). Control of *Listeria monocytogenes* and *Salmonella anatum* on the surface of smoked salmon coated with calcium alginate coating containing oyster lysozyme and nisin. *Journal of Food Science, 73*(2), M67–M71. https://doi.org/10.1111/j.1750-3841.2007.00633.x.

Dawson, P. L., Acton, J. C., Han, I. Y., Padgett, T., Orr, R., & Larsen, T. (1996). Incorporation of antibacterial compounds into edible and biodegradable packaging films. *Activities Report of the Research & Development Associates, 48*, 203–210.

Delgado, P., & Bañón, S. (2018). Effects of replacing starch by inulin on the physicochemical, texture and sensory characteristics of gummy jellies. *CyTA - Journal of Food, 16*(1), 1–10. https://doi.org/10.1080/19476337.2017.1327462.

Fiszman, S. M., & Damásio, M. H. (2000). Suitability of single-compression and TPA tests to determine adhesiveness in solid and semi-solid foods. *Journal of Texture Studies, 31*(1), 55–68. https://doi.org/10.1111/j.1745-4603.2000.tb00284.x.

Gill, A. O., & Holley, R. A. (2000a). Surface application of lysozyme, nisin, and EDTA to inhibit spoilage and pathogenic bacteria on ham and bologna. *Journal of Food Protection, 63*(10), 1338–1346. https://doi.org/10.4315/0362-028x-63.10.1338.

Gill, A. O., & Holley, R. A. (2000b). Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. *Food Research International, 33*(2), 83–90. https://doi.org/10.1016/S0963-9969(99)00152-0.

Glass, K. A., Golden, M. C., Wanless, B. J., Bedale, W., & Czuprynski, C. (2015). Growth of *Listeria monocytogenes* within a caramel-coated apple microenvironment. *MBio, 6*(5). https://doi.org/10.1128/mBio.01232-15. e01232-15.

Gustafson, R. E., & Ryser, E. T. (2017). Thermal inactivation and growth of *Listeria monocytogenes* during production and storage of caramel apples. *Food Control, 79*, 234–238. https://doi.org/10.1016/j.foodcont.2017.03.043.

Hartel, R. W., von Elbe, J. H., & Hofberger, R. (2018). Starches, proteins, pectin and gums. In R. W. Hartel, J. H. von Elbe, & R. Hofberger (Eds.), *Confectionery science and technology*. Springer.

Losso, J. N., Nakai, S., & Charter, E. A. (2000). Lysozyme. In A. S. Naidu (Ed.), *Natural food antimicrobial systems*. CRC Press.

Lusas, E. W., & Rhee, K. C. (1995). Soy protein processing and utilization. In D. R. Erickson (Ed.), *Practical handbook of soybean processing and utilization*. AOCS Press.

Mangalassary, S., Han, I., Rieck, J., Acton, J., & Dawson, P. (2008). Effect of combining nisin and/or lysozyme with in-package pasteurization for control of *Listeria monocytogenes* in ready-to-eat Turkey bologna during refrigerated storage. *Food Microbiology, 25*(7), 866–870. https://doi.org/10.1016/j.fm.2008.05.002.

Mishra, B. B., Gautam, S., & Sharma, A. (2012). Purification and characterization of polyphenol oxidase (PPO) from eggplant (*Solanum melongena*). *Food Chemistry, 134*(4), 1855–1861. https://doi.org/10.1016/j.foodchem.2012.03.098.

Monticello, D. J. (1990). Control of microbial growth with nisin/lysozyme formulations. *European Patent*, 0 374 823 B1 (pp. 5-8).

Moreira, M. R., Cassani, L., Martín-Belloso, O., & Soliva-Fortuny, R. (2015). Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *Journal of Food Science & Technology, 52*(12), 7795–7805. https://doi.org/10.1007/s13197-015-1907-z.

Moritaka, H., & Naito, S. (2002). Agar and gelatin gel flavor release. *Journal of Texture Studies, 33*(3), 201–214. https://doi.org/10.1111/j.1745-4603.2002.tb01345.x.

Morsy, M. K., Elsabagh, R., & Trinetta, V. (2018). Evaluation of novel synergistic antimicrobial activity of nisin, lysozyme, EDTA nanoparticles, and/or ZnO nanoparticles to control foodborne pathogens on minced beef. *Food Control, 92*, 249–254. https://doi.org/10.1016/j.foodcont.2018.04.061.

Murray, K., Moyer, P., Wu, F., Goyette, J. B., & Warriner, K. (2018). Inactivation of *Listeria monocytogenes* on and within apples destined for caramel apple production by using sequential forced air ozone gas followed by a continuous advanced oxidative process treatment. *Journal of Food Protection, 81*(3), 357–364. https://doi.org/10.4315/0362-028X.JFP-17-306.

Nattress, F. M., Yost, C. K., & Baker, L. P. (2001). Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. *International Journal of Food Microbiology, 70*(1–2), 111–119. https://doi.org/10.1016/S0168-1605(01)00531-1.

Palou, E., López-Malo, A., Barbosa-Cánovas, G. V., Welti-Chanes, J., & Swanson, B. G. (1999). Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. *Journal of Food Science, 64*(1), 42–45. https://doi.org/10.1111/j.1365-2621.1999.tb09857.x.

Park, J. W. (1994). Functional protein additives in surimi gels. *Journal of Food Science, 59*(3), 525–527. https://doi.org/10.1111/j.1365-2621.1994.tb05554.x.

Pereda, M., Ansorena, M. R., & Marcovich, N. E. (2017). Active biopackaging based on proteins. In M. A. Masuelli (Ed.), *Biopackaging*. CRC Press.

Perez-Gago, M. B., Serra, M., & del Río, M. A. (2006). Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biology and Technology, 39*(1), 84–92. https://doi.org/10.1016/j.postharvbio.2005.08.002.

Proctor, V. A., & Cunningham, F. E. (1992). The antimicrobial properties of lysozyme alone and in combination with other additives in vitro and in selected meat products. *Journal of Rapid Methods and Automation in Microbiology, 1*(4), 315–328. https://doi.org/10.1111/j.1745-4581.1992.tb00279.x.

Roberfroid, M. (2005). Inulin: A fructan. In M. Roberfroid (Ed.), *Inulin-type fructans: Functional food ingredients*. CRC Press.

Rojas-Graü, M. A., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: A review. *International Journal of Food Science and Technology, 44*(5), 875–889. https://doi.org/10.1111/j.1365-2621.2009.01911.x.

Salazar, J. K., Carstens, C. K., Bathija, V. M., Narula, S. S., Parish, M., & Tortorello, M. L. (2016). Fate of *Listeria monocytogenes* in fresh apples and caramel apples. *Journal of Food Protection, 79*(5), 696–702. https://doi.org/10.4315/0362-028X.JFP-15-442.

Serrano, M., Valverde, J. M., Guillén, F., Castillo, S., Martínez-Romero, D., & Valero, D. (2006). Use of *Aloe vera* gel coating preserves the functional properties of table grapes. *Journal of Agricultural and Food Chemistry, 54*(11), 3882–3886. https://doi.org/10.1021/jf060168p.

Silvetti, T., Morandi, S., Hintersteiner, M., & Brasca, M. (2017). Use of hen egg white lysozyme in the food industry. In P. Y. Hester (Ed.), *Egg innovations and strategies for improvements*. Academic Press.

Snyder, O. P. (2018). Foodservice operations: HACCP control programs. In Y. H. Hui, S. A. Sattar, K. D. Murrell, W. K. Nip, & P. S. Stanfield (Eds.), *Foodborne disease handbook, volume 2: Viruses, parasites, pathogens, and HACCP*. CRC Press.

Sozbilen, G. S., & Yemenicioğlu, A. (2020). Decontamination of seeds destined for edible sprout production from *Listeria* by using chitosan coating with synergetic lysozyme-nisin mixture. *Carbohydrate Polymer, 235*, 115968. https://doi.org/10.1016/j.carbpol.2020.115968.

Sudagidan, M., & Yemenicioğlu, A. (2012). Effects of nisin and lysozyme on growth inhibition and biofilm formation capacity of *Staphylococcus aureus* strains isolated from raw milk and cheese samples. *Journal of Food Protection, 75*(9), 1627–1633. https://doi.org/10.4315/0362-028X.JFP-12-001.

Takahashi, H., Kashimura, M., Miya, S., Kuramoto, S., Koiso, H., Kuda, T., et al. (2012). Effect of paired antimicrobial combinations on *Listeria monocytogenes* growth

inhibition in ready-to-eat seafood products. *Food Control, 26*(2), 397–400. https://doi.org/10.1016/j.foodcont.2012.02.005.

Teerakarn, A., Hirt, D. E., Acton, J. C., Rieck, J. R., & Dawson, P. L. (2002). Nisin diffusion in protein films: Effects of film type and temperature. *Journal of Food Science, 67*(8), 3019–3025. https://doi.org/10.1111/j.1365-2621.2002.tb08853.x.

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., et al. (2001). *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews, 14*, 584–640. https://doi.org/10.1128/CMR.14.3.584-640.2001.

Wagner, M., & McLauchlin, J. (2008). Biology. In D. Liu (Ed.), *Handbook of Listeria monocytogenes*. CRC Press.

Witono, J. R., Noordergraaf, I. W., Heeres, H. J., & Janssen, L. P. B. M. (2014). Water absorption, rete0ntion and the swelling characteristics of cassava starch grafted with polyacrylic acid. *Carbohydrate Polymers, 103*, 325–332. https://doi.org/10.1016/j.carbpol.2013.12.056.

Zhang, Q. (2019). Lactic acid bacteria and bacteriocins. In W. Chen (Ed.), *Lactic acid bacteria: Bioengineering and industrial applications*. Springer.

Zhang, Y. N., & Zhao, X. H. (2013). Study on the functional properties of soybean protein isolate cross-linked with gelatin by microbial transglutaminase. *International Journal of Food Properties, 16*(6), 1257–1270. https://doi.org/10.1080/10942912.2011.583705.