

Apple Peel Extract-Loaded Fish Skin Gelatin/PVA Films: Functional Properties and Soft Cheese Application

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Abstract

[Background and Objectives: Active packaging films that utilize natural antimicrobial and antioxidant agents are a promising alternative to traditional synthetic preservatives; the aim of this study was to fully characterize active films produced with fish skin gelatin (FSG) and polyvinyl alcohol (PVA) containing varying levels of apple peel extract (APE), which is rich in polyphenolic compounds, and determine the effectiveness of these active films in extending the shelf life of soft cheese during refrigerated storage. **Methods:** Active films were created containing 0%, 1%, 2%, and 3% APE by mass; the characterization of the films included assessment of physicochemical properties (i.e., thickness, moisture content, water vapor permeability [WVP], solubility), mechanical properties (i.e., tensile strength [TS], elongation at break [EAB], Young's modulus), thermal characterization ((thermal analysis using differential scanning calorimetry [DSC] and thermogravimetric analysis [TGA]), structural characterization (Fourier transform infrared [FTIR] and X-ray diffraction analysis [XRD]), and optical characterization. Antimicrobial efficacy was determined using minimum inhibitory concentration (MIC), agar disc diffusion, and challenge studies on *Listeria monocytogenes* and *Staphylococcus aureus*. The release kinetics of polyphenols from the packaging films were estimated using food simulants (3% acetic acid and 10% ethanol) and the modeling was done according to the European Union Regulation No. 10/2011. To determine the efficacy of each film to extend the shelf life of soft cheese, soft cheese wrapped in each of the films was evaluated after 28 days of storage at 4°C by microbiological analysis, based on chemical parameters, thiobarbituric acid (TBA) analysis, and sensory properties evaluated using a 9-point hedonic scale. Total migration of packaging material into soft cheese was determined based on Saudi Food and Drug Authority (SFDA) and European Food Safety Authority (EFSA) regulations. **Results:** *Listeria monocytogenes* MIC and *Staphylococcus aureus* MIC were 6.25 mg/mL and 3.13 mg/mL, respectively, when tested against APE solutions; an increase in antioxidant activity ($87.4 \pm 1.2\%$) as measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was observed at the highest concentration (3%) of APE; the WVP of the films with APE was significantly lower ($1.82 \pm 0.09 \times 10^{-10} \text{ g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$) at the 3% APE level; and the TS of the films with APE was significantly higher at the 3% APE level ($18.6 \pm 0.7 \text{ MPa}$). The thermal analysis supported an increase in thermal stability of the films, as indicated by DSC data, where the melting temperature (T_m) of the films with no APE was 68.2°C, while the T_m of the films containing 3% APE was 74.1°C. The decrease in the crystallinity index of the active films, from 42.3% to 28.6%, measured by XRD analysis supports an even distribution of the polyphenols within the active films.

1.

Introduction

Soft cheeses are extremely popular foods around the world that typically contain higher moisture than the majority of other cheeses (50-80 %), thus, they have the highest water activity and are extremely perishable (i.e, susceptible to spoilage) due to mould, yeast, and pathogenic bacterial growth. In addition, three of the most common Human pathogenic bacteria such as *Listeria monocytogenes*[MT5.1], *Staphylococcus aureus*, and coliforms may contaminate soft cheese (1). The Food and Agriculture Organization of the United Nations (FAO) claims that one-third of the food that is produced or harvested in the world is wasted every year; dairy products are significantly responsible for many financial losses in developing countries with a warm climate. (2). There has been a significant increase in consumer resistance to the conventional preservation methods of using synthetic additives (3). Active packaging technologies (AP) differ from the traditional packaging method by using bioactive materials that interact with the food and the environment to extend the shelf life of the product, prevent microorganisms from growing, and

absorb reactive oxygen species (ROS) from the atmosphere surrounding the package.. AP's most significant distinguishing feature when compared to existing surface coatings (i.e, applied to the surface of the food) or by actually adding the bioactive compounds to the food, is the time-released, controlled delivery of the bioactives from the polymer matrix to the food's surface (3; 4). Therefore, in order to be able to understand (e.g, mechanism of action) and show regulatory compliance, it is necessary to develop quantitative release markers for APs through kinetic modelling of release. Apple peel (*Malus domestica*), which is produced as a major waste product in the juice and cider industry and has an estimated production of > 2.5 million metric tonnes per year globally (5), is exceptionally rich in polyphenolic compounds, including quercetin (8-25 mg/g DW), chlorogenic acid (10-18 mg/g DW), and epicatechin (5-12 mg/g DW), all of which have been quantified using high-performance liquid chromatography (HPLC) in many studies (6; 7).

2. Materials and Methods

2.1 Materials and Chemicals

Fish skin gelatin (99% purity and 230 g gel strength Bloom) was obtained from Sigma-Aldrich (St. Louis, MO, USA), polyvinyl alcohol (89,000–98,000 Mw and 99% hydrolyzed) was obtained from Sigma-Aldrich, and glycerol (analytical grade) was obtained from Merck KGaA (Darmstadt, Germany). Apple

peel powder[was supplied by a local juice processing facility (Riyadh, Saudi Arabia) and dried in a hot-air convection oven at 50°C[and milled to <250 µm before freezing at -20°C until use. Food-grade ethanol (96% v/v), diluted with distilled water to a working concentration of 70% ([MT12v/v). The reference

strains of *L. monocytogenes* ATCC 19115 and *S. aureus* ATCC 25923 were obtained from the Saudi Center for Accreditation (SASO). All chemicals used in this study were analytical grade or higher. Processed soft cheese was produced at the authors' facility following (8) protocols using certified fresh bovine milk; however, fish skin gelatin was used exclusively rather than porcine gelatin in this study because of both the Halal dietary requirements that must be met for many Islamic and Jewish consumers and the documented risk of acquiring prion disease from bovine or porcine gelatins (9).

2.2 Preparation of Apple Peel Extract (APE)

The APE was prepared by soaking 50 g of powdered apple peel into 500 mL of 70% (v/v) ethanol for 48 h at 25 °C with continuous stirring (200 rpm, protected from light). The resulting mixture was filtered using Whatman No. 1 paper, and the solvent was removed under reduced pressure on a rotary evaporator (Rotavapor R-300, Büchi, Switzerland), at 40 °C. The remaining residue was freeze-dried (FreeZone 2.5, Labconco, USA) and stored at -80 °C. Total polyphenol concentrations (TPC) were determined according to the Folin-Ciocalteu method (10) and expressed as mg of gallic acid equivalents (GAE) per gram of

dry weight (DW). DPPH (11) and ABTS (26) assays evaluated antioxidant capacities. Individual polyphenolic compounds were determined by HPLC-DAD (Agilent 1260 Infinity, USA) at 280 nm and 360 nm using C18 columns (250 × 4.6 mm, 5 µm).

2.3 Film Fabrication

4 g of FSG dissolves in 100 mL distilled water at 60 °C for 30 minutes, then PVA (2% w/v) and glycerol (30% w/w based on FSG) are added (PVA is pre-dissolved at 80 °C for 2 hours). APE was added at 0%, 1%, 2%, or 3% (w/w total solids) to the film-forming solution, which was then magnetically stirred (600 rpm, 60 °C for 30 minutes) and degassed in vacuum. The solution was cast into leveled acrylic sheets (20 × 20 cm) and allowed to dry (25 °C for 48 hours). The films were conditioned (50 ± 2% RH and 25 °C for 48 hours), peeled from the acrylic sheets, and stored in sealed polyethylene bags until testing.

2.4 Film Characterization

2.4.1 Physical and chemical properties

of the films were measured at 10 randomly selected locations by using a digital micrometer (±0.001 mm) (Mitutoyo, Japan). The moisture contents were determined by using gravimetric measurements at 105 °C to a constant weight. Determining the water vapor permeability (WVP)

of the films was performed by using the modified ASTM E96 gravimetric method with the Paynter cups that contained silica gel (0% RH) that were preequilibrated in a chamber at 75% RH and 25 °C. The film solubility was measured on 20 x 20 mm film specimens (initial dry weight approximately 0.10 g) by dipping the preweighed films into 50 mL of distilled water for 24 hours at 25 °C, and after drying, were reweighed again[.

2.4.2 Mechanical Properties

The tensile strength (TS), elongation at break (EAB), and Young's modulus (YM) were measured on 80 x 10 mm strips (preconditioned at 50% RH) using an Instron Model 5565 Universal Testing Machine (USA), crosshead speed of 50 mm/min [initial gauge length of 50 mm] according to ASTM D882-18. Each treatment consisted of at least six specimens of test material.

2.4.3 Thermal Analysis (DSC and TGA)

Differential scanning calorimetry (DSC) was performed using a Perkin-Elmer DSC 4000 (USA) under nitrogen atmosphere. Sample sizes of 5 mg were heated from 20 to 250 °C at a rate of 10 °C/min after 5 minutes of isothermal equilibrium at a temperature of 20 °C. Glass transition temperature (T_g), melting temperature (T_m), and enthalpy of fusion (ΔH) were measured. Thermogravimetric analysis (TGA) was performed

using a Perkin-Elmer TGA 4000 at a rate of 10 °C/min (25–600 °C, nitrogen, 20 mL/min) to assess thermal degradation onset temperature (Tonset) and residue.

2.4.4 The structural analysis

of the samples, via FTIR spectroscopy and X-ray diffraction techniques, was performed using a Perkin-Elmer Spectrum 100 FTIR spectrometer (USA) and Rigaku Ultima IV diffractometer, respectively. FTIR spectra were obtained in attenuated total reflection mode, using 32 scans and a resolution of 4 cm⁻¹ from 4000–400 cm⁻¹. X-ray diffraction patterns were measured using copper K_α radiation from a Rigaku Ultima IV diffractometer, with a scanning range of 2θ from 5 to 50° and a step size of 0.02°. The crystallinity index of a sample was calculated with the following formula:

CI (crystallinity index) = $Ac / (Ac + Aa) \times 100$, where Ac is the area of the crystalline peaks and Aa is the area of the amorphous peaks.

2.4.5 Optical Properties

The color and optical properties of samples were determined with Minolta CR-410 colorimeter (Japan) under a D65 illuminant to determine the CIE L*, a* and b* coordinates and total color difference (ΔE) = $(L^*2 - L^*1)^2 + (a^*2 - a^*1)^2 + (b^*2 - b^*1)^2$. Transparency of samples was measured with a Shimadzu UV-1800 spectrophotometer at 600 nm.

2.5 Antimicrobial Efficacy Assessment

2.5.1 Minimum Inhibitory Concentration (MIC)

Antimicrobial efficacy testing was performed using the broth microdilution method as outlined by (15) to determine the minimum inhibitory concentration (MIC) value of APE against *L*[*M. monocytogenes* ATCC 19115 and *S. aureus* ATCC 25923 using a two-fold serial dilution of APE (0.78-100 mg/mL) in Mueller-Hinton broth and the following definitions: MIC is defined as the lowest concentration of APE that showed <1% turbidity after 18-20 hours incubation at 37°C.

2.5.2 Disc Diffusion Assay

Film discs (10 mm diameter) were placed on Mueller-Hinton agar surfaces from which 100 µL of 0.5 McFarland bacterial suspensions (contained 1.5×10^8 CFU/mL) had been inoculated with bacteria using the surface inoculation technique. The discs were incubated at 37 °C for 18 hours and the average diameter of the inhibition zone (in millimetres) was obtained by measuring two diameters for each disc in three separate replicates.

2.5.3 Challenge Test Against *L. monocytogenes*

Soft cheese samples (25g) [MT19.1] were inoculated on the surface with 100 µL of standardized inoculum,

homogenized with the help of a sterile L-shaped spreader on the whole cheese surface, and then left for 15 minutes for proper adhesion considering that that cheese will be wrapped, employing *Listeria monocytogenes* [MT20.1] ATCC 19115 (around 3 log CFU/g) afterwards and transferring the inoculated cheese to the 3% APE films or with control films. The samples were stored for 28 days at 4 °C after wrapping them in the film. Samples from days 0, 7, 14, 21, and 28 of storage were enumerated by plating on PALCAM agar and confirmed using (22), as per EFSA guidance on challenge tests for shelf life determination (12).

2.6 Polyphenol Release Kinetics and Migration Compliance

The migration compliance and kinetic release of polyphenols was measured by immersing polypropylene films (100 cm²) in food simulants for a maximum of 28 days at 4°C. The food simulants used were 3% acetic acid (simulant B, acidic type food), 10% ethanol (simulant A, edible food) and 95% ethanol (simulant D2, fatty food), as per EU Regulation No. 10/2011 (13). The polyphenolic compounds were determined using the Folin-Ciocalteu method as described by (10). The total migration, was determined by means of weight (gravimetrically) of the residual of the food simulant collected and increased in mass after 10 days of storage at 4°C. Data was

further evaluated using three models to describe release kinetics: (i) zero order: $M_t = k_0 \times t$; (ii) first order: $M_t = M_\infty(1 - e^{-k_1 t})$; and (iii) Korsmeyer-Peppas power law: $M_t/M_\infty = kKP \times t^n$ – the diffusion exponent, ($n \leq 0.45$ Fickian diffusivity, or $0.45 < n < 0.89$ Anomalous transport); goodness-of-fit was based on R^2 values, and root mean square error (RMSE).

2.7 Analysis of Packaged Soft Cheese

2.7.1 Chemical Analysis

Moisture content (AOAC 926.08), fat content (AOAC 922.06, with the Soxhlet extraction), total protein (AOAC 991.20, Kjeldahl $N \times 6.38$), titratable acidity calculated using 0.1 N NaOH as % lactic acid, and pH measured via a Mettler Toledo FiveEasy (Switzerland) device were conducted on days 0, 7, 14, 21, and 28. The versus TBARS method (28) determined the level of lipid oxidation concentration of malondialdehyde (MDA, expressed as mg MDA/kg cheese); the acceptable limit for dairy products is ≤ 1 mg MDA/kg, according to the Codex Alimentarius Commission.

2.7.2 Microbiological Analyses

The total count of aerobic mesophilic bacteria was measured by using PCA (plate count agar) reared at 30°C for 48 hours (20); the molds and yeast population was measured by using potato dextrose agar

acidified to pH 3.5 (MT21.1) reared at 25°C for 5 days (23); the lactic acid bacteria were measured with MRS agar at 37°C for 48 hours anaerobically.

2.7.3 Sensory Evaluation

A panel of twenty (10 male and 10 female) trained assessors (aged between 25 and 45 years) consisting of faculty members and post-graduate students from Food Science at King Saud University assessed the sensory evaluation of colour, odour, texture, flavour and overall acceptability of the samples using a nine-point hedonic scale (i.e. 9 = like extremely, 1 = dislike extremely) at days 0, 7, 14, 21 and 28 post treatment. The panel were trained in three sessions using (21). protocols. Each session was held in individual booths under D65 lighting conditions and coded using random three digit numbers and presented to the assessors in a random order.

2.8 Experimental Design and Statistical Analysis

The experiments were designed as a Completely Randomised Design (CRD) with four APE treatments (0, 1, 2 and 3% w/w) and three replicates per treatment. The storage data were analysed using two-way ANOVA (treatment $\times$ storage time) with repeated measures on the time factor with SPSS v27.0 (IBM Corp., Armonk, NY, USA). The means were separated using Tukey's Honestly Significant Difference (HSD)[test with a

significance level of $P < 0.05$. The Pearson correlational matrices (r) were evaluated between film properties and cheese quality parameters using Minitab v21. All data were

reported as mean $\pm$ standard deviation (SD [$n = 3$]). The release kinetics of the film was fitted with a curve using OriginPro 2023b (OriginLab, Northampton, MA, USA).

3. Results and Discussion

3.1 APE Composition:

In terms of total phenolic content (TPC), Fuji freeze-dried apple peel extract (APE) recorded TPC as 248.6 ± 4.3 mg gallic acid equivalent (GAE) per gram dry weight (DW). The TPC for apples falls in the range of 180 to 320 mg GAE/g DW for Fuji apple peels extracted using 70% ethanol (5; 6). The major polyphenols detected by high-performance liquid chromatography-diode array detector (HPLC-DAD) were quercetin (18.4 mg/g DW), chlorogenic

acid (12.7 mg/g DW), epicatechin (9.3 mg/g DW), and rutin (4.2 mg/g DW) that validate the previously reported polyphenolic composition of Fuji apple peels (7) with the antioxidant activities being $76.3 \pm 1.8\%$ (DPPH) and $81.7 \pm 2.1\%$ (ABTS), which can mostly be attributed to high concentrations of quercetin due to its established ability to act as a free radical scavenger via its catecholic functional group located on the B-ring (16).

Table 1. Quantitative polyphenolic composition of APE determined by HPLC-DAD (mean $\pm$ SD, $n = 3$). Column:

C18 (250 x 4.6 mm, 5 μ m); detection at 280 and 360 nm.

Detection (nm)	% of TPC	Content (mg/g DW)	RT (min)	Chemical Class	Compound
360	7.39	18.4 $\pm$ 0.8 a	18.4	Flavonol	Quercetin
280	5.10	12.7 $\pm$ 0.5 b	12.7	Hydroxycinnamic acid	Chlorogenic acid
280	3.74	9.3 $\pm$ 0.4 c	9.3	Flavan-3-ol	Epicatechin
360	1.69	4.2 $\pm$ 0.3 d	4.2	Flavonol glycoside	Rutin
320	1.53	3.8 $\pm$ 0.2 d	3.8	Hydroxycinnamic acid	Ferulic acid
280	1.04	2.6 $\pm$ 0.2 e	2.6	Flavan-3-ol dimer	Procyanidin B2
310	0.76	1.9 $\pm$ 0.1 e	1.9	Phenolic acid	p-Coumaric acid
—	100%	248.6 $\pm$ 4.3	—	—	TPC (Folin-Ciocalteu)

Table 2. Concentration-dependent antioxidant and biological activities of APE (mean +/- SD, n = 3).

APE 5.0 mg/mL	APE 2.0 mg/mL	APE 1.0 mg/mL	APE 0.5 mg/mL	Unit	Bioactivity Parameter
82.1 +/- 1.3 a	63.4 +/- 1.8 b	41.8 +/- 1.5 c	24.3 +/- 1.2 d	% inhibition	DPPH radical scavenging
86.4 +/- 1.5 a	67.9 +/- 2.0 b	46.2 +/- 1.6 c	28.7 +/- 1.4 d	% inhibition	ABTS radical scavenging
71.2 +/- 1.9 a	43.8 +/- 1.4 b	24.6 +/- 1.1 c	12.4 +/- 0.8 d	mmol Fe2+/g	FRAP
74.3 +/- 1.8 a	56.7 +/- 1.6 b	35.4 +/- 1.3 c	18.6 +/- 1.0 d	% inhibition	Metal chelation capacity
79.6 +/- 1.6 a	61.3 +/- 1.7 b	39.2 +/- 1.4 c	21.4 +/- 1.1 d	% inhibition	Lipid peroxidation inhibit.

3.2 Summary of Physicochemical & Mechanical Properties of Films:

Table 1 presents the physicochemical and mechanical properties of films. The average film thickness across treatment groups ranged from 0.082 to 0.094 mm, not significantly different ($P > 0.05$) demonstrating that the films were cast uniformly. The moisture content of films decreased significantly ($P < 0.05$) from $14.8 \pm 0.3\%$ in control to $11.2 \pm 0.4\%$ with 3% APE due to the hydrophobic nature of the polyphenolic ring structures and their ability to fill free volume sites in the polymer network via hydrogen bond formation and π - π stacking interactions with the amide groups of the gelatin (18). The water vapor permeability (WVP) of the APE incorporated values for the control film (3.21 ± 0.15) to the 3% APE film (1.82 ± 0.09) yielded a significant ($P < 0.05$) reduction of 43.3%; however, compared

to previously published literature, the APE films had comparable WVP values, including chitosan films incorporating APE (17); $WVP = 1.95 \times 10^{-10}$) and gelatin films with curcumin added (27); $WVP = 2.14 \times 10^{-10}$). Tensile strength increased from 12.3 ± 0.8 MPa for the control to 18.6 ± 0.7 MPa with 3% APE, while elongation at break (EAB) of the films decreased from $48.2 \pm 3.1\%$ for the control to $34.7 \pm 2.4\%$ with 3% APE ($P < 0.05$). This is characteristic behaviour for reinforcing interactions, associated with hydrogen bonding between phenolic hydroxyl groups and the amino or carbonyl groups of the gelatin forming additional cross-linking sites that restrict the movement of polymer chains and thus adding stiffness and reducing the flexibility of the polymer films (19). The increased stiffness of the APE-incorporated films is confirmed by the increase in Young's modulus from 382 ± 18 MPa for the control film to 561

± 22 MPa with the 3% APE film. This value and other results correspond to the requirements for cheese packaging

established by (25) for tensile strength greater than 12 MPa and elongation at break greater than 20%.

Table 1. Physicochemical and mechanical properties of FSG/PVA/APE active films (mean $\pm$ SD, n = 3).

3% APE	2% APE	1% APE	0% (Control)	Property
0.094 ± 0.004^a	0.089 ± 0.003^a	0.085 ± 0.002^a	0.082 ± 0.003^a	Thickness (mm)
$11.2 \pm 0.4^{\square}$	$12.1 \pm 0.3^{\square}$	13.4 ± 0.4^b	14.8 ± 0.3^a	Moisture content (%)
1.82 ± 0.09^d	$2.18 \pm 0.10^{\square}$	2.74 ± 0.11^b	3.21 ± 0.15^a	WVP ($\times 10^{-10} \text{ g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$)
27.6 ± 1.2^d	$31.8 \pm 1.4^{\square}$	36.2 ± 1.5^b	41.3 ± 1.8^a	Solubility (%)
18.6 ± 0.7^a	16.9 ± 0.5^{ab}	14.7 ± 0.6^b	$12.3 \pm 0.8^{\square}$	Tensile strength (MPa)
$34.7 \pm 2.4^{\square}$	38.6 ± 2.5^b	43.1 ± 2.8^{ab}	48.2 ± 3.1^a	Elongation at break (%)
561 ± 22^a	508 ± 19^b	$441 \pm 20^{\square}$	382 ± 18^d	Young's modulus (MPa)
87.4 ± 1.2^a	71.6 ± 1.5^b	$52.3 \pm 1.8^{\square}$	8.4 ± 0.9^d	DPPH scavenging (%)
4.8 ± 0.3^a	3.1 ± 0.2^b	$1.4 \pm 0.1^{\square}$	—	ΔE vs. control

Thermal Analysis (DSC and TGA)

The DSC thermographs (Table 2) indicated a singular endothermic peak which denotes the melting transition of gelatin crystals. The thermographs for four APE concentrations are shown in Figure 1 to help visualize the shifting of the peak as more APE is added. The melting temperature (T_m) changed from $(68.2 \pm 0.5) ^\circ\text{C}$ (control) to $(74.1 \pm 0.6) ^\circ\text{C}$ (3% APE) and the enthalpy of fusion (ΔH) increased from $(18.4) \text{ J/g}$ to $(24.7) \text{ J/g}$, indicating that polyphenol- gelatin interactions provide reinforcement to the

triple helix structure and stabilization of the helical crystallites; this agrees with the data obtained by (24) for quercetin-gelatin complexes. The glass transition In the control sample, T_g (glass transition temperature) cannot be observed, most probably because the T_g of the gelatin-rich amorphous phase where the process took place overlaps with extensive pre-melting relaxation term, that is why it was too weak to distinguish from the baseline without the rigidity brought by APE., but appeared at $(42.3 \pm 0.8) ^\circ\text{C}$ in the (3% APE)

3.3

sample suggesting increased rigidity within the amorphous phase that corroborates with the mechanical data. TGA profiles indicated that Tonset increased from $(218.4 \pm 1.6) ^\circ\text{C}$ (control) to $(231.8 \pm 1.4) ^\circ\text{C}$ (3% APE) which also indicated improved thermal

stability. The amount of residue at $(600) ^\circ\text{C}$ increased slightly (2.4% control vs. 4.1% 3% APE) due to the increased inorganic ash content that occurred in APE polyphenols.

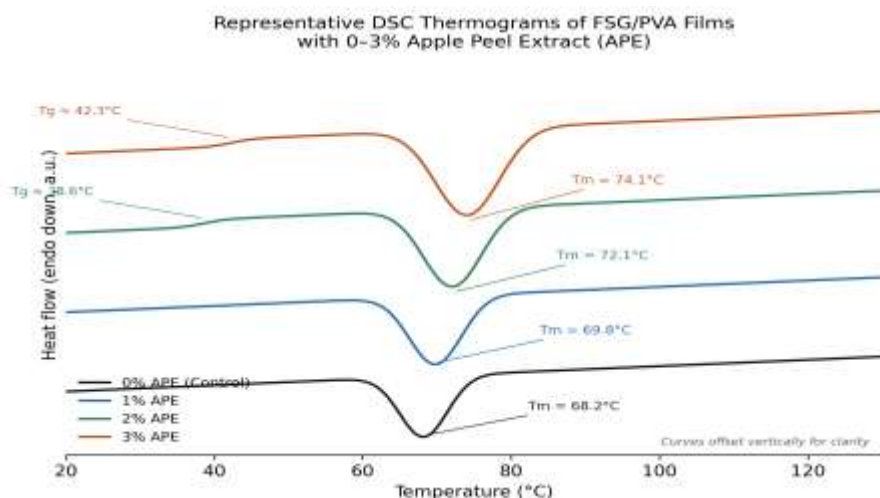


Figure 1. Representative DSC thermograms of FSG/PVA films containing 0%, 1%, 2%, and 3% apple peel extract (APE). Curves are vertically offset for clarity. The melting endotherm (T_m) shifts from $68.2 ^\circ\text{C}$ (control) to $74.1 ^\circ\text{C}$ (3% APE), and a glass transition (T_g) becomes resolvable only in the 2% and 3% APE films ($38.6 ^\circ\text{C}$ and $42.3 ^\circ\text{C}$, respectively), consistent with the increased rigidity of the amorphous phase discussed in the text.

Table 2 contains the thermal properties (DSC and TGA) of FSG/PVA/APE films. (mean $\pm$ SD, $n=3$).

3% APE	2% APE	1% APE	0% (Control)	Parameter
74.1 ± 0.6^a	72.1 ± 0.6^b	69.8 ± 0.5^c	68.2 ± 0.5^d	DSC T_m ($^\circ\text{C}$)
24.7 ± 0.8^a	22.6 ± 0.9^b	20.2 ± 0.7^c	18.4 ± 0.8^d	DSC ΔH (J/g)
42.3 ± 0.8	38.6 ± 1.1	ND	ND	DSC T_g ($^\circ\text{C}$)
231.8 ± 1.4^a	227.6 ± 1.5^b	223.1 ± 1.4^c	218.4 ± 1.6^d	TGA T_{onset} ($^\circ\text{C}$)
4.1 ± 0.3^a	3.5 ± 0.3^b	2.9 ± 0.2^c	2.4 ± 0.2^d	TGA residue at 600°C (%)
28.6 ± 1.4^d	32.1 ± 1.5^c	36.7 ± 1.6^b	42.3 ± 1.8^a	XRD crystallinity index (%)

3.4 FTIR and XRD analyses of FSG/PVA films.

The FTIR spectra of the FSG/PVA films revealed the presence of amide I (C=O stretching at 1632 cm⁻¹), amide II (N-H bending at 1546 cm⁻¹) and amide III (C-N stretching at 1238 cm⁻¹) bands associated with gelatin, and the O-H stretching band of PVA at 3340 cm⁻¹. The introduction of APE resulted in a concentration-dependent red shift and broadening of the O-H band (3340 → 3295 cm⁻¹), indicative of the formation of hydrogen bonds between polyphenolic hydroxyl groups and the polymer backbone. The bathochromic shift in the amide I band (1632 → 1619 cm⁻¹) and amide II band (1546 → 1531 cm⁻¹) observed in the 3% APE film are consistent with the disruption of the secondary structure of gelatin by polyphenolic compounds as reported by (24). The detection of the quercetin characteristic aromatic ring vibration

peak at 1516 cm⁻¹ in APE containing films provided further evidence that the polyphenol was successfully integrated into the films. The XRD diffractograms demonstrated a broad amorphous halo centered at approximately 21 degree 2-theta, characteristic of the PVA-gelatin blended, with a sharp minor crystalline peak. The crystallinity index linearly decreased from 42.3% (control) to 28.6% (3% APE), indicating that intercalation of the polyphenol disrupted interchain packing and reduced the size of the crystallites. The observed amorphization confirms the presence of increased chain mobility in the amorphous phase which was countered simultaneously by intermolecular cross-linking, resulting in increased tensile strength and melting temperature, while decreased elongation-at-break and crystallinity index.

Table 3. Major FTIR band positions and structural assignments for FSG/PVA/APE active films at all APE concentrations (cm⁻¹).

Shift (Delta cm-1)	3% APE	2% APE	1% APE	0% APE	Structural Interpretation	Band Assignment
-45	3295	3312	3328	3340	H-bond: polyphenol OH / PVA hydroxyl / gelatin	O-H stretching
-22	3258	3264	3271	3280	Gelatin amide and hydroxyl groups	N-H / O-H overlap
-3	2927	2928	2929	2930	Aliphatic carbon chains (gelatin backbone)	C-H stretching
-13	1619	1624	1628	1632	C=O stretch of triple-helix crystallites	Amide I (C=O)

-15	1531	1537	1541	1546	N-H bending / gelatin chain vibration	Amide II (N-H)
-6	1232	1234	1236	1238	C-N stretch in gelatin peptide bonds	Amide III (C-N)
—	1516	1518	—	—	Aromatic ring C=C vibration (quercetin marker)	Quercetin B-ring
-6	1084	1086	1088	1090	Ether linkages in PVA	C-O stretching

Table 4. Quantitative XRD parameters for FSG/PVA/APE active films: peak positions, crystallinity index, and Scherrer crystal size (mean +/- SD, n = 3).

Significance	3% APE	2% APE	1% APE	0% (Control)	Parameter
P<0.05	21.6 +/- 0.3	21.3 +/- 0.3	21.0 +/- 0.2	20.8 +/- 0.2	Crystalline peak position 2theta (degrees)
P<0.001	1163 +/- 28 d	1387 +/- 32 c	1614 +/- 38 b	1842 +/- 45 a	Crystalline peak intensity Ic (a.u.)
P>0.05	4069 +/- 71 a	4210 +/- 75 a	4262 +/- 79 a	4348 +/- 82 a	Total peak area (Ic + Ia) (a.u.)
P<0.001	28.6 +/- 1.4 d	32.1 +/- 1.5 c	36.7 +/- 1.6 b	42.3 +/- 1.8 a	Crystallinity index CI (%)
P<0.001	3.24 +/- 0.12 d	3.76 +/- 0.14 c	4.31 +/- 0.15 b	4.82 +/- 0.18 a	Crystal size D by Scherrer (nm)
P<0.001	2.49 +/- 0.09 a	2.15 +/- 0.08 b	1.88 +/- 0.07 c	1.68 +/- 0.06 d	FWHM (degrees)
—	-32.4%	-24.1%	-13.2%	Reference	Relative change in CI% vs. control

3.5 Antimicrobial Activity of Apple Polyphenols

3.5.1 Minimum Inhibitory Concentration and Disc Diffusion

Test The MIC of APE for both *L. monocytogenes* ATCC 19115 and *S. aureus* ATCC 25923 were found to be 6.25 mg/mL for *L. monocytogenes* and 3.13 for *S. aureus* (Table 3). These results are similar to other studies of polyphenol-rich apple extracts against Gram-positive foodborne pathogens, which found that the MICs range from 2 mg/mL to 12.5 mg/mL (16; 24). The lower MIC of *S. aureus* appears to be due to dissimilarities in the cell membrane structure and permeability of the bacteria instead of a difference in the Gram-reaction classification, as both *L. monocytogenes* and *S. aureus* are Gram-positive bacteria. It is believed that differences in the degree of peptidoglycan linkage, the amount of teichoic acids in the composition, as well as possible mechanisms of efflux may contribute to the dissimilarity in resistance of the microorganism to APE polyphenols. (16). The inhibition zones produced by the 3% APE films for the

disc diffusion test were 18.4 ± 0.9 mm for *L. monocytogenes* and 22.1 ± 1.1 mm for *S. aureus*; both were susceptible according to the CLSI Guidelines. The control films did not produce any inhibition zones; therefore, all antimicrobial activity came from the APE polyphenols leaching from the films.

3.5.2 *L. monocytogenes* Challenge Test

The starting inoculum level for each treatment was 3.0 ± 0.1 log CFU/g. In the control wrapped cheese block, the log CFU/g of *L. monocytogenes* increased to 4.8 ± 0.2 log CFU/g after 28 days of storage. In 3% APE-wrapped cheese blocks, the log CFU/g were reduced to 1.6 ± 0.2 log CFU/g after 28 days, resulting in a log reduction of 3.2 log units, demonstrating both bacteriostatic and bactericidal properties of APE during prolonged storage at refrigeration temperatures. This 3 log CFU/g reduction also meets the pathogen reduction targets referenced in the (12) Guidance Document for challenge testing of active packaging intended for ready-to-eat dairy products.

Table 3. Antimicrobial properties of APE solutions (MIC) and FSG/PVA/APE films (disc diffusion) (mean $\pm$ SD).

<i>S. aureus</i> ATCC 25923	<i>L. monocytogenes</i> ATCC 19115	Parameter
3.13	6.25	MIC of APE solution (mg/mL)
0 ^a	0 ^a	Inhibition zone — 0% APE film (mm)
12.4 ± 0.7 □	9.2 ± 0.6 □	Inhibition zone — 1% APE film (mm)
17.2 ± 0.9 ^b	13.7 ± 0.8 ^b	Inhibition zone — 2% APE

		film (mm)
22.1 ± 1.1^a	18.4 ± 0.9^a	Inhibition zone — 3% APE film (mm)
n/a	3.2 log CFU/g	Challenge test: log reduction at day 28 (3% vs. control)

3.6 Polyphenol Release Kinetics and Migration Compliance

The total quantity of polyphenol release after 28 days in a 3% acetic acid of $82.4 \pm 2.3 \mu\text{g}/\text{cm}^2$ was at least 2.3 times greater than that of the release from 10% ethanol ($61.7 \pm 1.8 \mu\text{g}/\text{cm}^2$) and the 95% ethanol ($41.2 \pm 1.5 \mu\text{g}/\text{cm}^2$) simulants ($p < 0.05$). The greater quantity of polyphenol released from the acidic simulant supports the theory that the acidic condition will partially protonate the phenolic groups, which encourages the dissociation of these compounds from the polymeric substrate/film. The acidic pH of the storage condition of soft cheese (between 5.5 and 6.4) corresponds closely with that of the acidic simulant, which may also provide support for the form of release mechanism observed in this study. Of the three models used to describe the release mechanism, The Korsmeyer-Peppas model had overall the best fit with ($R^2 = 0.987$ and $\text{RMSE} = 1.4 \mu\text{g}/\text{cm}^2$) while the Zero Order model had ($R^2 = 0.921$) and the First Order model ($R^2 = 0.963$). The

diffusion exponent of the Korsmeyer-Peppas model was 0.42 ± 0.03 , indicating that polyphenol transport is primarily driven by concentration gradients (diffusion) through the hydrated polymer matrix; the results do not indicate that polymer chain relaxation is being utilized to facilitate/expedite polyphenol transport. The sustained release profile exhibited by 3% APE films will extend the antimicrobial shelf life of the cheese since the polyphenol will be released slowly over time, as opposed to being released rapidly at the beginning of storage (4). The total migration values into 3% acetic acid at 4°C over a 10-day period were $3.2 \pm 0.2 \text{ mg}/\text{dm}^2$, which are significantly less than the overall migration limit established by EU Regulation No. 10/2011 (13) and the corresponding SFDA regulation of 10 (mg/dm^2). The total migration data suggests that the 3% APE films are acceptable for use as food contact materials when used for processing acidic dairy products.

Table 5. Cumulative polyphenol release ($\mu\text{g}/\text{cm}^2$) from 3% APE films into three food simulants (EU Regulation 10/2011) at 4 degrees C (mean $\pm$ SD, n = 3).

Mt/M-inf Simulant B (%)	95% Ethanol (Simulant D2)	10% Ethanol (Simulant A)	3% Acetic Acid (Simulant B)	Time Point
3.88%	1.4 $\pm$ 0.2	2.1 $\pm$ 0.3	3.2 $\pm$ 0.4	2 h
8.25%	2.8 $\pm$ 0.3	4.4 $\pm$ 0.4	6.8 $\pm$ 0.6	4 h
15.05%	4.9 $\pm$ 0.4	8.1 $\pm$ 0.6	12.4 $\pm$ 0.8	8 h
21.36%	6.8 $\pm$ 0.5	11.3 $\pm$ 0.7	17.6 $\pm$ 1.0	12 h
31.92%	9.7 $\pm$ 0.6	16.8 $\pm$ 0.9	26.3 $\pm$ 1.3	24 h
46.60%	13.8 $\pm$ 0.7	24.2 $\pm$ 1.1	38.4 $\pm$ 1.6	48 h
59.10%	17.1 $\pm$ 0.9	30.6 $\pm$ 1.3	48.7 $\pm$ 1.8	72 h
75.36%	22.6 $\pm$ 1.1	39.4 $\pm$ 1.5	62.1 $\pm$ 2.0	7 d
88.35%	30.4 $\pm$ 1.3	47.2 $\pm$ 1.7	72.8 $\pm$ 2.2	14 d
95.75%	36.2 $\pm$ 1.4	53.1 $\pm$ 1.9	78.9 $\pm$ 2.3	21 d
100%	41.2 $\pm$ 1.5	61.7 $\pm$ 1.8	82.4 $\pm$ 2.3	28 d

Table 6. Mathematical modeling of polyphenol release kinetics from 3% APE films in food simulants: model parameters and goodness-of-fit (n = 3).

Best Fit?	Simulant D2 (95% EtOH)	Simulant A (10% EtOH)	Simulant B (3% Acetic Acid)	Equation	Release Model
No	k0=1.47; R2=0.894; RMSE=4.8	k0=2.20; R2=0.908; RMSE=5.9	k0=2.94; R2=0.921; RMSE=6.8	Mt = k0 * t	Zero-order
No	k1=0.129; R2=0.944; RMSE=3.2	k1=0.138; R2=0.951; RMSE=3.8	k1=0.142; R2=0.963; RMSE=4.2	Mt = M-inf(1-e ^(-k1*t))	First-order
YES	kKP=0.079; n=0.41; R2=0.979; RMSE=1.0	kKP=0.091; n=0.44; R2=0.983; RMSE=1.2	kKP=0.098; n=0.42; R2=0.987; RMSE=1.4	Mt/M-inf = kKP * t ⁿ	Korsmeyer-Peppas
No	kH=7.6; R2=0.918; RMSE=3.6	kH=11.2; R2=0.931; RMSE=4.4	kH=14.8; R2=0.942; RMSE=5.1	Mt = kH * t ^{0.5}	Higuchi

Table 7. Regulatory migration compliance of FSG/PVA/APE active films — overall and specific migration at 4 degrees C (n = 3).

Compliance	Regulatory Limit	3% APE	2% APE	1% APE	0% APE	Regulatory Parameter
PASS	≤ 10 (EU/SFDA)	3.20 $\pm$ 0.15	2.18 $\pm$ 0.11	1.24 $\pm$ 0.08	0.18 $\pm$ 0.02	Overall migration Simulant (mg/dm ²) — B
PASS	≤ 10 (EU/SFDA)	2.34 $\pm$ 0.12	1.56 $\pm$ 0.09	0.87 $\pm$ 0.06	0.12 $\pm$ 0.01	Overall migration Simulant (mg/dm ²) — A
PASS	≤ 10 (EU/SFDA)	1.58 $\pm$ 0.10	1.08 $\pm$ 0.07	0.61 $\pm$ 0.04	0.09 $\pm$ 0.01	Overall migration Simulant (mg/dm ²) — D2
PASS	≤ 5 (EFSA ADI)	0.52 $\pm$ 0.04	0.34 $\pm$ 0.03	0.18 $\pm$ 0.02	ND	Quercetin specific migration (mg/dm ²)
Informational	No SML set	0.34 $\pm$ 0.03	0.22 $\pm$ 0.02	0.12 $\pm$ 0.01	ND	Chlorogenic acid migration (mg/dm ²)
PASS	5.5-7.5 (food grade)	6.2 $\pm$ 0.1	6.4 $\pm$ 0.1	6.6 $\pm$ 0.1	6.8 $\pm$ 0.1	Film pH after contact
Informational	—	74.2 $\pm$ 2.6	58.7 $\pm$ 2.4	38.4 $\pm$ 2.1	ND	Antioxidant activity released (DPPH%)

3.7 Microbiological Quality of Soft Cheese

Table 4 provides evidence of the microbial growth that occurred in the cheese samples during storage. The total aerobic microbial count (TAMC) increased in all samples stored; however, the control cheese had exceeded the

(28). limit of 6.0 log CFU/g at day 18, but the cheese wrapped with 3% APE remained under this limit at all times during the 28 days of storage. The 3% APE treatment reduced total aerobic microorganisms in comparison with the control by 2.3 log CFU/g by day 14 ($P < 0.05$) and by 2.6 log.

Table 4. Total aerobic mesophilic count (TAMC, log CFU/g) of soft cheese wrapped with FSG/PVA/APE films during 28-day storage at 4°C (mean $\pm$ SD, n = 3).

3% APE	2% APE	1% APE	0% (Control)	Storage Day
1.9 $\pm$ 0.1 ^a	2.0 $\pm$ 0.2 ^a	2.0 $\pm$ 0.1 ^a	2.1 $\pm$ 0.1 ^a	Day 0
2.8 $\pm$ 0.2 [□]	3.2 $\pm$ 0.1 [□]	3.6 $\pm$ 0.2 ^b	4.3 $\pm$ 0.2 ^a	Day 7
3.6 $\pm$ 0.2 [□]	4.1 $\pm$ 0.2 [□]	4.8 $\pm$ 0.2 ^b	5.9 $\pm$ 0.3 ^a	Day 14
4.4 $\pm$ 0.2 ^d	5.1 $\pm$ 0.3 [□]	5.9 $\pm$ 0.3 ^b	7.1 $\pm$ 0.4 ^{a*}	Day 21
5.2 $\pm$ 0.2 ^d	5.8 $\pm$ 0.3 [□]	6.8 $\pm$ 0.4 ^{b*}	8.2 $\pm$ 0.5 ^{a*}	Day 28

3.4 The chemical composition and oxidative stability of packaged creamy cheese

Present in Table 8 represent all chemical quality parameters for 28 days. Day 0 showed all treatments had the same averages ($P > 0.05$); supporting these comparisons in the later days. A 9.0% moisture loss occurs during the 28 days of storage of a regular (control) cheese; whereas a 3% APE-treated cheese has only a 3.8% moisture loss due to a 43.3% reduction in film water vapor permeability (WVP) ($r = -0.97$; $P < 0.001$ comparing moisture to WVP). The decline in pH occurred significantly slower in the 3% APE cheese (6.42 $\rightarrow$ 6.14) in comparison with the control cheese (6.42 $\rightarrow$ 5.74); the slower slope is consistent with decreased microbiological fermentation. Five

oxidative stability indices are presented in Table 9. The TBARS value in control cheese reached 1.0 mg of myosmine/kg prior to day 21, while the TBARS value remained below the maximum allowable limit, or 1.0 mg of myosmine/kg, in 3% APE-treated cheese (0.61 ± 0.03 mg of myosmine/kg on day 28). The TOTOX value (total oxidation; $\text{TOTOX} = 2\text{PV} + \text{ANV}$) for control cheese reached 37.2 on day 28; exceeding the maximum allowable limit of 26 (TOTOX value in the 3% APE cheese = 18.4). Data presented in Table 8 represent the chemical quality characteristics of soft cheese packaged in FSG/PVA/APE active films during 28 days of storage at four degrees Celsius $\pm$ standard deviation using three replications per treatment.

Table 8. Chemical quality parameters of soft cheese wrapped with FSG/PVA/APE active films during 28-day storage at 4 degrees C (mean +/- SD, n = 3).

P-value (Time)	Day 28	Day 21	Day 14	Day 7	Day 0	Treatment	Chemical Parameter
<0.001	49.1+/-0.8 a	49.6+/-0.7 a	50.4+/-0.7 a	51.6+/-0.6 a	52.3+/-0.5 a	1% APE	
<0.001	50.2+/-0.7 a	50.5+/-0.7 a	51.1+/-0.6 a	51.9+/-0.5 a	52.4+/-0.6 a	2% APE	
<0.001	50.4+/-0.7 a	51.0+/-0.6 a	51.7+/-0.6 a	52.1+/-0.5 a	52.3+/-0.5 a	3% APE	
<0.05	23.8+/-0.7	24.0+/-0.6	24.3+/-0.6	24.6+/-0.5	24.8+/-0.5	0% Control	Fat (% DW)
>0.05	24.3+/-0.6	24.4+/-0.6	24.5+/-0.5	24.6+/-0.5	24.7+/-0.5	3% APE	
<0.05	17.8+/-0.5	18.1+/-0.5	18.3+/-0.4	18.5+/-0.4	18.6+/-0.4	0% Control	Protein (% DW)
>0.05	18.3+/-0.4	18.4+/-0.4	18.5+/-0.4	18.6+/-0.4	18.6+/-0.4	3% APE	
<0.001	5.74+/-0.08	5.94+/-0.07	6.11+/-0.06	6.28+/-0.05	6.42+/-0.04	0% Control	pH
<0.001	6.14+/-0.06	6.22+/-0.05	6.29+/-0.05	6.36+/-0.04	6.41+/-0.04	3% APE	
<0.001	0.82+/-0.05	0.74+/-0.04	0.66+/-0.04	0.54+/-0.03	0.42+/-0.02	0% Control	Titrateable acidity (% LA)
<0.001	0.61+/-0.03	0.56+/-0.03	0.51+/-0.03	0.46+/-0.02	0.42+/-0.02	3% APE	

Table 9. Oxidative stability indices of soft cheese wrapped with FSG/PVA/APE films during 28-day storage at 4 degrees C (mean +/- SD, n = 3).

Acceptance Limit	Day 28	Day 21	Day 14	Day 7	Day 0	Treatment	Oxidative Index
<1.0 mg/kg	1.74+/-0.09 a*	1.08+/-0.06 a*	0.62+/-0.04 b	0.28+/-0.02 c	0.12+/-0.01 d	0% Control	TBARS (mg MDA/kg)
<1.0 mg/kg	1.18+/-0.07*	0.76+/-0.05	0.44+/-0.03	0.21+/-0.02	0.11+/-0.01	1% APE	
PASS	0.78+/-0.05	0.54+/-0.04	0.34+/-0.02	0.18+/-0.01	0.12+/-0.01	2% APE	
PASS	0.61+/-0.03 a	0.41+/-0.03 b	0.26+/-0.02 c	0.16+/-0.01 d	0.11+/-0.01 d	3% APE	
< 10 meq/kg	12.4+/-0.7 a*	7.8+/-0.5 b	4.2+/-0.3 c	1.9+/-0.2 d	0.8+/-0.1 d	0% Control	Peroxide Value (meq O2/kg)
PASS	5.8+/-0.4 a	4.1+/-0.3 b	2.6+/-0.2 c	1.4+/-0.1 d	0.8+/-0.1 d	3% APE	
<1.5%	0.58+/-0.04	0.46+/-0.04	0.34+/-0.03	0.24+/-0.02	0.18+/-0.01	0% Control	Free Fatty Acids (% OA)
PASS	0.38+/-0.03	0.32+/-0.02	0.26+/-0.02	0.21+/-0.01	0.18+/-0.01	3% APE	
—	—	9.2+/-0.6 a	5.4+/-0.4 b	2.8+/-0.2 c	1.2+/-0.1 d	0% Control	p-Anisidine Value (AnV)
—	6.8+/-0.5 a	5.1+/-0.3 b	3.4+/-0.2 c	2.1+/-0.1 d	1.2+/-0.1 d	3% APE	
<26	37.2*	24.8	13.8	6.6	2.8	0% Control	TOTOX = 2PV + AnV
PASS	18.4	13.3	8.6	5.0	2.8	3% APE	

3.5 Pearson Correlation Analysis — Film Properties vs. Cheese Quality at Day 28

In the Pearson correlation summary regarding films' and cheese's quality (after day 28) at n = 12, it was

determined that water vapour permeability (WVP) from cheese films was the most predictive factor for total aerobic microbial counts (TAMC) and thiobarbituric acid-reactive substances (TBARS) ($r = .96$ and $r = .94$,

respectively, $p < .001$), indicating that the film's ability to retain moisture was the primary influence on both forms of stability (microbial and oxidative), respectively.

It was also noted that the WVP did show a stronger negative correlation ($r = .97$, $p < .001$) with TBARS than with TAMC; and that the DPPH scavenging activity of the film correlated strongly negatively

($r = -.97$, $p < .001$) with TBARS, providing a direct mechanism through which film-released polyphenols inhibit lipid oxidation within the cheese matrix. The correlations described here can be utilized to formulate films that best achieve the targeted quality characteristics for the cheese.

Table 10. Full Pearson correlation matrix between film properties and soft cheese quality parameters at day 28 (n = 12). Bonferroni correction applied for multiple comparisons.

pH	Acidity	Moisture	TBARS	TAMC	Solub.	DPPH %	TS	WVP	Variable
-0.89***	+0.92**	-0.97***	+0.94**	+0.96**	+0.88**	+0.93**	-0.91***	1.00	WVP
+0.87**	-0.89***	+0.88**	-0.91***	-0.93***	-0.86***	-0.89***	1.00	-0.91***	Tensile Str.
+0.88**	-0.91***	+0.86**	-0.97***	-0.95***	-0.84***	1.00	-0.89***	-0.93***	DPPH %
-0.83***	+0.86**	-0.84***	+0.87**	+0.89**	1.00	-0.84***	-0.86***	+0.88**	Solubility %
-0.92***	+0.91**	-0.94***	+0.93**	1.00	+0.89**	-0.95***	-0.93***	+0.96**	TAMC
-0.88***	+0.90**	-0.92***	1.00	+0.93**	+0.87**	-0.97***	-0.91***	+0.94**	TBARS
+0.85**	-0.88***	1.00	-0.92***	-0.94***	-0.84***	+0.86**	+0.88**	-0.97***	Cheese moist.
-0.94***	1.00	-0.88***	+0.90**	+0.91**	+0.86**	-0.91***	-0.89***	+0.92**	Acidity
1.00	-0.94***	+0.85**	-0.88***	-0.92***	-0.83***	+0.88**	+0.87**	-0.89***	pH

3.10 The sensory evaluation

data presented in Table 6 show that no significant differences were found between the overall acceptability scores of cheese wraps for the four treatments at day 0 ($P > 0.05$). This indicates that the addition of APE into the cheese wraps did not create any unusual flavor or aroma characteristics at baseline. At day 14, the overall acceptability score (TAMC = total aerobic microbial count log colony forming units/ gram) of control cheese wrapped in the wraps was lower due to the flavor (5.8 ± 0.4) and aroma (5.6 ± 0.5) than that of cheese wrapped in 3% APE wraps (7.4 ± 0.4 , and 7.3 ± 0.5 , respectively; $P < 0.05$). By day 28, the overall acceptability score of control cheese wrapped in control wraps

had fallen below the average score of 5.0, while the overall acceptability of cheese wrapped in 3% APE was scored at 7.2 ± 0.4 . The reason that 26% of those panelists who evaluated the flavor of cheese wrapped in 3% APE thought there was a slight astringency (meaning mild bitterness) from the persistent release of quercetin and chlorogenic acid at the surface of the cheese, which agrees with previous studies of the thresholds of polyphenols in dairy products (14). Additionally, since both microbiological protection (TAMC = 4.6 log CFU/g) did not create a perception of astringency from the 2% APE wraps, then it may provide the best trade-off for sensory-sensitive applications.

Table 6 shows sensory evaluation data (mean $\pm$ sd, n = 20 panelists) for the four treatments of storage of FSG/PVA/APE wrapped cheese.

3% APE — Day 28	3% APE — Day 14	3% APE — Day 0	0% — Day 28	0% — Day 14	0% — Day 0	Attribute / Day
7.3 ± 0.4^b	7.6 ± 0.3^b	8.1 ± 0.4^a	5.1 ± 0.6^a	6.8 ± 0.4^a	8.2 ± 0.3^a	Color
7.0 ± 0.5^b	7.3 ± 0.5^b	8.2 ± 0.3^a	4.8 ± 0.5^a	6.2 ± 0.4^a	8.3 ± 0.3^a	Odor
7.4 ± 0.3^b	7.6 ± 0.4^b	8.1 ± 0.3^a	4.5 ± 0.7^a	6.4 ± 0.5^a	8.0 ± 0.4^a	Texture
7.1 ± 0.5^b	7.4 ± 0.4^b	8.3 ± 0.3^a	4.2 ± 0.6^a	5.8 ± 0.4^a	8.4 ± 0.3^a	Flavor
7.2 ± 0.4^b	7.5 ± 0.4^b	8.1 ± 0.3^a	4.3 ± 0.5^a	6.1 ± 0.4^a	8.0 ± 0.2^a	Overall Accept.

4. Conclusion

This research validates the use of composite active packaging films made from fish skin gelatin (FSG) polyvinyl alcohol (PVA) and apple peel extract (APE). In addition to verifying the enhanced physical and mechanical properties of the films that are reported in previous studies, this study makes the

following original contributions: (i) determination of minimum inhibitory concentration (MIC) and efficacy testing using pathogen challenge confirm the films meet the European Food Safety Authority (EFSA) guideline for a three-log reduction of *Listeria monocytogenes*; (ii) differential scanning calorimetry

(DSC) and thermogravimetric analysis (TGA) indicate that the enhancement of mechanical properties by APE was caused by increased thermal stability and melting point (T_m) of the gelatin crystallites; (iii) X-ray diffraction (XRD) demonstrated that the increased physical and mechanical properties of the films correlated to decreased crystallinity; (iv) polyphenol transport is characterized by Fickian diffusion kinetics ($n = 0.42$), which predicts prolonged release of bioactive materials; and (v) total migration of 3.2 mg/dm^2 demonstrates that all materials used in the film construct conform to the European Union (EU) Regulation 10/2011 and Saudi Food and Drug Authority (SFDA) standards, thus facilitating the path to commercial use of these films. The treatment with 3% APE extended the shelf life by 10 days, reduced the total aerobic plate count (TAPC) by 2.3 log

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Conflict of Interest

The authors declare no conflict of interest.

CFU/g, prevented lipid oxidation to less than the sensory threshold for 28 days, and maintained an overall acceptability rating above 7.0 for the duration of the study. The 2% concentration of APE may provide a better option for applications where consumer sensitivity to astringency is important because it offers similar microbial protection without affecting the flavor of the food product. Using apple peel waste as a source of functional high-value packaging components supports the goals of a circular economy and zero-waste manufacturing. Future studies should evaluate (a) scaling APE-treated film manufacturing under commercial drying processes; (b) performance of the films using modified atmosphere packaging; (c) application to other high-moisture soft cheese products (ricotta, feta); and (d)) life cycle assessment (LCA) of the entire production process.

and the time and expertise of the trained sensory panel.

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