



Enhancing the quality and shelf life of strawberries: Study of pectin-based films and application of coatings with natural deep eutectic solvents and *Moringa oleifera* leaf extract

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ABSTRACT

This study investigates the dual application of Natural Deep Eutectic Solvents (NADES), composed of choline chloride and maltose (4:1 M ratio), as an eco-friendly medium for extracting bioactive compounds from *Moringa oleifera* leaf flour and as a plasticizer in pectin films. The NADES extract (MNE) showed high efficiency in extracting phenolic compounds (36.25 ± 0.14 mg GAE/g), with antioxidant capacities of 0.97 ± 0.03 mmol TE/g (ORAC) and 0.96 ± 0.21 PI (ABTS). Pectin-based films were prepared with and without NADES or MNE and evaluated for thickness, optical properties, water vapor permeability (WVP), and microstructure. Films containing NADES or MNE exhibited higher WVP (2.327 ± 0.075 and 2.180 ± 0.032 g·m⁻¹·s⁻¹·Pa⁻¹ × 10⁻¹⁰, respectively) compared to control films without plasticizer (1.269 ± 0.051 × 10⁻¹⁰ g m⁻¹ s⁻¹ Pa⁻¹), and demonstrated improved structural homogeneity. To assess practical use, the films were applied as edible coatings on strawberries. Coated fruits showed reduced weight loss and decay, and better retention of firmness, acidity, soluble solids, and anthocyanins over 11 days at 4 °C. These results highlight NADES-based coatings as a sustainable approach to enhance food preservation. Future research should aim to optimize these formulations for broader food applications and industrial scalability.

1. Introduction

Strawberries hold a prominent status among the world's most consumed fruits, renowned for their rich reservoir of essential nutrients including vitamins, minerals, folate, and an array of phytochemical compounds. Extensive research, as demonstrated by [1] underscores the multifaceted health advantages linked to strawberry consumption. These benefits encompass notable support for cardiovascular health, potent antiproliferative properties, and promising neurological implications. Such findings illuminate strawberries as not only a beloved dietary staple but also a compelling source of therapeutic potential. The short postharvest lifespan of strawberries, coupled with the rising demand for safe and high-quality products, presents a pressing concern for

producers striving to meet consumer expectations while minimizing losses. Hence, a range of innovative technologies has been developed to address this challenge, including modified atmosphere packaging, pressurized inert gases, electron beam irradiation, ultraviolet light, and cold plasma [2]. Notably, considerable focus has been directed toward the utilization of edible coatings. These coatings, characterized by their thin layer application onto product surfaces, serve as a protective barrier against moisture loss, gaseous exchange, and microbial proliferation, thereby effectively extending the shelf life of perishable goods [3].

Edible films and coatings are crafted from natural sources, including proteins such as gelatin, collagen, casein, and whey proteins, as well as lipids like waxes, oils, acetylated glycerides, and fatty acids. Polysaccharides such as chitosan, cellulose, gums, starch, and pectin are also

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commonly used. Moreover, these films and coatings can be produced from blended materials offering versatility and adaptability to various food preservation needs.

Pectin is one of the polysaccharide constituents of cell walls of higher plants with complex structures that comprise homogalacturonan, xylogalacturonan, and rhamnogalacturonans I and II, recognized as safe and used in the pharmaceutical, cosmetic, and food industries [4]. Its diverse characteristics, such as low cost, formation of transparent coatings, availability, and gas barrier properties, make it a main compatible material for fruit and vegetable coatings [5]. Several studies report the successful application of edible coatings based on pectin and other polymers to extend the shelf life of fruits and vegetables [6–9]. On the other hand, the incorporation of additives such as plasticizers, nanoparticles, essential oils, and plant extracts in the coating solutions improves the quality and increases the shelf life of the product. *Moringa oleifera* Lam. (Moringaceae), also called the miracle tree and grown in tropical and subtropical regions, has a diverse range of bioactive compounds that have proven to be good agents against various health disorders and have a vast potential for food applications [10].

Therefore, the use of *Moringa* leaf extracts in food packaging applications has received special attention from several researchers [11–17]. However, solvent residues from traditional organic solvents used in the extraction of bioactive compounds, being toxic, restrict their incorporation in edible films and coatings; hence, natural deep eutectic solvents (NADES) could replace the conventional solvents as green alternatives. Defined as an eutectic mixture of mainly two components, the first is the hydrogen bond acceptor (HBA), and the second is the hydrogen bond donor (HBD), made of natural constituents (organic acids, amino acids, choline derivatives, sugars, etc), presenting a high melting point depression at specific compositions and becoming liquids at room temperature [18,19].

NADES have emerged as a sustainable alternative to conventional organic solvents, particularly for the extraction of bioactive compounds due to their low toxicity, biodegradability, and tunable physicochemical properties. Moreover, NADES components can be chosen from generally recognized as safe (GRAS) substances, making them especially attractive for applications in food and pharmaceutical products. Despite growing interest in NADES as green extractants, few studies have explored their potential dual functionality, serving not only as extractants but also as functional additives or plasticizers in polymeric matrices. This dual application could significantly simplify production steps and reduce environmental impact, representing a novel strategy in the development of active biodegradable films.

However, the literature lacks a comprehensive approach that combines the use of NADES for both bioactive compound recovery and direct incorporation into biopolymer-based films. Most prior research has treated extraction and film development as separate processes, frequently using synthetic plasticizers or additional cross-linking agents after the extraction step.

In addition to their potential as extracting solvents for bioactive compounds [20–24], NADES demonstrate high hydrogen-bonding capacity, enabling them to efficiently dissolve cell walls and facilitate strong intermolecular interactions with cellulose chains [25]. Moreover, NADES have been shown to act effectively as plasticizing agents [26–28].

A study by Wu, et al. (2020) explored the use of ultrasonic-assisted extraction (UAE) with specific deep eutectic solvents (DES) to optimize the extraction of phenolic and flavonoid compounds, as well as antioxidant activity, from *Moringa oleifera* leaves [29]. Their findings revealed that DES-based UAE outperformed other extraction methods in isolating phenolic compounds such as gallic acid, orientin, quercetin, kaempferol, and catechin. These compounds exhibited high antioxidant activity, contributing to the overall potency of the extracts. In a related study [30], the efficacy of various DES formulations for polyphenol extraction from *Moringa oleifera* leaves was investigated. The ultrasonic-assisted DES extraction method demonstrated higher efficiency than

70% ethanol, with seven different DES formulations showing superior performance. Furthermore, Jaglan et al. (2024) optimized the extraction process for *Moringa oleifera* flower by evaluating six different DES combinations at molar ratios of 1:1, 1:2, and 1:3 [31].

Several edible coatings and packaging films are already available for fresh produce preservation, including synthetic polymers such as polyethylene (PE) and polyvinyl chloride (PVC), as well as biopolymer-based materials derived from chitosan, alginate, starch, or cellulose. While synthetic films typically offer superior barrier properties (e.g., water vapor permeability below $1.0 \times 10^{-10} \text{ g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$), they are non-biodegradable and not suitable for direct food contact without regulation. The biopolymer films, although biodegradable, often require chemical crosslinkers or additives to reach acceptable mechanical strength and moisture resistance [32]. In contrast, the pectin-based films developed in this work, enriched with NADES or *Moringa oleifera* leaves extract, present a biodegradable, bioactive alternative. Although pectin-based films generally exhibit higher water vapor permeability (WVP) compared to synthetic alternatives, they offer significant advantages in terms of biodegradability and can be enhanced with bioactive compounds to improve the preservation of perishable fruits such as strawberries. As shown in previous studies that incorporated natural extracts or functional additives into polysaccharide-based films to extend shelf life and reduce microbial spoilage [32,33].

The selection of a NADES, composed of choline chloride and maltose, for this study is driven by its potential as a green, sustainable medium for the efficient extraction of bioactive compounds and its additional functionality as a plasticizer in pectin-based edible films. Previous research of Braham et al. (2022) demonstrated that NADES composed of choline chloride and lactic acid or glycerol efficiently extracted phenolic compounds from *Moringa oleifera* leaves, identifying ten compounds with high antioxidant capacity [34]. Among these, NADES with glycerol showed a higher capacity for polyphenolic extraction.

Building on this foundation, choline chloride with maltose was selected to further enhance the extraction process due to the unique hydrogen bonding interactions of maltose, which may increase solubility and extraction yield. Moreover, its dual role as a plasticizer can improve the structural and functional properties of pectin-based films, addressing the challenge of extending the shelf life of perishable fruits like strawberries while maintaining their quality. This dual-purpose approach not only advances the green extraction methodology but also contributes to the development of sustainable food preservation solutions.

Regarding regulatory compliance, all components used in our coatings (pectin, choline chloride, and maltose) are considered food-grade and permitted under both U.S. FDA and European EFSA frameworks. Pectin is listed as E440(i) by EFSA and recognized as GRAS by the FDA. Choline chloride is also listed as GRAS (Food Additive Number. E 1001 (iii), approved as a human nutrient under 21 CFR 182.8252), and maltose is widely used as a sweetener and carbohydrate source in food systems. Recent studies have demonstrated that NADES composed entirely of such edible constituents can be applied directly to food surfaces without requiring solvent removal, offering a promising pathway toward natural, safe food-grade solvent systems [18,21]. These regulatory aspects support the feasibility of the proposed coatings for real-world food applications.

Therefore, the objective of this work was to develop multifunctional pectin-based films incorporating NADES both as a green extractant for *Moringa oleifera* leaves phenolics and as a plasticizer. The study evaluates the structural, barrier, mechanical, and functional properties of the resulting films and examines their application as an edible coating for fresh fruit preservation.

2. Materials and methods

2.1. Materials

A commercial high-methoxyl (HM) pectin (Pectin CU201-HM; degree of methylation, DM: 70–74%; molecular mass: 85 kDa) was kindly supplied by Herbstreith & Fox KG (Germany). D-(+)-maltose monohydrate (Panreac Applichem; molar mass: 360.32 g mol⁻¹) and choline chloride (ChCl) (Sigma-Aldrich; molar mass: 139.62 g mol⁻¹) were used as received. Deionized water was used throughout all experiments.

2.2. Preparation of NADES and *Moringa oleifera* leaf extract in NADES (MNE)

The natural deep eutectic solvent (NADES) was synthesized by combining dried choline chloride (acting as the hydrogen bond acceptor) with maltose (serving as the hydrogen bond donor) at a precise molar ratio of 4:1, a method outlined in Radošević et al. (2016) [21]. To optimize its properties, 20% water was added, a crucial step known to reduce viscosity, enhance mass transfer rates, and consequently, elevate extraction yields. The mixture underwent heating at 80 °C while being stirred until achieving a uniform, transparent liquid consistency.

On the other hand, the preparation of *Moringa oleifera* leaf extract (MNE) followed a protocol outlined in Braham et al. (2020) and Oomah et al. (2010) [35,36]. Briefly, 1 g of *M. oleifera* leaf flour was combined with 40 ml of NADES solvent. Following a 2-h extraction period at 30 °C, under continuous stirring at 900 rpm, the extract underwent centrifugation at 11,000g for 30 min. The resulting filtrates were then stored in the freezer for subsequent analysis, ensuring the preservation of key components for further investigation.

2.3. Determination of phenolic compounds and antioxidant capacity in *Moringa oleifera* leaf extract in NADES (MNE)

Total phenol content was determined using the Folin-Ciocalteu method as described by Škerget et al. (2005) [37], and the results were expressed as mg gallic acid equivalents (GAE) per g of sample. Total flavonoids were measured by a colorimetric method with AlCl₃ reagent according to Djeridane et al. (2006) [38] and expressed as mg quercetin equivalents (QE) per g of sample. The antioxidant capacity was estimated by the 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-yl (DPPH) assay and the ferric reducing antioxidant power (FRAP) following the method of Horszwald & Andlauer, (2011) [39]. The oxygen radical absorption capacity assay (ORAC) was done as described by Huang et al. (2002) [40]. Trolox was used as a standard for the preparation of calibration curves, and the results were expressed as mmol trolox equivalents (TE) per g of sample. The 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity was determined according to Gião et al. (2007) [41] and the results were expressed as a percentage of inhibition (PI). All analyses were carried out in triplicate.

A microplate reader (BioTek Instruments Inc., San Diego, CA USA) was used to perform DPPH, FRAP and ORAC assays. To perform the total phenols and flavonoids determination, as well as the ABTS radical scavenging capacity, an UV/Vis spectrometer V-630 Bio from Jasco was used.

2.4. Preparation of film-forming/coating solutions

Pectin solutions (P) were prepared by dissolving 2 g of pectin powder in 100 g of distilled water under stirring (600 rpm) at room temperature under continuous magnetic stirring until complete solubilization was achieved. At the concentration used, pectin is a water-soluble polysaccharide; therefore, heating was not required for its dissolution. P-NADES and P-MNE solutions were prepared by adding 1% (w/w,

relative to the dry mass of pectin) of NADES or MNE to the pectin solution and stirring for several minutes at room temperature, to homogenise the resulting solutions.

2.4.1. Cryogenic scanning electron microscopy (Cryo-SEM)

Cryo-SEM micrographs of the samples, 1–3-mm³ of each solution, were taken using a JEOL JSM 630F (JEOL Ltd., Japan) equipped with a cryogenic preparation chamber (Gatan ALTO 2500).

2.5. Films preparation

Pectin-based films were prepared by the knife-coating technique. Briefly, 60 g of the film-forming solutions were spread at 0.3 m.s⁻¹ on acrylic plates using a film applicator (Sheen, model 1132 N, Cambridge, UK). Then, the films underwent a 24-h drying period at 40 °C and 60% relative humidity (RH) in an environmental test chamber (Binder KMF 115), and finally they were carefully peeled off their supporting substrate.

2.6. Films characterization

2.6.1. Thickness (d)

The film thickness was measured, using a digital micrometer (Absolute Digimatic Indicator, model ID-F150, Mitutoyo Co., Kanagawa, Japan), at ten different positions on each sample with an accuracy of 1 μm.

2.6.2. Colour and opacity

The colour parameters and opacity of the different pectin films were evaluated using a Colour Lite spectrophotometer (Colour Lite GmbH, sph860, Katlenburg-Lindau, Germany) after calibration with white and black standard tiles. Colour parameters were expressed as L* (lightness), a* (red-green), and b* (yellow-blue), and the total difference in colour (ΔE*) was calculated using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Where ΔL*, Δa*, and Δb* parameters represent the difference between the colour of the white standard tile and that of the samples.

The opacity of the films was determined according to the equation:

$$\text{Opacity}(\%) = 100 \cdot \left(\frac{Y_b}{Y_w} \right) \quad (2)$$

Where Y_b is the opacity of each sample on the black standard and Y_w is the opacity on the white standard. Colour and opacity were measured in triplicate for each film specimen.

2.6.3. Water vapor permeability (WVP)

The water vapor permeability (WVP) of films was determined following the ASTM standard method ASTM, A. (2016).E96/E96M-16 [42]. Film samples (discs with an 80 mm diameter) were conditioned in an environmental test chamber (Binder KMF 115) at 25 °C and RH = 53% for at least 72 h. Subsequently, they were tightly sealed on the top of permeation cells containing anhydrous calcium chloride (RH = 2%) and placed in a container filled with distilled water (RH = 100%, constant temperature = 25 °C). Air convection was used to facilitate water diffusion. The weight of the permeation cells was recorded periodically, and WVP (g.m⁻¹.s⁻¹.Pa⁻¹) was determined in triplicate and calculated as follows:

$$WVP = \frac{\Delta m \cdot x}{A \cdot \Delta t \cdot \Delta p} \quad (3)$$

Where Δm represents the weight gain (g), x the film thickness (m), and A the area (0.003 m²) exposed for a time Δt (s) to a partial water vapor pressure Δp (Pa).

2.6.4. Scanning electron microscopy (SEM)

The surface and cross-sectional morphology of film samples were examined using an FEI Quanta 400 FEG scanning electron microscope. These analyses were conducted at CEMUP (Centro de Materiais da Universidade do Porto-PT), following the methodology outlined in [26].

2.7. Edible coating application to strawberries

The process of applying edible coatings to strawberries involved several steps. Firstly, strawberries were obtained from a local market, specifically *Supermercado El Corte Inglés* in Porto, Portugal. Only fruits with a uniform size, exhibiting 90% surface red coloration, and free from physical damage or fungal infections were chosen. These selected strawberries were then gently washed with tap water and thoroughly drained.

The strawberries were categorized into four groups: Group C comprised strawberries without any coating, serving as the control; Group P consisted of strawberries coated with a 2% pectin solution; Group P-NADES included strawberries coated with a 2% pectin solution containing 1% of NADES (ChCl: maltose, 4:1) and Group P-MNE encompassed strawberries coated with a 2% pectin solution containing 1% of moringa NADES extract.

Subsequently, the strawberries were immersed in their respective coating solutions for 1 min, followed by air drying at room temperature. Finally, the coated strawberries were stored for 11 days at 4 °C in a chamber. Analyses were conducted on days 1, 3, 5, 7, 9, and 11, with weight measurements performed on day zero.

2.8. Quality attributes of strawberry fruits

2.8.1. Colour parameters

The Colour Lite spectrophotometer (Colour Lite GmbH, model sph860, Katlenburg-Lindau, Germany) was employed to assess the surface colour of the strawberries. This allowed for the determination of the CIE Lab* colour parameters. The results, representing the average of seven replicates from each group on each day of storage, were then expressed accordingly.

2.8.2. Visual appearance, weight loss and firmness

Visual appearance, weight loss, and firmness of the samples were assessed during storage. Visual inspections of the samples were conducted at each day of analysis, and photographs of the strawberries were taken using a Nikon D3200 camera, Japan.

Weight loss was determined by weighing the same strawberries from each group on day zero and at each subsequent day of analysis. The results were expressed as the percentage of initial weight lost.

Firmness analysis was conducted following the method outlined by Liu et al. (2018) [43], utilizing a TA-XT2 texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 6 mm cylindrical probe (P/6). The penetration depth was set at 10 mm, and the test speed was 2 mm/s. A minimum of ten measurements were taken for each sample, and the maximum penetration force (N) was considered as the measure of firmness.

2.8.3. pH and titratable acidity

The strawberries were cut into small pieces and blended thoroughly using a hand-held blender. Subsequently, the pH of the blended strawberry mixture was measured using a calibrated pH meter. To determine the titratable acidity, 5 g of the puree were mixed with 50 ml of hot distilled water (at 80 °C) for half an hour, then filtered. The resulting solution was titrated to reach a pH of 8.1 using 0.1 mol/L NaOH, and the acidity was expressed as a percentage of citric acid [44].

2.8.4. Total soluble solids content and ripening index

Total soluble solids content (°Brix) of strawberry juices was recorded at 25 °C using a digital refractometer (Atago, Japan). Therefore, the

ripening index (°Brix/% citric acid) was calculated as a ratio of total soluble solids and titratable acidity [45]. All analyses were performed in triplicate.

2.8.5. Total anthocyanin content

The anthocyanin content of samples was quantified using the method reported by Khalifa et al. (2016) [46]. Briefly, 5 g of strawberry puree was extracted with 40 ml of acidified ethanol for 2 h at room temperature, and the absorbance of the extract was measured at 535 nm with a UV/Vis spectrometer from Jasco, model V-630 Bio. Measurements were carried out in triplicate and expressed as mg per 100 g of sample.

2.9. Statistical analyses

Statistical analyses were performed using the software STATISTICA, v. 8.0 (StatSoft, Tulsa, Oklahoma). An analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) post-test was applied to compare mean values among groups at a confidence interval of 95%. Data were presented as mean ± standard deviation.

3. Results and discussion

3.1. Determination of phenolic compounds and antioxidant capacity of MNE

The phenolic compounds and antioxidant capacity of moringa NADES extract were evaluated. Table 1 presents the findings. The ChCl: maltose NADES with a 4:1 ratio with 20% water content demonstrated efficient extraction of polyphenols, resulting in a total phenolic content (TPC) of 36.25 ± 0.14 mg GAE/g sample. This effectiveness can be attributed to factors such as the polarity and viscosity of the NADES system, as well as the extraction method employed [47].

The extraction efficiency of NADES is known to depend strongly on the nature of its components, particularly the hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). In this study, choline chloride (HBA) was paired with maltose (HBD), a disaccharide with multiple hydroxyl groups capable of forming extensive hydrogen bonding networks. This structural feature likely enhances the solubilization of polar phenolic compounds from the *Moringa oleifera* leaves.

Compared to other NADES systems such as choline chloride:lactic acid or choline chloride:glycerol, the choline chloride:maltose combination demonstrates a higher viscosity but offers greater hydrogen bonding capacity, which may contribute to improved stabilization and extraction of polyphenols. Previous reports have shown that NADES containing lactic acid tend to extract phenolics with lower molecular weight, while sugar-based NADES like the one used here may be more effective at extracting and stabilizing anthocyanins and other flavonoids due to selective polarity matching and complex formation [48]. In line with this, choline chloride–fructose (1:2) NADES has also been applied to extract phenolic compounds from oil palm leaves, demonstrating higher efficiency and stability in eutecto gel formulation [49]. Moreover, research by Z. Wei et al. (2015) demonstrated the excellent extractability of polar and weak polar phenolic compounds from *Cajanus cajan* leaves using NADES composed of choline chloride and maltose

Table 1
Phenolic content and antioxidant capacity of MNE extracted using ChCl: maltose (4:1) with 20% water.

Test	Results*
Total phenols (mg GAE/g sample)	36.25 ± 0.14
Total flavonoids (mg QE/g sample)	2.05 ± 0.03
FRAP (mmol TE/g sample)	0.39 ± 0.02
DPPH (mmol TE/g sample)	0.54 ± 0.01
ORAC (mmol TE/g sample)	0.97 ± 0.03
ABTS (PI)	0.96 ± 0.21^f

* Data are presented as mean ± standard deviation of three replicates.

comparing to other sugar-based NADES like choline chloride- glucose and choline chloride- sucrose [24].

Thus, the choice of maltose as the HBD in this formulation likely played a key role in the observed TPC and antioxidant capacity values.

Furthermore, the *Moringa oleifera* leaf NADES extract (MNE) exhibited significant antioxidant capacity, with ABTS at 0.96 ± 0.21 PI, ORAC at 0.97 ± 0.03 , and DPPH at 0.54 ± 0.01 mmol TE/g sample. However, it showed a lower ability to reduce iron (FRAP at 0.39 ± 0.02 mmol TE/g sample) compared to scavenging peroxy, DPPH, or ABTS. According to Jurić et al. 2021, the FRAP assay showed a strong correlation with the total flavonoids content, whereas a weaker correlation was observed with the DPPH assay [50]. This suggests that the antioxidant activity is primarily attributed to flavonoids and phenolic compounds. Previous studies have also highlighted the efficient extraction and high antioxidant capacity of *Moringa oleifera* extracts using deep eutectic solvents. For instance, reports by different authors [29,34,51,52] have underscored these points.

Additionally, Radošević et al. (2016) suggest that extracts obtained using natural deep eutectic solvents can be directly incorporated into products for human consumption, avoiding the need for costly downstream purification steps [21]. As a result, the *Moringa* NADES extract was utilized for strawberry coating, indicating its potential application in food products.

3.2. Morphological characteristics of film-forming/coating solutions

Before the drying process that leads to film formation, significant structural changes can occur. The cryo-SEM technique enables the observation and morphological characterization of solution structures in their native state. Fig. 1 shows the cryo-SEM findings, highlighting distinct network structures, meshes, layouts, and bonding patterns within the solutions. These structural differences are evidently influenced by variations in the solution composition.

The P-NADES sample demonstrates a polymeric network that is more compact and organized compared to the P sample, which exhibits an irregular structure. This irregularity is likely due to pectin molecules interacting with amorphous water [53].

Incorporating *Moringa* Leaves extract (P-MNE) into the solution further modifies the morphological structure of the polymers, resulting in a more regular arrangement with pore diameters ranging from 1 to 12 μm . In contrast, the P-NADES sample features larger pore diameters, ranging from 4 to 16 μm . This variation in network density is likely attributed to the bioactive components extracted from *Moringa* leaves, which influence the polymer structuring process. These findings highlight the significant role of natural extracts in tailoring the microstructural properties of polymeric materials, potentially enhancing their functionality for specific applications.

3.3. Film properties

3.3.1. Visual film appearance and optical properties

The prepared films (with and without NADES and/or MNE) were visually transparent. As shown in Fig. 2 the films exhibited a homogeneous structure, free from cracks or pores. They were also flexible, allowing them to be easily peeled off from the support dish and handled without difficulty.

Analysis of the optical properties, presented in Table 2, showed that all samples had high lightness, indicated by an L^* value of 92. Regarding the chromatic parameters a^* and b^* , the film containing NADES (P-NADES) exhibited the lowest a^* value and a slight increase in the b^* value. However, the addition of *Moringa oleifera* leaf extract in NADES (MNE) did not significantly affect the chromatic parameters compared to the P-NADES film (Table 2). The P-NADES film displayed the highest total colour difference value. This result is consistent with the findings of Jakubowska et al. (2021), who noted that the incorporation of deep eutectic solvents as plasticizers into chitosan films notably influenced colour parameters, particularly the b^* values [54]. The ΔE values, which represent the total colour difference compared to a reference (likely the control film P), range from 3.76 to 4.08. These values are slightly above the common visual acceptability threshold of $\Delta E \approx 3$, suggesting that colour differences are noticeable but may still be acceptable depending on the application. Regarding opacity, all films showed similar values with no significant differences between them, suggesting their potential



Fig. 2. Pectin edible film.

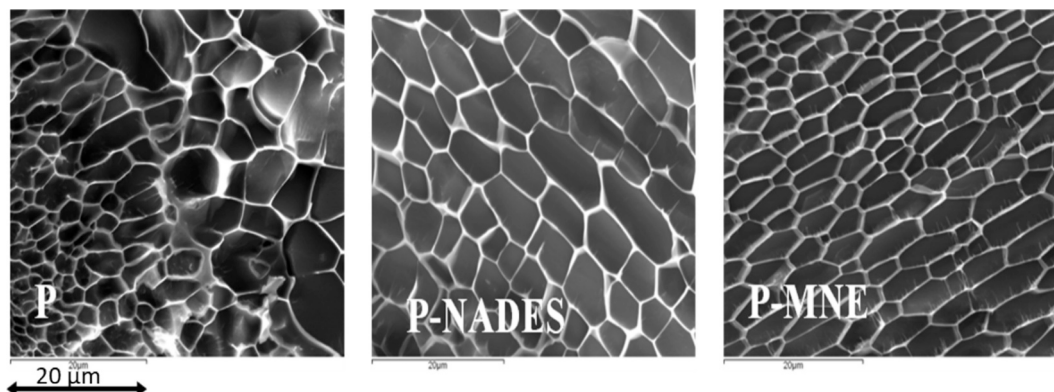


Fig. 1. Cryo-SEM images of the studied pectin solutions for P-film (control), P-NADES films, P-MNE films.

Table 2

Colour parameters and opacity of the different pectin films.

Film	L^*	a^*	b^*	ΔE^*	Opacity(%)
P	92.48 ± 0.12 ^{ab}	0.46 ± 0.05 ^a	2.91 ± 0.19 ^a	3.76 ± 0.05 ^a	0.48 ± 0.04 ^a
P-NADES	92.24 ± 0.24 ^a	0.24 ± 0.05 ^b	3.25 ± 0.05 ^b	4.08 ± 0.05 ^b	0.47 ± 0.02 ^a
P-MNE	92.37 ± 0.23 ^{ab}	0.45 ± 0.04 ^a	3.11 ± 0.07 ^{ab}	3.86 ± 0.13 ^a	0.51 ± 0.02 ^a

All values are given as means ± standard deviation. Means with different superscript letters in the same column indicate significant differences ($P < 0.05$).

for use in coating applications, as they do not alter the appearance of the coated materials.

3.3.2. Scanning electron microscopy (SEM)

The surface images of the films, captured at 5000× magnification, are shown in Fig. 3. The SEM images reveal a compact and homogeneous surface microstructure for films without NADES or MNE. Fig. 3 includes SEM micrographs of both cross-sectional (a, b, and c) and surface (d and e) views of the films.

The P films displayed a smooth surface (Fig. 3d) but had a heterogeneous cross-sectional structure (Fig. 3a). The P-MNE films exhibited some surface irregularities (Fig. 3e), which were attributed to increased porosity, and a relatively loose cross-sectional structure was also observed (Fig. 3b). Similarly, the P-NADES films were characterized by a loose cross-sectional structure (Fig. 3c).

3.3.3. Thickness and barrier (water vapor permeability (WVP)) properties

The results presented in Table 3 reveal significant differences ($P < 0.05$) in film thickness among the samples. Specifically, films containing NADES or MNE showed higher thickness values compared to those prepared solely with pectin. The increase in film thickness observed in samples containing NADES or MNE can be attributed to several factors linked to the physicochemical properties of these components. NADES are known to have high viscosity due to their unique hydrogen-bonding network and interactions between their constituents. When incorporated into the film matrix, these properties can contribute to increased

Table 3

Films thickness (d) and water vapor permeability (WVP) of pectin films.

Films	d (mm)	WVP ($\text{g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$) $\times 10^{-10}$
P	0.018 ± 0.003 ^a	1.269 ± 0.051 ^a
P-NADES	0.026 ± 0.005 ^b	2.327 ± 0.075 ^c
P-MNE	0.025 ± 0.005 ^b	2.180 ± 0.032 ^b

All values are given as means ± standard deviation. Means with different superscript letters in the same column indicate significant differences ($P < 0.05$).

molecular interactions and structural reinforcement, resulting in a thicker film.

Furthermore, NADES may promote the retention of water or other solvent molecules, leading to swelling of the matrix and an increase in its apparent thickness. Additionally, MNE (if it contains lipophilic or amphiphilic molecules) may also contribute to a more complex microstructure, enhancing the film's dimensional stability and thickness. These findings align with previous studies, which have shown that additives like NADES can modify the film-forming process by altering the matrix's intermolecular interactions and physical properties [53,54].

The water vapor permeability (WVP) data presented in Table 3 indicate that the P-NADES film exhibits the highest WVP value, while the film prepared with pectin alone presents the lowest. This suggests that the presence of NADES increases the water vapor permeability, whereas phenolic extracts of *Moringa* display a capability to mitigate it. According to Gouveia et al. (2019), the increase in WVP observed in

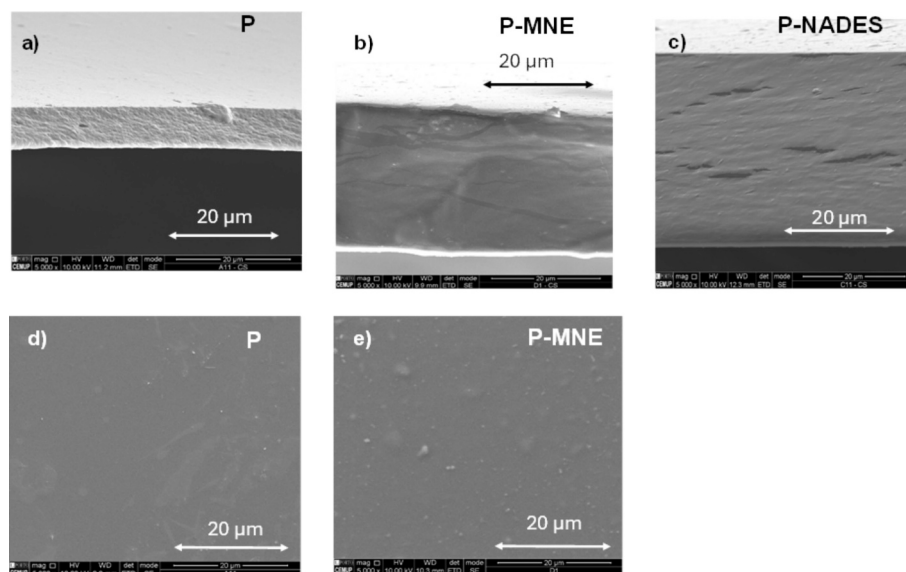


Fig. 3. SEM micrographs of the cross sections of (a) P film (control), (b) P-MNE films, (c) P-NADES films and surfaces of (d) P film and (e) P-MNE films.

films containing deep eutectic solvents can be attributed to the hydrophilic nature of their constituents and the inclusion of choline chloride in the eutectic mixture, facilitating the creation of interstitial spaces and thus allowing a higher diffusion rate of water molecules [27]. On the other hand, and following our results, various studies [55–57] have reported a reduction in WVP for films incorporating bioactive compounds. These studies propose that the formation of cross-links between phenolic compounds and film molecules diminishes the free volume within the film matrix, resulting in a more compact structure that impedes the diffusion of water vapor.

Although lower WVP values are generally preferred for packaging dry products, in the case of fresh produce, moderate WVP can be beneficial. High water vapor barrier properties may lead to condensation inside the packaging, creating a humid microenvironment that

favors microbial growth [58]. On the other hand, a film with slightly higher WVP allows for controlled moisture exchange, reducing the risk of water accumulation and maintaining firmness and freshness.

In this study, the P-NADES film exhibited a WVP level that, while slightly elevated, may have contributed positively to moisture balance during strawberry storage. This functional permeability is aligned with the needs of high-respiration fruits, where complete water retention is neither necessary nor desirable and may also help limit excessive weight loss by avoiding both dehydration and condensation-driven deterioration.

3.4. Edible coating application and quality attributes of strawberries fruits

Strawberries are prized for their vivid chromaticity, distinct

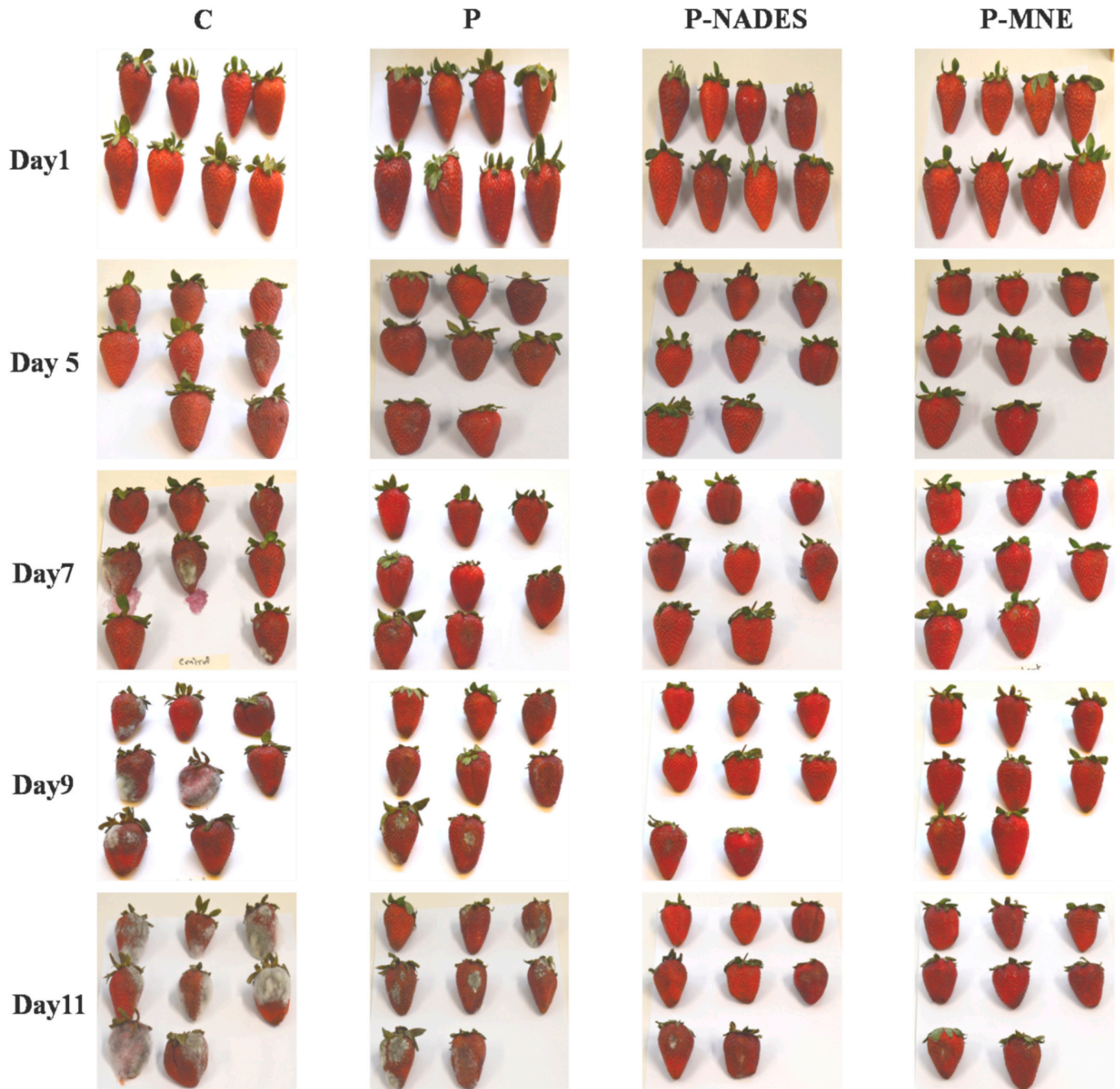


Fig. 4. Visual appearance of samples stored at 4 °C for 11 days. C (control), P (strawberries coated with only pectin), P-NADES (strawberries coated with pectin containing NADES) and P-MNE (strawberries coated with pectin containing moringa NADES extract).

olfactory profile, and palatable taste properties, making them highly desirable to consumers. A comprehensive understanding of these attributes is critical to assessing the holistic quality and commercial viability of strawberry fruits. Herein, we outline discernible characteristics evident in both coated and uncoated strawberries.

3.4.1. Visual appearance

A visual assessment was performed to evaluate the impact of different coatings on strawberries. Images of the samples throughout the storage period are presented in Fig. 4. The samples were inspected for signs of fungal growth, including brown spots, visible grey, white, or black mold, and softening in infected areas. On the first day of the experiment, both control and coated strawberries appeared similar showing that the coating application didn't affect the visual appearance of strawberries, which is following the optical properties results (Table 4).

Throughout the storage period, edible coatings demonstrated their effectiveness in inhibiting fungal growth, extending the shelf life of strawberries. Notably, strawberries coated with P-NADES and P-MNE exhibited the longest shelf life, remaining free from fungal growth for up to 9 days.

3.4.2. Physical properties

3.4.2.1. Colour parameters (fruit colour). As depicted in Table 4, the colour parameters of coated and uncoated strawberries exhibit no statistically significant variances ($P > 0.05$) on the first analysis day.

This indicates that the diverse coating solutions effectively preserve the strawberries' original colour, an essential factor for consumer acceptance.

3.4.2.2. Weight loss percentage. An increase in weight loss with a distinct percentage was observed for all samples during storage (Fig. 5A); hence, the coating solutions P-MNE and P-NADES reduced the mass loss of strawberry fruits more or less a half compared to control samples; however, coated samples with only pectin showed a weight loss slightly lower than the control. Previous investigations [9,59–61] reported that coated strawberries had lower weight loss than uncoated ones. Other studies [13,14] demonstrated that coatings containing *Moringa* leaf extract were the most effective treatments for reducing weight loss, which could be explained by the capacity of phenolic compounds to improve water retention. In addition, they can retard some enzyme activities during the ripening stage which affects water loss and the softening of fruits.

3.4.2.3. Fruit firmness. Fruit firmness is a critical quality attribute that significantly influences consumer purchasing decisions and plays a vital role in determining the shelf life and market value of most fresh fruits. Factors such as harvest maturity, relative humidity, and storage temperature primarily impact fruit firmness.

On the first day of analysis, the firmness values of coated strawberries were higher than those of the control sample, as illustrated in Fig. 5B. This aligns with findings by Mannozi et al. (2018), which reported that coatings enhance the rigidity of fruit skins [62]. During storage time (11 days at 4 °C), firmness decreased for all samples with a slow rate for coated strawberries. For instance, the firmness of strawberries coated with the P-NADES solution decreased from 5.92 ± 0.10 N to 4.39 ± 0.23 N; while the control sample showed a sharper decrease, from 5.17 ± 0.44 N to 1.56 ± 0.13 N. The higher firmness in coated strawberries can be attributed to reduced moisture loss, consistent with the weight loss results (Fig. 5A).

Additionally, enzymatic hydrolysis of the cell wall likely contributed to the softening of strawberries over time.

Table 4
Colour parameters of control and coated strawberries during storage at 4 °C.

Storage time (days)	L^*			a^*			b^*					
	L^* (C)	L^* (P)	L^* (P-NADES)	L^* (P-MNE)	a^* (C)	a^* (P)	a^* (P-NADES)	a^* (P-MNE)	b^* (C)	b^* (P)	b^* (P-NADES)	b^* (P-MNE)
1	29.11 ± 2.02 ^a	29.74 ± 1.37 ^a	29.31 ± 0.87 ^a	29.16 ± 0.91 ^a	24.11 ± 1.82 ^a	24.64 ± 1.88 ^a	24.59 ± 2.12 ^a	24.24 ± 2.17 ^a	16.33 ± 1.60 ^a	16.08 ± 1.60 ^a	16.41 ± 1.16 ^a	16.66 ± 1.50 ^a
3	31.08 ± 1.80 ^a	31.43 ± 1.25 ^a	31.81 ± 2.35 ^a	31.98 ± 1.28 ^a	24.84 ± 1.93 ^a	24.25 ± 2.08 ^a	24.63 ± 2.26 ^a	24.98 ± 2.25 ^a	16.87 ± 1.35 ^a	16.99 ± 1.27 ^a	16.81 ± 1.47 ^a	16.45 ± 1.46 ^a
5	30.09 ± 0.97 ^a	30.69 ± 1.25 ^a	30.16 ± 0.72 ^a	31.11 ± 1.55 ^a	26.67 ± 2.18 ^a	25.25 ± 1.69 ^a	25.67 ± 1.28 ^a	25.96 ± 2.05 ^a	16.26 ± 1.56 ^a	16.34 ± 1.17 ^a	16.53 ± 1.54 ^a	16.51 ± 1.63 ^a
7	26.48 ± 2.08 ^b	28.47 ± 0.84 ^b	30.22 ± 1.27 ^a	30.34 ± 0.87 ^a	26.35 ± 1.88 ^a	26.15 ± 2.37 ^a	25.93 ± 1.92 ^a	26.37 ± 2.12 ^a	16.83 ± 1.43 ^a	16.71 ± 1.21 ^a	16.41 ± 1.55 ^a	16.82 ± 1.31 ^a
9	23.61 ± 1.76 ^c	26.51 ± 0.89 ^b	29.79 ± 1.07 ^a	30.43 ± 0.59 ^a	17.11 ± 0.90 ^c	22.69 ± 2.01 ^b	26.71 ± 2.34 ^a	26.93 ± 1.68 ^a	15.66 ± 1.32 ^a	16.50 ± 1.39 ^a	16.09 ± 1.08 ^a	16.28 ± 0.95 ^a
11	24.77 ± 1.16 ^c	27.97 ± 1.21 ^a	29.87 ± 1.47 ^b	29.74 ± 0.57 ^b	22.38 ± 1.23 ^b	21.75 ± 1.77 ^b	25.61 ± 2.07 ^a	24.98 ± 1.83 ^a	15.85 ± 1.48 ^a	16.28 ± 1.52 ^a	16.45 ± 1.53 ^a	16.71 ± 1.20 ^a

All values are given as means ± standard deviation. Means in the same line with different superscript letters indicate significant differences ($P < 0.05$) within the same storage period. The lightness of uncoated and coated strawberries with pure pectin decreased progressively from 29.11 ± 2.02 to 24.77 ± 1.16 and 29.74 ± 1.37 to 27.97 ± 1.21 , respectively, resulting from oxidation reactions and moisture loss while P-NADES and P-MNE showed nearly stable lightness during storage. The redness values (a^*) increased slightly for both control and coated strawberries during the first week of storage due to the maturity process. Afterwards, the a^* values of uncoated samples and those coated with only pectin decreased, which could be due to anthocyanin degradation, increasing of CO_2 and decreasing of O_2 during mold growth [8,58]. The b^* values remained relatively stable throughout the storage period for all samples, with no statistically significant changes observed over time ($P > 0.05$).

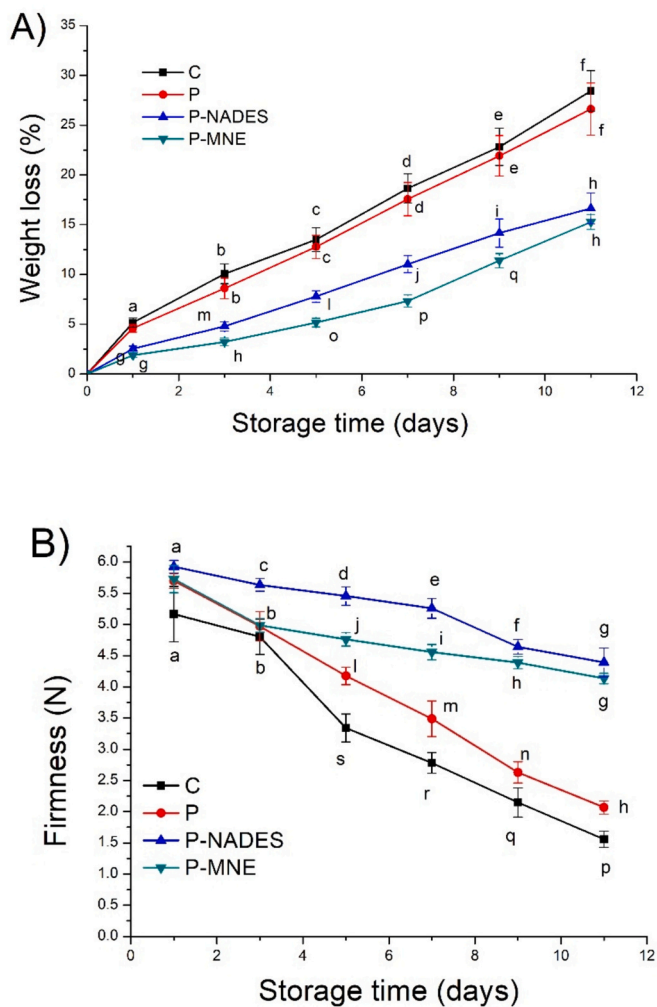


Fig. 5. Effect of coating on weight loss (A), and firmness (B) of strawberries during storage time at 4 °C. C (control), P (strawberries coated with only pectin), P-NADES (strawberries coated with pectin containing NADES) and P-MNE (strawberries coated with pectin containing *Moringa* NADES extract). Values are expressed as mean \pm SD ($n = 3$). Identical letters in the figure indicate no significant differences among treatments at the same time point ($P > 0.05$), according to Tukey's test. Different letters indicate that treatments differ significantly at that time point ($P < 0.05$).

3.4.3. Chemical properties

3.4.3.1. Titratable acidity and pH. Fig. 6 (A and B) illustrates a consistent decrease in titratable acidity and a corresponding increase in pH across all samples. Notably, coated strawberries with P-MNE and P-NADES exhibit a slower rate of change compared to the others.

No significant differences ($P > 0.05$) were observed among all samples for both pH and titratable acidity on the first day of analysis. During storage, the acidity reduction for the control sample was from 0.91 ± 0.02 to 0.52 ± 0.02 (% citric acid) significantly ($P < 0.05$) higher than that of the coated strawberries with P-MNE and P-NADES, which decreased from 0.92 ± 0.04 to 0.69 ± 0.02 (% citric acid) and from 0.88 ± 0.01 to 0.65 ± 0.02 (% citric acid), respectively. Moreover, the coated strawberries with P presented a decrease in acidity from 0.91 ± 0.03 to 0.47 ± 0.02 (% citric acid). On the other hand, the pH values of coated samples with P-MNE and P-NADES achieved 3.36 ± 0.01 and 3.39 ± 0.01 at the end of storage time, while control and coated strawberries with P increased to 3.47 ± 0.03 and 3.51 ± 0.02 , respectively. The variations in acidity and pH levels could be attributed to metabolic reactions occurring within the fruits during storage or to using organic

acids in the respiratory process [63].

The observed changes in pH and titratable acidity have important implications for fruit preservation. Lower pH values are known to inhibit the activity of several spoilage-related enzymes, such as polygalacturonase and pectin methyl esterase, which are involved in cell wall degradation and softening. In addition, more acidic environments limit the proliferation of spoilage microorganisms, particularly pH-sensitive fungi and bacteria. The films containing NADES-extracted phenolics (P-MNE) contributed to the maintenance of acidity and stabilization of pH, which may be linked to the presence of organic acids and phenolic compounds with known antimicrobial activity. These findings suggest a potential synergistic effect between the coating composition and fruit metabolism, contributing to the extended shelf life observed in subsequent analyses.

3.4.3.2. Total soluble solids content and ripening index. The total soluble solids content remained relatively constant during storage for coated strawberries with P-MNE and P-NADES. However, both the control and coated samples with P showed a slight decrease, as depicted in Fig. 6C. This trend aligns with previous research findings [59,60,63]. Changes in titratable acidity and total soluble solids content serve as indicators of strawberry maturity. As illustrated in Fig. 6D, the ripening index advanced consistently throughout the storage period for all samples. However, control and coated strawberries with P exhibited a faster progression, suggesting that the addition of MNE or NADES slowed down metabolic activity and delayed ripening.

3.4.3.3. Total anthocyanin content. The phenylalanine ammonia-lyase enzyme is responsible for synthesising anthocyanins in fruits and vegetables; they are a group of antioxidants that provide the red colour of ripe strawberries [64]. As Fig. 6E shows, the total anthocyanin content increased slightly from the third day to the seventh day of storage which can be attributed to the biosynthesis of these compounds during strawberry ripening [65]. Therefore, the highest anthocyanin content reached 13.64 ± 0.23 mg/100 g for the control sample, and the lowest value was 12.71 ± 0.13 mg/100 g for strawberries coated with P-MNE. From day 9, the anthocyanin content of uncoated strawberries and coated ones with P dropped sharply, with values of 3.74 ± 0.17 mg/100 g and 5.27 ± 0.36 mg/100 g, respectively, while P-MNE and P-NADES coatings restricted the decrease of anthocyanins in samples.

4. Conclusions

The incorporation of choline chloride and maltose-based natural deep eutectic solvents (NADES), with or without *Moringa oleifera* leaf extract (MNE), into pectin-based films and coatings represents a promising and environmentally sustainable strategy for improving the shelf life and quality of highly perishable fruits. The dual role of NADES as both an extraction medium for *Moringa oleifera* bioactives and a plasticizer in edible films and their application into pectin-based films and coatings represents a promising and environmentally sustainable strategy for improving the shelf life of highly perishable fruits and the properties of films.

NADES demonstrated high efficacy in the extraction of bioactive polyphenols, which exhibited significant antioxidant activity. When integrated into pectin matrices, NADES improved key structural and functional characteristics, including increased film thickness and enhanced microstructural homogeneity.

The application of these functional coatings to strawberries yielded marked improvements in postharvest preservation, including significant reductions in weight loss, delayed ripening, maintenance of fruit firmness, and stabilization of anthocyanin content. The presence of MNE further augmented these effects by enhancing the coatings' ability to minimize moisture loss and metabolic activity during storage. These findings highlight the potential of NADES- and MNE-based formulations

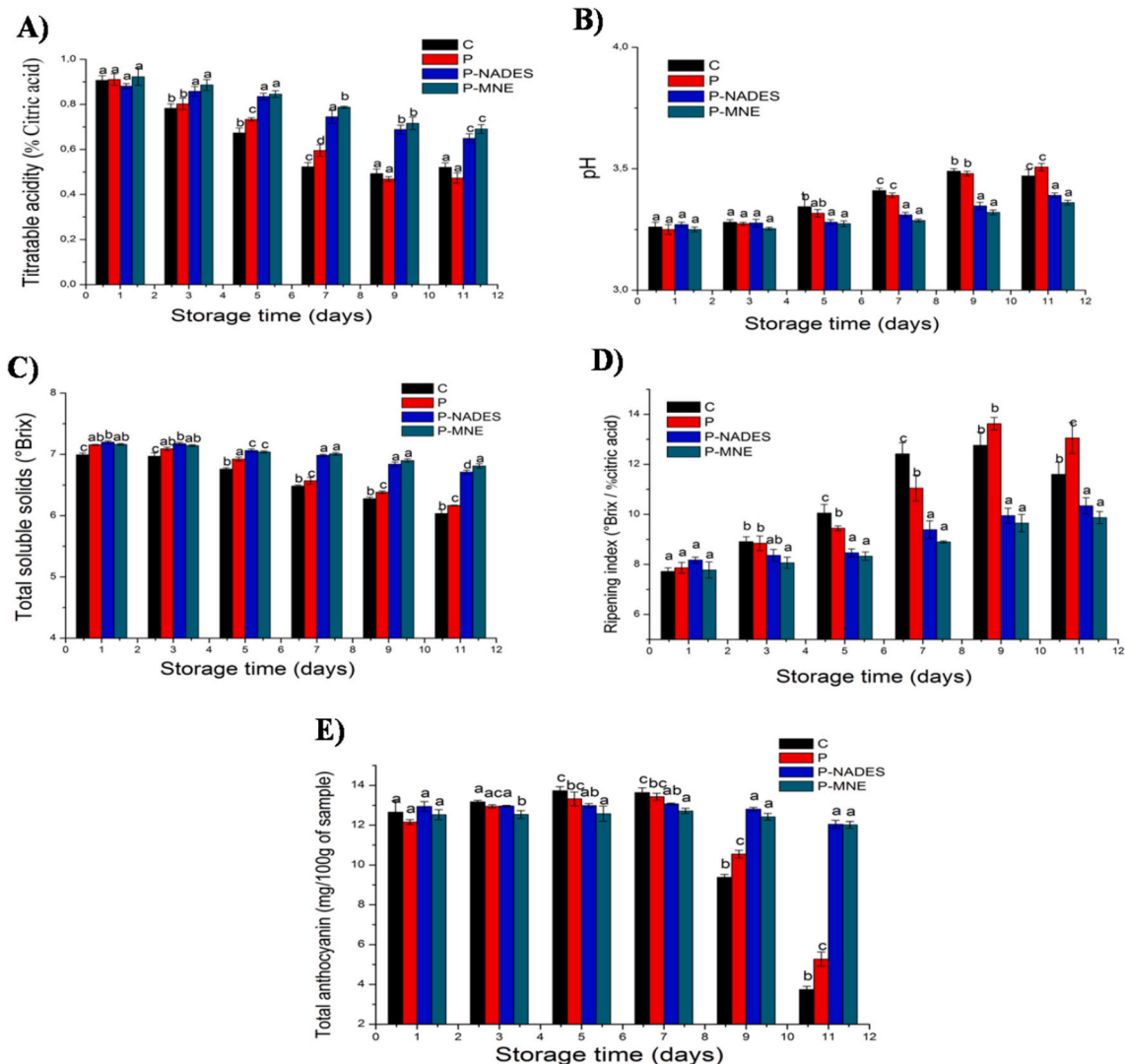


Fig. 6. Changes in titratable acidity (A), pH (B), total soluble solids (C), ripening index (D) and total anthocyanins (E) of control (C) and coated strawberries with pectin alone (P), pectin containing NADES (P-NADES) and pectin containing moringa NADES extract (P-MNE) during storage. Different letters denote significant differences ($P < 0.05$) among treatments at each time point, according to Tukey's test.

as innovative, sustainable alternatives to conventional fruit preservation technologies.

Despite the promising results, this study has some limitations. The extraction process using NADES was conducted under laboratory-scale conditions, and its scalability for industrial applications remains to be evaluated. In addition, while the dual functionality of NADES as both extractant and plasticizer was demonstrated, the potential long-term stability of these films/coating under variable storage conditions (e.g., temperature, humidity) requires further investigation.

The interactions between specific phenolic compounds and the biopolymer matrix were inferred through FTIR data, but more detailed structural analyses such as NMR or XRD could provide deeper insight into the mechanisms involved. Moreover, the sensory properties and consumer acceptance of these films, especially when used as edible coatings, were not addressed in this study.

Future research should explore the effect of varying NADES compositions on film properties, as well as the behavior of the films in real food systems over extended storage periods. Biodegradability assessments under composting or marine conditions would also be valuable to confirm their environmental impact. Also, future investigations should prioritize the optimization of these formulations for industrial scalability, alongside evaluating their performance across diverse food matrices

to broaden their applicability within the food preservation sector.

CRedit authorship contribution statement

Fazia Braham: Writing – original draft, Investigation, Data curation. **Hiléia K.S. Souza:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Júlia M.C. S. Magalhães:** Writing – review & editing, Validation, Supervision, Conceptualization. **Farid Zaidi:** Writing – review & editing, Validation, Supervision, Conceptualization. **Maria P. Gonçalves:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hileia K.S. Souza reports financial support was provided by REQUIMTE LAQV Porto. Maria P Goncalves reports a relationship with REQUIMTE LAQV Porto that includes: board membership. The authors have no conflicts of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The authors do not have permission to share data.

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