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A Data Science Approach to Colorimetric and pH Analysis for Freshness Monitoring

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Abstract

Polysaccharide-based films represent a sustainable alternative for the development of intelligent packaging systems capable of monitoring food quality through colorimetric changes. In this study, films formulated with chitosan, starch, glycerol, and *Hibiscus sabdariffa* extract were evaluated as potential freshness indicators for pork meat. The films were prepared using different extract concentrations and stored at 6 and 25 °C before and after contact with pork samples for 36 h. Their colorimetric response was quantified through digital image analysis using the RGB color model combined with the K-means clustering algorithm. The relationships among the experimental variables were investigated using descriptive statistics, Spearman correlation analysis, and feature relevance metrics, including Information Gain, Gain Ratio, and Analysis of Variance (ANOVA). The results demonstrated that polymer blend concentration was the most influential factor in differentiating film performance, whereas storage time and extract concentration significantly affected both the colorimetric response and pH values. Multivariable analysis revealed distinct patterns associated with film stability and the physicochemical changes induced by contact with pork meat during storage. These findings highlight the potential of integrating polysaccharide-based materials with data science approaches to support the design, optimization, and performance evaluation of intelligent packaging systems for real-time freshness monitoring of meat products. Beyond the specific application evaluated, the proposed methodology demonstrates how data science tools can support the objective assessment of intelligent biodegradable films, facilitating their future implementation in real-time food quality monitoring systems.

Keywords: chitosan; *Hibiscus sabdariffa*; multivariable analysis; machine learning

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Academic Editor: Azizur Rahman

Received: 26 July 2026
Revised: 17 September 2026
Accepted: 20 September 2026
Published: 24 September 2026

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1. Introduction

Plastic pollution of the environment, including soil, water, and air, has become a major global concern, particularly because of the increasing accumulation of micro- and nanoplastics. The widespread use of conventional petroleum-based plastics in food packaging is one of the main contributors to this problem. The toxic effects, limited biodegradability, inadequate recycling practices, and long-term persistence of food packaging waste have received increasing scientific attention in recent years. Consequently, considerable research has focused on the development of sustainable, cost-effective, and biodegradable materials that can reduce the dependence on conventional plastic packaging while maintaining the quality and safety of food products. Among the alternatives investigated, polysaccharides, proteins, and lipids have attracted considerable interest because of their biodegradability and potential to form functional films.

Chitosan and starch are polysaccharides with properties that make them promising materials for food packaging applications, including biodegradability, edibility, gas barrier capacity, and suitable mechanical properties. Chitosan is produced through the deacetylation of chitin, one of the most abundant natural polysaccharides. Chitin is primarily obtained from the exoskeletons of crustaceans, including shrimp, crab, and lobster, and is also present in insects, corals, and fungi [1–3]. Despite its advantageous characteristics, chitosan has limitations related to its mechanical and thermal stability. These limitations can be addressed by combining chitosan with other polymers, such as starch, to modify and improve the structural and functional properties of the resulting films.

Starch is widely distributed in cereals such as rice, wheat, and maize, as well as in fruits and other plant sources. Rice starch is characterized by relatively small granules, a smooth texture, and a characteristic amylose-to-amylopectin ratio, which may provide advantageous properties when incorporated into chitosan-based matrices. These characteristics can contribute to the flexibility, moisture resistance, and viscoelasticity of chitosan–starch films [4]. The properties of these matrices can also be modified through the incorporation of organic acids and plasticizing agents. Acetic acid facilitates the dissolution of chitosan and can promote the formation of a more compact film structure [2]. In addition, the type and concentration of the acid used in the chitosan solution may influence the stability of the starch components; a 1% (*v*/*v*) acetic acid solution in water has been reported as suitable for chitosan–starch systems [4]. Glycerol acts as a plasticizing agent by increasing polymer-chain mobility, which can reduce crystallinity and modify the thermal, mechanical, and electrical properties of the resulting films [5].

Although chitosan–starch matrices can provide suitable structural properties, the incorporation of bioactive and functional compounds may further expand their potential applications in active and intelligent food packaging. In this context, roselle (*Hibiscus sabdariffa*) is of particular interest because its calyces contain a variety of bioactive compounds, including phenolic acids such as protocatechuic and gallic acids; organic acids such as ascorbic and citric acids; flavonoids such as quercetin and catechins; and anthocyanins, particularly delphinidin-3-sambubioside and cyanidin-3-sambubioside. Roselle calyces also contain pectin, mucilage, and minerals such as potassium, calcium, magnesium, iron, manganese, and zinc, together with comparatively lower amounts of sugars and starch [6–9]. The presence of these compounds has been associated with several biological activities, including antimicrobial and antiparasitic effects. Extracts of *H. sabdariffa* have been investigated for their activity against microorganisms such as *Mycobacterium tuberculosis* and *Escherichia coli*, as well as members of the genus *Bacillus* [10].

Roselle is one of approximately 300 species belonging to the genus *Hibiscus*, which is part of the Malvaceae family. Other economically important members of this family include cotton (*Gossypium hirsutum*), cacao (*Theobroma cacao*), ceiba (*Ceiba pentandra*), and Panama

tree (*Sterculia apetala*). The plant is also known by several common names, including jamaica, hibiscus, patwa, zobo, and Florida cranberry [6,11–13]. Its calyces are predominantly red and can develop under a range of environmental conditions, including different soil types, altitudes from sea level to approximately 1400 m, and temperatures of approximately 22–25 °C (72–77 °F). In Mexico, roselle is mainly cultivated in the states of Guerrero, Michoacán, and Oaxaca [12,14]. These characteristics, together with the chemical composition of its calyces, make roselle a promising natural source of functional compounds for incorporation into biodegradable polymeric films.

The incorporation of natural extracts into polysaccharide-based films can provide additional functionality beyond the passive barrier properties of conventional packaging materials. In particular, the presence of anthocyanins and other pH-sensitive compounds in roselle extracts may provide a basis for developing films capable of exhibiting visible color changes in response to changes in the surrounding environment. Such behavior is relevant to intelligent packaging systems, in which changes in the physicochemical conditions of packaged food can be monitored through a visual or instrumental response. For meat products, changes in pH during storage are associated with biochemical and microbiological processes that can alter product quality. Therefore, a biodegradable film containing a natural color-responsive extract could potentially serve as an indicator for monitoring changes associated with meat freshness.

The evaluation of color changes in intelligent packaging materials can be complemented by digital image analysis and data-driven methods. The RGB color model provides a quantitative representation of image color information, while clustering methods such as K-means can be used to identify and classify color patterns. In addition, statistical and feature relevance approaches can help determine which experimental variables contribute most strongly to the observed responses. Combining material characterization with data science may therefore provide a systematic approach for evaluating the effects of formulation, extract concentration, storage conditions, and exposure to food products on the performance of biodegradable indicator films.

Based on this approach, the present study investigated chitosan–starch films prepared with acetic acid and glycerol and incorporating different concentrations of roselle (*Hibiscus sabdariffa*) extract as potential intelligent packaging materials for pork meat. The films were evaluated before and after contact with pork samples under storage conditions of 6 and 25 °C for 36 h. Colorimetric changes were quantified through digital image analysis using the RGB color model and the K-means clustering algorithm. The relationships among formulation, storage conditions, exposure time, color characteristics, and pH were further examined using descriptive statistics, Spearman correlation, Information Gain, Gain Ratio, and Analysis of Variance (ANOVA). This integrated approach was used to identify the variables most strongly associated with the observed changes and to assess the potential of chitosan–starch–roselle films as biodegradable materials for freshness assessment in meat packaging.

Data Science Theory

The increasing complexity of experimental datasets generated during the development and characterization of intelligent packaging materials has highlighted the need for advanced data-driven approaches capable of identifying hidden patterns and relationships among multiple variables. Conventional statistical methods, such as Analysis of Variance (ANOVA) and correlation analysis, provide valuable information regarding the significance and association between individual factors; however, these approaches may be insufficient when complex interactions occur among material composition, environmental conditions, and physicochemical responses. In this context, machine learning techniques offer comple-

mentary strategies for extracting meaningful information from multidimensional datasets without requiring predefined relationships among variables [15].

Among unsupervised machine learning approaches, clustering algorithms have gained increasing attention due to their ability to classify experimental observations according to their intrinsic similarities. K-means clustering is one of the most widely applied partition-based clustering techniques, in which data points are grouped into a predefined number of clusters by minimizing the within-cluster variance and maximizing the similarity among observations belonging to the same group. The algorithm iteratively assigns each observation to the nearest centroid based on a distance metric, commonly the Euclidean distance, and updates the centroid position until cluster stability is achieved. This process enables the identification of natural patterns within datasets where the underlying structure is not previously known [15].

In the context of intelligent packaging materials, clustering approaches provide an effective strategy for analyzing complex responses generated by polysaccharide-based films exposed to different environmental stimuli. The performance of these systems depends on multiple interacting variables, including polymer composition, concentration of bioactive compounds, storage temperature, exposure time, and changes in pH caused by food deterioration. Therefore, unsupervised learning methods such as K-means clustering are particularly suitable for identifying groups of films exhibiting similar response behaviors based on colorimetric variations and physicochemical changes, without requiring previous classification criteria.

Compared with traditional classification approaches, K-means clustering presents several advantages for the analysis of intelligent polysaccharide films. Unlike supervised machine learning algorithms, clustering does not require predefined labels or assumptions regarding the expected response of the films, making it particularly suitable for exploratory analysis of novel biomaterials. Additionally, K-means allows the simultaneous evaluation of multiple variables, providing a global perspective of film behavior under different experimental conditions. This characteristic is especially relevant for intelligent packaging materials, where the final performance results from the interaction between material composition and external stimuli rather than from a single independent factor.

Therefore, the integration of K-means clustering with digital image analysis and physicochemical measurements provides a robust data science framework for evaluating intelligent polysaccharide-based films. This approach enables the identification of complex response patterns associated with environmental stimuli and supports the development of predictive strategies for the optimization of biodegradable packaging materials. The main aim of this study was to evaluate the performance of chitosan–starch films enriched with roselle extract for monitoring meat freshness and to investigate the relationships among pH, exposure temperature, exposure time, film blend composition, and color response using a data science approach.

2. Materials and Methods

2.1. Materials

For film synthesis:

- Chitosan, medium molecular weight, deacetylation around 85%, Sigma-Aldrich (Toluca, Mexico).
- Rice starch, Sigma-Aldrich.
- Glacial acetic acid, 99.9% purity, Fermont (Monterrey, Mexico).
- Glycerol, 99.7% purity, Fermont.
- Roselle calyces, purchased in a local fruit market (Tampico, Mexico).

2.2. Synthesis of Films

The roselle calyces were washed with distilled water (Hycel) to eliminate impurities. The aqueous extracts were prepared using distillated water (boiling at 100 °C per 15 min, and later it was allowed to cool to room temperature). The extracts were filtered with a Büchner funnel. The extracts' concentrations were 0.2% m/v (2 g of roselle calyces in 1000 mL of water) and 0.8% m/v (8 g of roselle calyces in 1000 mL of water). This is a simple and cheap method for obtaining compounds of interest such as anthocyanins.

The chitosan solution at 2% (m/v) was prepared using a glacial acetic acid solution (1% m/v) due to the solubility of chitosan in slightly acidic aqueous media. The starch solution (2% m/v) preparation requires the maintenance of the solution at 90 ± 2 °C for 20 min with constant stirring. The solutions must be at room temperature (25 °C approximately) after blending. Each film blend contains 40 mL of chitosan solution, 40 mL of rice starch solution and 0.2 mL of glycerol (film plasticizer). This base polymer blend formulation corresponds to the control matrix previously characterized chemically, mechanically, and morphologically by Lozano-Navarro et al. [16]. The extracts were added at three different levels relative to the total weight of the chitosan–starch blend: 0.4, 1.6, and 4 mL (corresponding to 0.5%, 2%, and 5% v/v, respectively). The blend was maintained under magnetic stirring for 5–10 min and subsequently poured into a polystyrene tray.

The indoor drying was carried out at a controlled temperature (25 °C); the trays were covered with brown paper to avoid contact with light (natural and/or artificial) and dust. Drying under these conditions could result in films with fewer molecular changes, higher tensile strength, a smoother surface, and better preservation of antioxidant compounds. After 2 weeks, the films were peeled off the polystyrene trays and stored until use [17–20]. The experimental design and the film nomenclature are presented in Figure 1.

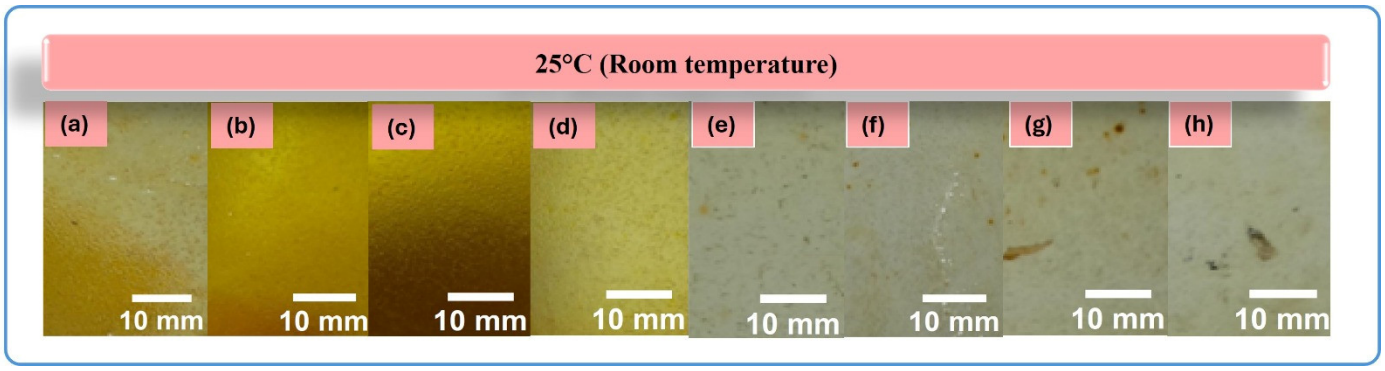

Figure 1. Films obtained: (**a**) H2B0S1 (Blank1), (**b**) H2B1S1, (**c**) H2B2S1, (**d**) H2B3S1, (**e**) H1B0S1 (Blank2), (**f**) H1B1S1, (**g**) H1B2S1, (**h**) H1B3S1, where H: extract concentration at 0.2% (w/v) (H1) and 0.8% (w/v) (H2); B: added volume at 0 mL (B0), 0.5 mL (B1), 2 mL (B2), and 5 mL (B3); and S: temperature and time conditions: 25 °C and 0 h (S1), 25 °C and 36 h (S2), and 6 °C and 36 h (S3).

2.3. pH Measurement

The anthocyanin meat preservation mechanisms and pork meat stability to storage were analyzed by pH films. The measurements were carried out using Macherey-Nagel pH tests strip. The pH strip test was collocated on the pork meat previously covered by roselle-polymer films in glass Petri dishes after 36 h of storage at 6 or 25 °C (42.8 or 77 °F), as shown in Figures 2 and 3.

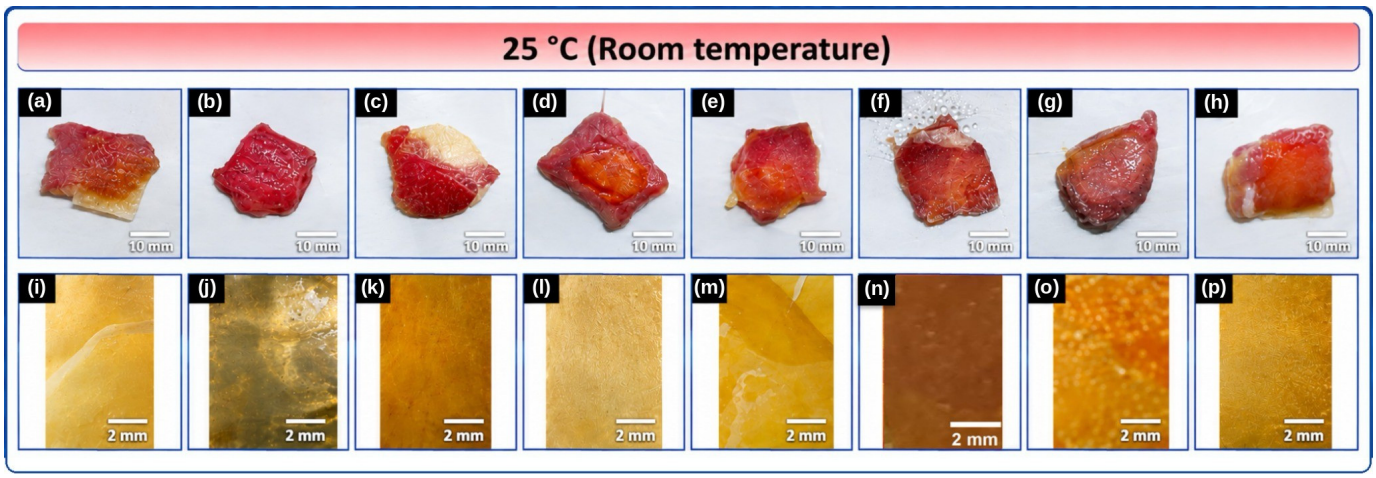

Figure 2. Color change in the films after exposure to pork meat: (**a**–**h**) sample appearance and (**i**–**p**) corresponding film colors for: (**a**,**i**) H1B0S2 (Blank3), (**b**,**j**) H1B1S2, (**c**,**k**) H1B2S2, (**d**,**l**) H1B3S2, (**e**,**m**) H2B0S2 (Blank4), (**f**,**n**) H2B1S2, (**g**,**o**) H2B2S2, (**h**,**p**) H2B3S2. Where H denotes the extract concentration: 0.2% (*w*/*v*) (H1) and 0.8% (*w*/*v*) (H2); B denotes the added volume: 0 mL (B0), 0.5 mL (B1), 2 mL (B2), and 5 mL (B3); and S denotes the temperature and exposure time conditions: 25 °C and 36 h (S2).

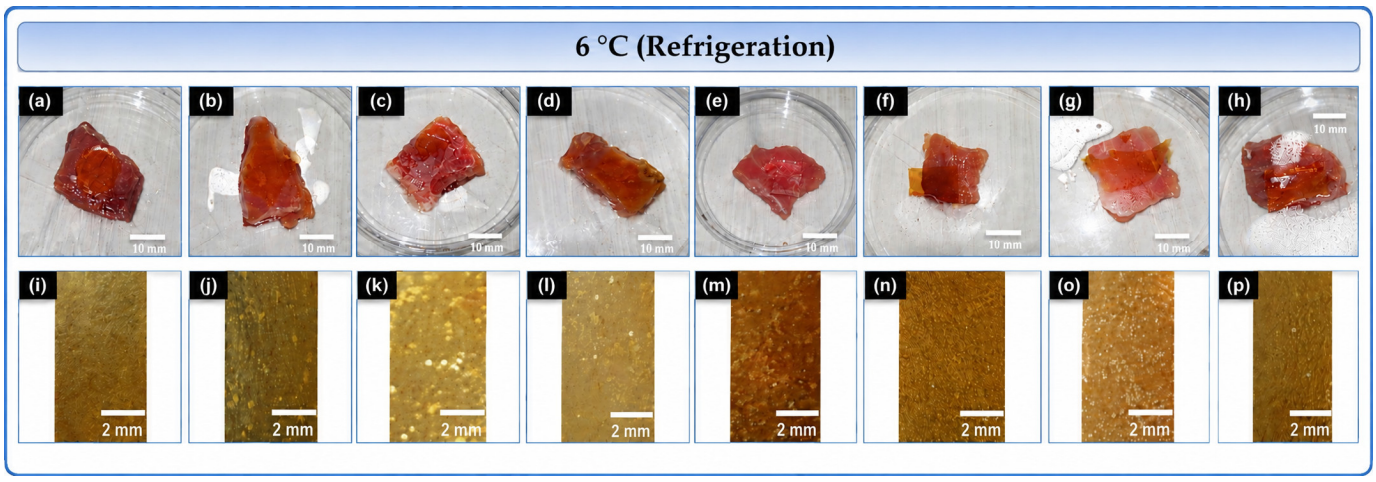

Figure 3. Color change in the films after exposure to pork meat: (**a**–**h**) sample appearance and (**i**–**p**) corresponding film colors for: (**a**,**i**) H1B0S3 (Blank5), (**b**,**j**) H1B1S3, (**c**,**k**) H1B2S3, (**d**,**l**) H1B3S3, (**e**,**m**) H2B0S3 (Blank6), (**f**,**n**) H2B1S3, (**g**,**o**) H2B2S3, (**h**,**p**) H2B3S3. Where H denotes the extract concentration: 0.2% (*w*/*v*) (H1) and 0.8% (*w*/*v*) (H2); B denotes the added volume: 0 mL (B0), 0.5 mL (B1), 2 mL (B2), and 5 mL (B3); and S denotes the temperature and exposure time conditions: 6 °C for 36 h (S3).

2.4. Evaluation of Film Coloration

The RGB (Red–Green–Blue) color model is based on the additive combination of three primary light components captured by digital imaging sensors. Each channel represents the intensity of reflected light within a specific region of the visible spectrum: red (620–750 nm), green (495–570 nm), and blue (450–495 nm). The red channel quantifies the amount of radiation reflected in the red region and is associated with the overall intensity of red-colored components. The green channel is closely related to variations in reflectance and absorption in the central region of the visible spectrum and often is considered one of the most sensitive channels for detecting subtle color differences due to the high visual resolution associated with this wavelength range. The blue channel is particularly sensitive to physicochemical transformations because chemical degradation, oxidation reactions, and structural modifications can alter the interaction between the material and incident

light, leading to noticeable changes in blue light reflectance. Therefore, variations in RGB values provide quantitative information concerning changes in light absorption, reflection, and scattering behavior. Since physicochemical transformations within the film matrix may modify its optical properties, RGB parameters provide objective, reproducible descriptors of color evolution and material response under different environmental conditions [21].

Color variations in films based on chitosan, starch, and hibiscus extract were quantitatively evaluated through digital image analysis. Standardized photographs were acquired before and after exposure under controlled illumination and fixed geometry to ensure measurement consistency.

The coloration of covered pork meat samples was studied visually and using an analyzer software called Image Color Summarizer (v0.77, Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada). The samples were photographed before and after 36 h meat storage. The films were collocated on a white background. The images were captured using a macro mode 5 megapixel camera with full HD, using artificial fluorescent lights avoiding other light sources and the distance between camera and samples was 3–5 cm approximately (Figure 2). The coloration changes were analyzed to understand the role of roselle-polymer films in meat preservation or spoilage [22].

Representative RGB values for each sample were calculated using weighted averages based on cluster proportions. This approach enabled a quantitative description of color evolution under different experimental conditions, including variations in hibiscus extract concentration, polymer composition, exposure time, temperature, and contact with pork meat.

The methodology provides a reproducible colorimetric descriptor of film response, suitable for statistical comparison and subsequent correlation with physicochemical changes in the material. Equations (1)–(3) were used to calculate the final values for each color channel (red, green, and blue).

$$R = ((RC1 * \%P1) + (RC2 * \%P2))/2 \quad (1)$$

$$G = ((GC1 * \%P1) + (GC2 * \%P2))/2 \quad (2)$$

$$B = ((BC1 * \%P1) + (BC2 * \%P2))/2 \quad (3)$$

where

R = red;
RC1 = red value in cluster 1;
RC2 = red value in cluster 2;
%P1 = percentage of pixels in cluster 1;
G = green;
GC1 = green value in cluster 1;
GC2 = green value in cluster 2;
%P2 = percentage of pixels in cluster 2;
B = blue;
BC1 = blue value in cluster 1;
BC2 = blue value in cluster 2.

For the evaluation of film coloration and pH changes, measurements were acquired as a single representative observation (n = 1) serving as a non-destructive screening tool for trend assessment; consequently, statistical dispersion parameters were not computed for these metrics.

3. Results and Discussion

3.1. Descriptive Statistical Analysis of Color and pH Data

To establish an initial quantitative characterization of the system, a descriptive statistical analysis was performed on the experimental and colorimetric variables obtained from chitosan–starch films containing hibiscus extract. Measures of central tendency and dispersion corresponding to formulation variables, experimental conditions, RGB channels, and pH of the films, with and without contact with pork meat, are summarized in Table 1.

Table 1. Statistical analysis of the dataset.

Statistic	Polymer Blend Concentration	Hibiscus Concentration	Temperature	Time	Red Channel	Green Channel	Blue Channel	Film pH	pH of Films Exposed to Meat
Count	24.000000	24.000000	24.000000	24.000000	24.000000	24.000000	24.000000	24.000000	24.000000
Mean	1.875000	0.500000	18.666667	24.000000	70.340417	57.674583	28.120417	6.125000	6.500000
Std	1.990466	0.306452	9.149325	17.335563	9.024482	12.990416	17.173156	0.946963	1.215838
Minimum	0.000000	0.200000	6.000000	0.000000	53.050000	35.430000	2.550000	5.000000	5.000000
25%	0.375000	0.200000	6.000000	0.000000	62.142500	45.937500	14.630000	5.000000	5.750000
50%	1.250000	0.500000	25.000000	36.000000	70.855000	57.300000	24.850000	6.000000	6.000000
75%	2.750000	0.800000	25.000000	36.000000	75.812500	70.237500	37.395000	7.000000	7.250000
Maximum	5.000000	0.800000	25.000000	36.000000	93.230000	76.950000	62.580000	8.000000	9.000000

MixConc = concentration of the polymer blend; HibConc = concentration of hibiscus extract (% *m*/*v*); Red, Green, and Blue = red, green, and blue color channels; pH film + meat corresponds to the pH of the films after contact with pork meat.

The blue and green channels increase proportionally, suggesting a direct relationship between these two-color components, where both tend to intensify simultaneously. The red and green channels also exhibit a proportional relationship, indicating that an increase in the intensity of one channel is accompanied by an increase in the other. As hibiscus concentration increases, the intensity of the blue channel decreases, which may reflect color degradation or a concentration-dependent color change in films. Over time, the red channel intensity tends to decrease, which could be associated with changes in the color stability of the films. Similarly, the blue channel intensity also decreases with time, suggesting a deterioration or aging process of the material. When the polymer blend concentration is higher, all three-color channels tend to exhibit slightly higher values. These preliminary trends suggest that polymer blend composition contributes not only to the structural characteristics of the films but also to their optical response. This observation anticipates the subsequent correlation and feature relevance analyses, where polymer blend concentration emerges as one of the variables with the greatest influence on film differentiation (for more details, see Section 3.5).

3.2. Spearman Correlation Analysis

To identify the relationships among variables within the dataset and interactions between attributes, Spearman correlation analysis was first performed. The Spearman correlation coefficient measures the strength and direction of a monotonic relationship between two variables, whether increasing or decreasing, without requiring linearity. It is suitable for both ordinal and continuous data and is robust to the presence of outliers. Table 2 presents the evaluation of the correlation type between variables based on the values obtained using Spearman equation.

Table 2. Range classification for Spearman correlation coefficient values (*p*).

Correlation Coefficient Range (*p*)	Correlation Strength	Interpretation
0.00–0.19	Very weak	Minimal correlation
0.20–0.39	Weak	Slight tendency
0.40–0.59	Moderate	Noticeable association
0.60–0.79	Strong	Clear relationship
0.80–1.00	Very strong	Highly pronounced association

In addition, the *p*-value was calculated to determine whether the observed correlations were statistically significant. A *p*-value lower than 0.05 indicates that the correlation is statistically significant, whereas a *p*-value greater than 0.05 suggests that the observed correlation may be attributable to random variation. The most relevant correlations among the descriptive attributes of the films during their evaluation are discussed below.

Correlations Associated with pH Variations

Spearman correlation analysis revealed several significant associations involving film pH and other experimental variables, including storage time, polymer blend concentration, temperature, and color parameters. The most relevant correlations identified are discussed below, highlighting the factors that may influence pH behavior and its relationship with film properties.

- Time vs. pH of films with and without meat exposure.
  As exposure time increases, the pH of both films stored alone and films exposed to meat tends to increase, suggesting that pH may be associated with maturation processes or chemical changes occurring over time.
- Polymer blend concentration vs. film pH.
  Higher polymer blend concentrations are associated with lower pH values in the films, which may indicate chemical interactions between the blend components and the film matrix.
- pH of films with and without meat exposure vs. green channel intensity.
  Higher pH values are associated with a slight reduction in green channel intensity, suggesting that pH variations may influence the color characteristics of the films.
- Temperature vs. pH of films with and without meat exposure.
  Films stored at room temperature tend to exhibit slightly higher pH values. However, when films are exposed to meat, the association between pH and temperature becomes negligible.
- pH of films without meat exposure vs. pH of films exposed to meat.
  The pH values of films stored without meat tend to increase proportionally with those of films exposed to meat, indicating a positive relationship between both measurements across the different film formulations. Table 3 summarizes the most relevant correlations identified in the dataset.

Overall, these correlations indicate that film response cannot be attributed to a single experimental factor. Instead, storage time, polymer composition, and environmental conditions interact to simultaneously influence pH and colorimetric behavior, highlighting the multivariable nature of intelligent packaging systems (Section 3.5).

Table 3. Most relevant correlations in the dataset.

Spearman Correlation Coefficient (*p*)	Attribute 1	Attribute 2	Correlation Type
0.78	Blue Channel	Green Channel	Strong positive
0.766	Red Channel	Green Channel	Strong positive
−0.698	Blue Channel	Hibiscus Concentration	Strong negative
−0.651	Time	Green Channel	Strong negative
0.526	Time	pH of Films Exposed to Meat	Moderate positive
−0.397	Hibiscus Concentration	Green Channel	Moderate negative
0.382	Time	Film pH	Moderate positive
−0.348	Polymer Blend Concentration	Film pH	Moderate negative
−0.332	Red Channel	Time	Moderate negative
0.332	Temperature	Green Channel	Moderate positive
−0.306	Green Channel	Film pH	Moderate negative
−0.294	Blue Channel	Time	Moderate negative

3.3. Attribute Relevance Analysis

Once the relationships between variables had been identified, feature relevance metrics were applied to determine which attributes contributed the most to differentiating film behavior. The relevance of each attribute was assessed using Information Gain, Gain Ratio, and ANOVA metrics (Table 4). These measures provide insight into the contribution of each attribute to the classification process within the dataset, allowing the identification of the variables with the greatest discriminatory power for differentiating the film formulations evaluated in this study.

Table 4. Rank analysis of the dataset attributes.

Attribute	Inf. Gain	Gain Ratio	ANOVA	Interpretation
Polymer Blend Concentration	0.811	0.406	9.821	🟢 Highly relevant. This attribute showed the highest importance across all metrics for differentiating film formulations.
Film pH	0.19	0.103	2.825	🟡 Moderately relevant. It provides a substantial contribution to sample discrimination.
pH of Films Exposed to Meat	0.029	0.015	0.145	🟠 Very low relevance.
Blue Channel	0.027	0.014	0.086	🔴 Minimal relevance.
Red Channel	0.027	0.014	0.05	🔴 Minimal relevance.
Green Channel	0.027	0.014	0.013	⚫ No contribution to data differentiation.
Hibiscus Concentration	0	0	0	⚫ No contribution to data differentiation.
Temperature	0	0	0	⚫ No contribution to data differentiation.

The feature relevance analysis indicated that polymer blend concentration was the attribute with the greatest discriminatory power among the evaluated film formulations, whereas hibiscus extract concentration, storage time, and temperature contributed only marginally to the classification when film identity was used as the target variable. These findings do not imply that these variables lack influence on the physicochemical properties of the material; rather, their ability to differentiate among film formulations was lower than that of the polymer matrix composition.

An additional noteworthy finding is that the variables describing the physical properties of the films, such as opacity, transparency, and thickness, contained greater discriminatory information than the experimental conditions themselves. This suggests that the final physicochemical characteristics of the films integrate the combined effects of formulation

and processing variables, making them more informative descriptors for distinguishing film formulations than the individual experimental factors.

Interestingly, these findings complement rather than contradict the Spearman correlation analysis. While Spearman identifies pairwise associations between variables, feature relevance metrics evaluate the ability of each attribute to discriminate among film formulations. Consequently, hibiscus concentration may strongly influence specific optical properties, particularly the blue channel, while contributing little to the overall classification of film identity.

3.4. Machine Learning-Based Analysis of Film Responses to Environmental Stimuli

The multivariable responses of the films to environmental stimuli were further investigated using an unsupervised learning approach based on K-means clustering to identify response patterns that could not be detected through pairwise statistical analyses.

Figure 4 presents the heatmap generated from the clustering analysis applied to the RGB color attributes of the films. This approach enables visualization of color variations as a function of polymer blend concentration, hibiscus extract concentration, storage temperature, and exposure time.

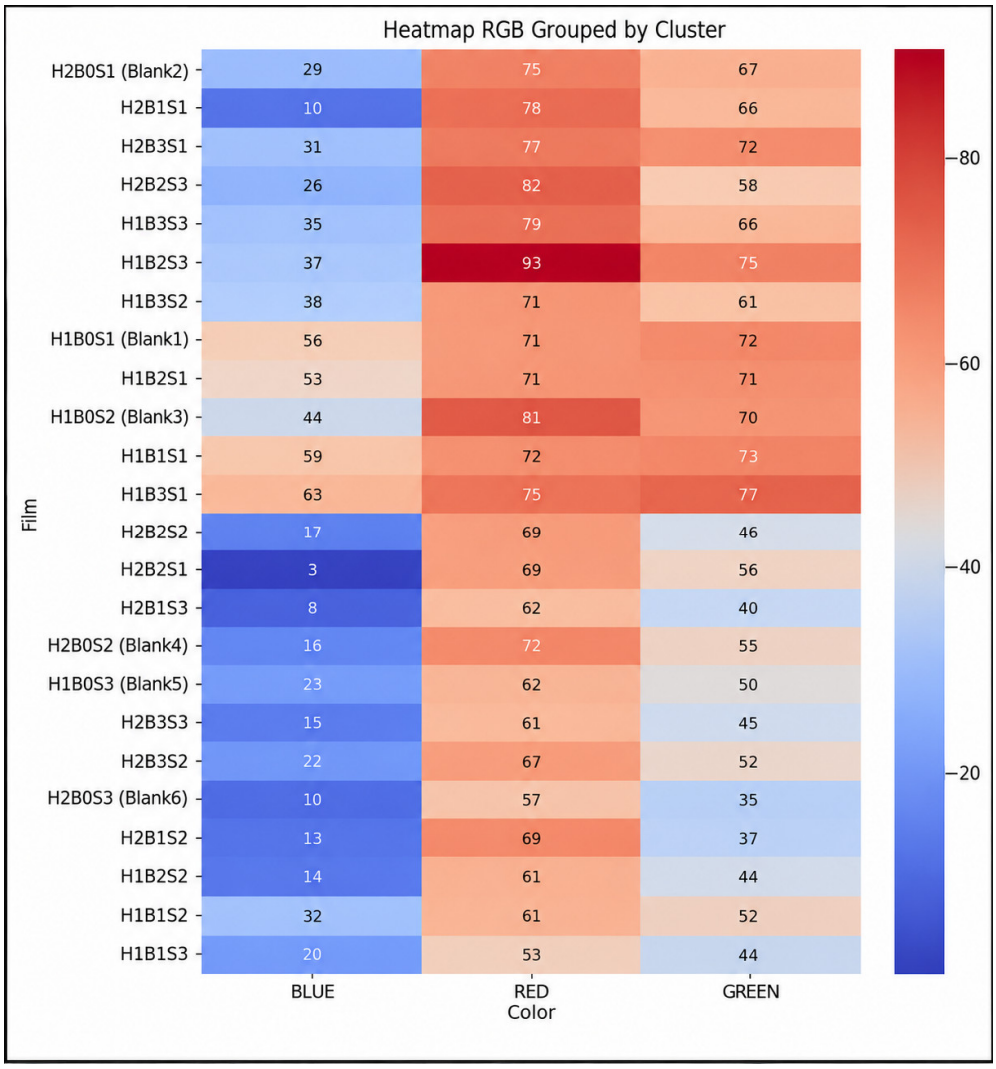

Figure 4. Heatmap of clusters generated from the RGB color analysis of the films.

3.4.1. K-Means Clustering Analysis of RGB Variations

The red channel predominated in most film formulations, reaching values of up to 93. This behavior may be associated with the presence of hibiscus extract; however, storage time, temperature, and contact with pork meat influenced the intensity of this channel. This trend is also reflected in the statistical analysis (Figure 5), where the red channel exhibited the highest mean value (70.34) compared with the green (57.67) and blue (28.12) channels. In addition, the red channel showed moderate variability, with a standard deviation of 9.02, indicating relatively stable color intensity across film formulations and storage conditions.

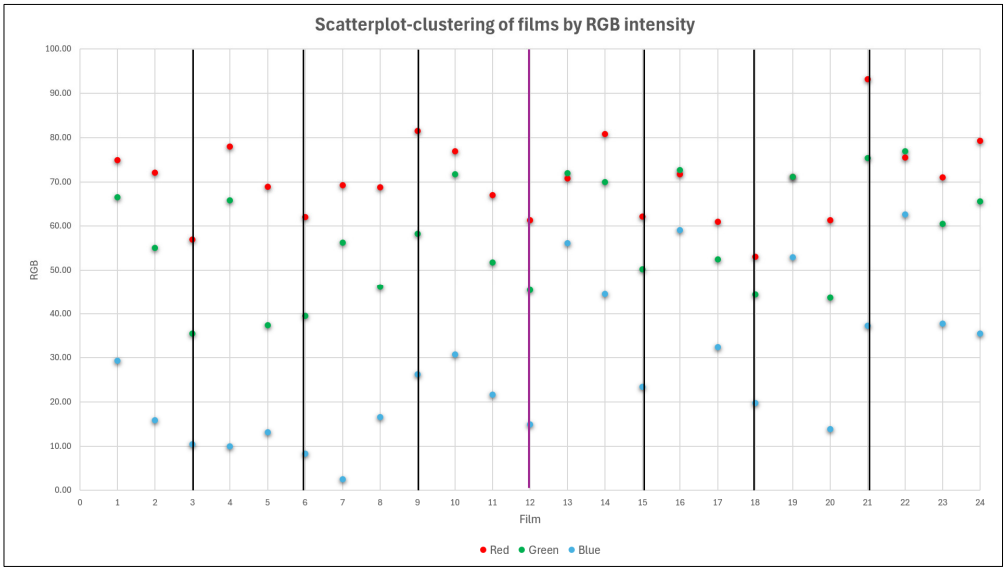

Figure 5. Scatter plot shows the clustering of film samples based on the distribution of RGB color channel values.

In contrast, the blue channel exhibited substantial variation among the films, with a mean value of 28.12 and a high relative dispersion of 60.03. These results indicate the presence of both low and high intensity values depending on the formulation and storage conditions, making this channel one of the most sensitive parameters for distinguishing differences among films.

The green channel remained at intermediate intensity levels in most samples, with a mean value of 57.67. Nevertheless, considerable variability was observed among formulations. The interquartile range (IQR) analysis revealed that the green channel was the most discriminative color parameter, highlighting its ability to differentiate among the evaluated films.

This behavior is consistent with the feature relevance analysis, which identified color-related variables as informative descriptors of film behavior, and supports the use of digital image analysis as a reliable tool for monitoring physicochemical changes in intelligent packaging materials.

According to the clustering analysis performed to facilitate the interpretation of film behavior in terms of appearance based on RGB color analysis, several trends were identified (Table 5).

Overall, highest values corresponded to the red channel, followed by the green channel and, finally, the blue channel. The lowest hibiscus extract concentration (0.2% m/v) resulted in a greater dispersion of values across the three-color channels and was associated with lower blue and green channel intensities. This behavior may be attributed to the high absorption capacity of anthocyanins within these regions of the visible spectrum. As the concentration of these compounds increases, light absorption at blue and green wavelengths

becomes more pronounced, reducing reflectance and consequently decreasing the recorded values in these channels [23].

Table 5. Trends in RGB color channel behavior as a function of temperature, component concentrations, and exposure time.

Component	Trend with Temperature/Meat Exposure	Trend with Exposure Time/Meat Exposure	Trend with Hibiscus Concentration	Trend with Polymer Blend Concentration
Blue Channel	Higher at 25 °C/decreases upon exposure.	Higher without exposure.	Higher at 0.2% (*m*/*v*)	Stable
Red Channel	Stable/decreases upon contact.	Stable/decreases upon contact.	Stable	Stable
Green Channel	Higher at 25 °C/decreases upon exposure.	Higher without exposure.	Higher at 0.2% (*m*/*v*)	Stable

More specifically, hibiscus extract concentration influenced the blue channel values of the films. At the highest concentration (0.8% *m*/*v*), blue channel values were lower and exhibited reduced dispersion, whereas at the lowest concentration (0.2% *m*/*v*), values were relatively higher and showed considerably greater variability. This behavior is consistent with the absorption of blue light by anthocyanin pigments present in hibiscus extract, which impart red–purple hues to the films and consequently reduce blue light reflectance. No clear or significant effect of polymer blend concentration on the blue channel was observed. Except for formulation 2, lower storage temperatures resulted in reduced blue channel values compared with those observed under room-temperature conditions. In addition, prolonged exposure time (36 h) and contact with pork meat were associated with a decline in blue channel intensity, suggesting progressive color degradation during storage.

The red channel was the predominant color component across all film formulations. Unlike the blue channel, red intensity did not show substantial changes with increasing hibiscus extract concentration. This behavior may be attributed to the limited effect of anthocyanins on the red region of the visible spectrum or to saturation of the red channel resulting from the presence of these pigments. Cooling and contact with pork meat appeared to increase data dispersion relative to the initial room-temperature conditions, although no pronounced trend was observed. Nevertheless, red channel values tended to be slightly lower under these conditions. As storage time increased, red intensity decreased and exhibited greater variability than at the beginning of the experiment. This trend suggests that contact with pork meat, which contains enzymes and compounds capable of promoting anthocyanin degradation or oxidation, may contribute to the loss of red coloration in the films.

In contrast, the green channel appeared to be strongly influenced by hibiscus extract concentration. Increasing the concentration of hibiscus extract resulted in a marked reduction in green channel intensity. This behavior may be attributed to the stronger absorption of light by anthocyanins in the green region of the visible spectrum, which decreases reflectance and consequently lowers the recorded values of this channel.

The observed decrease in the R (red), G (green), and B (blue) values of chitosan–starch films containing hibiscus extract after contact with pork meat may be attributed to an overall loss of color brightness and saturation associated with several physicochemical phenomena. Pigments present in hibiscus extract, particularly anthocyanins, are responsible for the visible coloration of the films and contribute to the RGB color components [24]. The interaction between the films and pork meat may promote oxidation, hydrolysis, or

reactions involving proteins and lipids, leading to pigment degradation and consequently reducing color intensity across all RGB channels.

On the other hand, the absorption of meat exudates, including water, lipids, and dissolved proteins, may alter the optical properties of the films by decreasing light reflectance. As a result, lower RGB values are recorded, giving the films a darker and more brownish appearance. Overall, clustering revealed that the films exhibit a visible chromatic response upon interaction with pork meat, highlighting their potential as colorimetric indicators of changes occurring during storage [25].

Beyond confirming the colorimetric sensitivity of the films, the clustering analysis demonstrates that machine learning provides an objective framework for detecting complex response patterns associated with food freshness. This integrated strategy may facilitate the future development of predictive intelligent packaging systems capable of supporting automated freshness monitoring.

3.4.2. K-Means Clustering Analysis of pH Variations

K-means clustering was applied to evaluate the behavior of the films in terms of pH changes during exposure to pork meat over time and under refrigerated storage conditions (Figure 6).

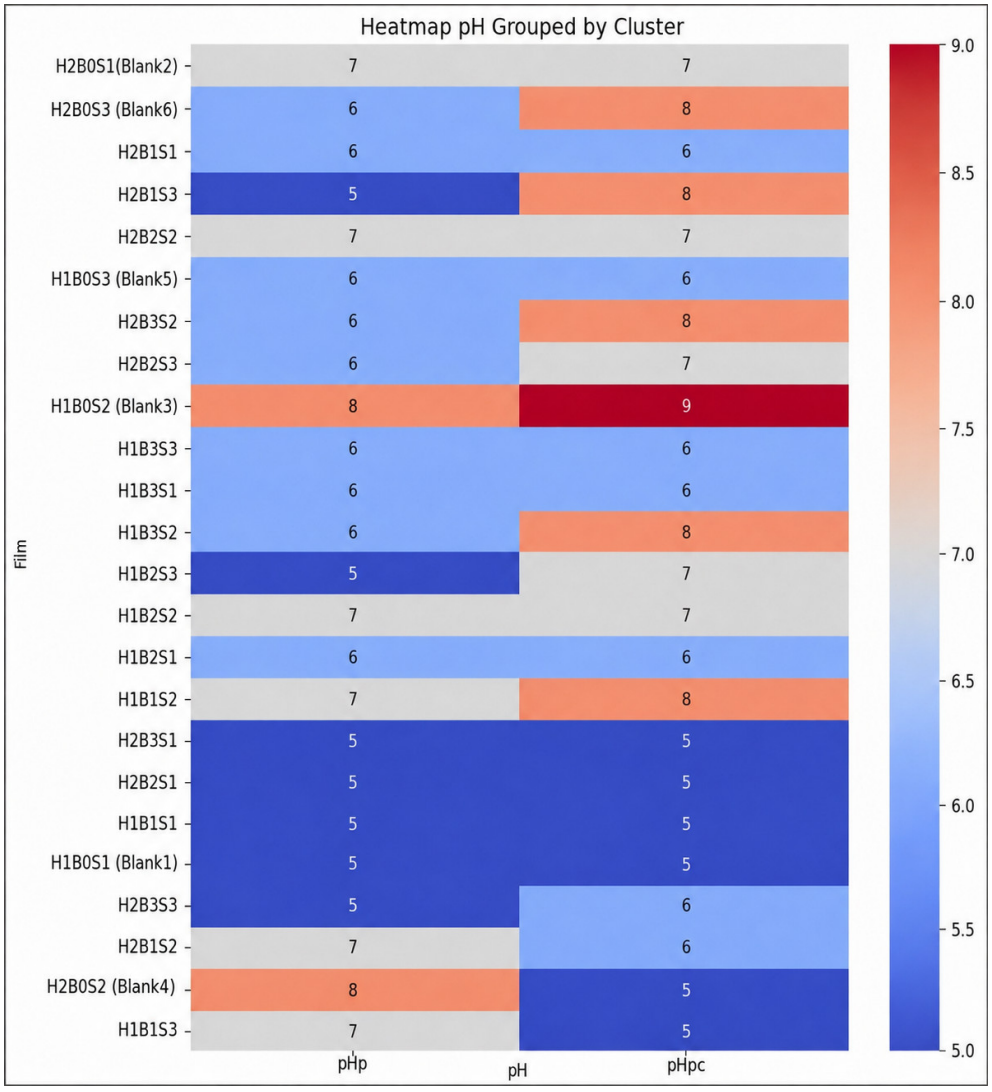

Figure 6. Heatmap of clusters generated from the pH values of the films before and after exposure to pork meat.

Effect of Hibiscus Extract and Polymer Blend Concentrations on Film pH

Overall, no substantial variations in film pH were observed as a function of hibiscus extract concentration, either before or after exposure to pork meat.

In contrast, polymer blend concentration showed a noticeable relationship with film pH prior to meat exposure. As polymer blend concentration increased, pH values tended to decrease and exhibited lower dispersion. Conversely, the control samples (films without polymer blend) displayed relatively higher pH values and greater variability. Following exposure to pork meat, this relationship became less evident, suggesting that the influence of polymer blend concentration on film pH was reduced under these conditions.

Effect of Meat Contact and Exposure Time on Film pH

Overall, the films exhibited lower pH values prior to contact with pork meat. The highest pH value recorded was 8.0, corresponding to control samples containing 0.2% and 0.8% (*m*/*v*) hibiscus extract, stored at 25 °C for 36 h.

Regarding exposure time, films subjected to 36 h of contact with pork meat tended to exhibit more pronounced pH changes, indicating a greater degree of interaction between the film matrix and the meat. This behavior suggests that prolonged contact may promote chemical reactions leading to pH increases, potentially associated with protein degradation and the formation of basic compounds during storage.

Among the 24 film formulations evaluated, 12 showed no pH variation after contact with pork meat. Of the remaining samples, nine exhibited an increase in pH, whereas three showed a decrease. Exposure time was identified as the factor most strongly associated with pH increases, as films maintained in contact with pork meat for 36 h generally exhibited pH increases ranging from 1 to 2 units. Notably, five of the films that showed an increase in pH contained 0.8% (*m*/*v*) hibiscus extract and were stored under refrigerated conditions (6 °C).

Effect of Temperature on Film pH

The pH values of the films exhibited a distribution closer to the mean under refrigerated conditions (6 °C), indicating a narrower pH range at lower temperatures. In contrast, films stored at room temperature showed greater pH dispersion. This behavior suggests that reduced temperatures contribute to greater pH stability, whereas the broader distribution observed at room temperature may reflect the intrinsic characteristics of the different film formulations.

Overall, film pH tended to decrease with decreasing temperature. The estimated pH gradient was approximately 0.0295 pH units per degree Celsius within the temperature range evaluated (6–25 °C), indicating a gradual reduction in pH as temperature decreased. This trend suggests that temperature influences the chemical stability of the system, potentially through interactions among the polymer matrix, starch, hibiscus extract, and acetic acid.

Consistent with these observations, films exposed to pork meat at 25 °C generally exhibited higher pH values than those stored under refrigerated conditions. This behavior suggests that higher temperatures may favor processes leading to pH increases after meat contact possibly because of enhanced enzymatic or microbial activity and the subsequent formation of basic compounds.

Figure 7 illustrates the pH changes observed in the films after contact with pork meat using a bar chart representation. Films showing no pH variation are identified with blue markers, whereas films exhibiting pH changes are represented by red bars.

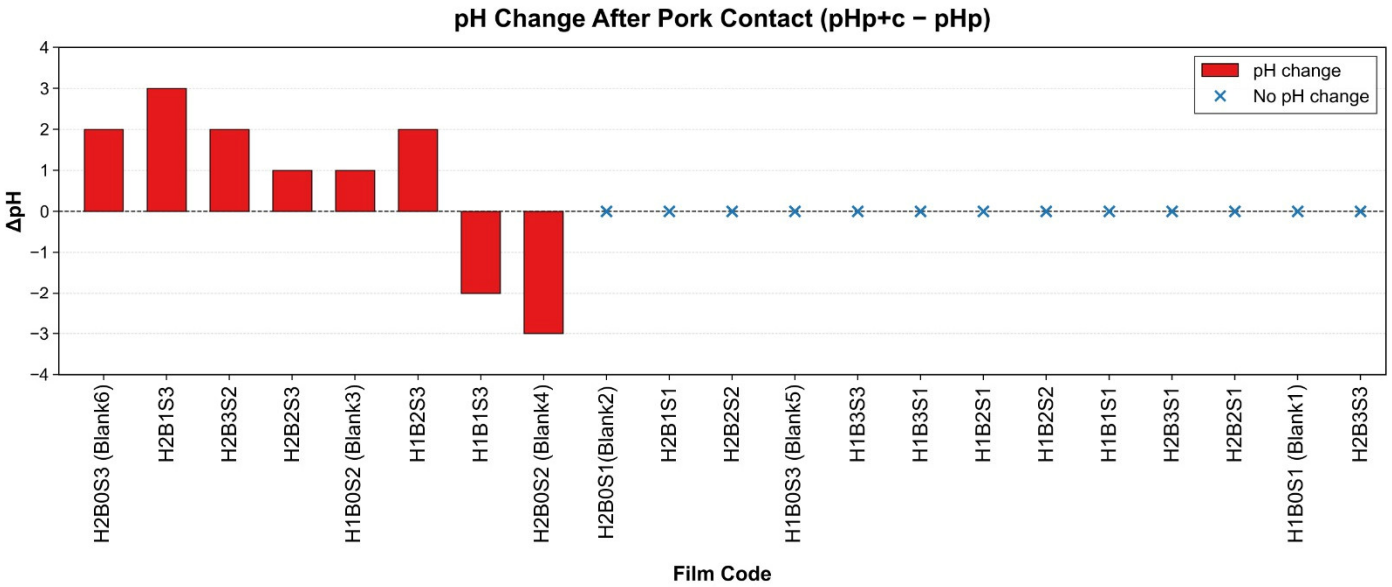

Figure 7. Graphical representation of films with and without pH changes after contact with pork meat.

Overall, clustering revealed that formulations containing higher polymer blend concentrations (5 and 2) exhibited greater stability frequency. Film stability did not appear to be strongly influenced by the hibiscus extract concentration, as stable responses were observed at both extract levels. Regarding storage temperature, no significant effect was observed at either 6 or 25 °C on the stability of films without pH variation. Under more demanding storage conditions (6 °C and 36 h), only specific formulations (blend concentration 5 or white films, combined with 0.2 or 0.8% m/v hibiscus extract) maintained their stability.

3.5. Relationship Between Film Properties and Data Science

The dark red coloration of films after synthesis is enhanced by the presence organic acid (acetic acid), polysaccharides (chitosan and starch), flavonoids and non-flavonoid phenolic compounds, which induces the anthocyanin co-pigmentation [26,27]. The acidic conditions prompt the red color intensity of extract (acetic acid 1% solution) [28,29].

The films' browning after meat storage could be attributed to light and time exposure and temperature increment that diminish the aqueous extract's monomeric anthocyanins [29]. Another key factor of browning is the presence of oxygen [30–32]. The pH and color modifications were studied due to their relationship with meat spoilage. The pH of fresh pork meat is approximately 6.0, and an increase in pH is associated with the presence of microorganisms and ammonia [33]. Myoglobin provides pork muscle with a purple coloration. When meat is exposed to oxygen, its color changes to bright red due to oxymyoglobin production. The meat color depends on animal conditions such as age, diet, and exercise. The presence of light and the contact of oxygen with myoglobin and oxymyoglobin produce metmyoglobin, which confers a brownish coloration to pork meat. The biochemical mechanism linking pH variation, color changes, and myoglobin stability has been extensively established in meat model systems. Acidic environments accelerate the oxidation of oxymyoglobin to metmyoglobin, whereas pH values close to neutrality preserve oxymyoglobin stability [34]. These color changes are not related to meat spoilage (Figure 8) [35,36]. Meat browning is not necessarily indicative of putrefaction; it also happens at refrigeration or freezing. Putrefaction is determined by the presence of fetid odor, stickiness, and viscous meat appearance [35]. It is important to consider the presence of greenish or grayish coloration, fat with yellow coloration, and mold as authentic signs of meat spoilage [37]. It is also known that roselle anthocyanins could be capable of reducing

Fe^{+3} to Fe^{+2} [38]; therefore, a wider study about pork meat storage using polysaccharide films enriched with roselle extract is recommended.



Figure 8. Myoglobin reaction during meat storage.

4. Conclusions

In this study, biodegradable films based on chitosan, starch, and *Hibiscus sabdariffa* extract exhibited pH and color-sensitive responses under different storage conditions and during contact with pork meat, confirming their potential as functional materials for the development of intelligent packaging systems aimed at freshness monitoring.

Descriptive statistical analysis and Spearman correlation revealed that the film response was governed by the interaction among polymer matrix composition, storage time, and environmental conditions. Exposure time exerted a pronounced influence on the increase in pH and the reduction in the intensity of the RGB color channels, whereas the concentration of *Hibiscus sabdariffa* extract showed a strong association with the blue channel, highlighting the sensitivity of anthocyanins to environmental changes.

Attribute relevance analysis demonstrated that the polymer blend concentration was the variable with the greatest discriminative power among the evaluated formulations. Furthermore, the physical properties of the films, including thickness, transparency, and opacity, provided greater discriminatory information than the experimental conditions themselves, suggesting that the final material properties integrate the combined effects of formulation composition and processing conditions.

In addition, the unsupervised K-means clustering algorithm enabled the identification of multivariate patterns in both the colorimetric response and pH variations that were not evident through conventional statistical analyses. Cluster analysis confirmed that the green and blue color channels were the most responsive to changes induced by H. sabdariffa extract concentration, storage time, and contact with pork meat. Likewise, pH clustering indicated that refrigerated storage promoted greater chemical stability of the films, whereas interaction with pork meat represented the primary factor associated with the observed pH increase.

Based on the data science analysis, higher extract concentrations are associated with more intense film coloration and lower pH values. An increase in pH is more pronounced after 36 h than at 0 h of meat exposure, while the highest exposure temperature (25 °C) is associated with higher pH values. Both trends could be related to microbial growth and chemical changes associated with ammonia production. Higher pH values are associated with changes in anthocyanin coloration and greater absorption of green light, resulting in a decrease in the green light captured by the camera sensor, as indicated by lower values in the green channel.

Collectively, these findings demonstrate that the integration of data science tools, including correlation analysis, attribute relevance metrics, and unsupervised machine learning, provides a robust methodological framework for the objective characterization of intelligent biodegradable films. This approach facilitates the identification of complex response patterns and establishes a solid foundation for the future development of predictive models to support the design and optimization of intelligent food packaging systems for real-time freshness monitoring.

Author Contributions: Conceptualization, N.P.D.-Z. and C.V.-S.; methodology, C.V.-S., D.B.C.-P., C.G.M.-H. and L.M.S.-O.; methodology—data analysis, N.P.D.-Z., J.I.L.-N. and V.L.-P.; software, V.L.-P. and F.R.-V.; validation, A.C.G.-C.; formal analysis, N.P.D.-Z., J.I.L.-N., V.L.-P. and M.A.A.-L.; investigation,

C.G.M.-H. and A.C.G.-C.; resources, N.P.D.-Z.; data curation, V.L.-P. and F.R.-V.; writing—original draft preparation, N.P.D.-Z., J.I.L.-N. and V.L.-P., writing—review and editing, N.P.D.-Z., J.I.L.-N., F.R.-V. and V.L.-P.; visualization, V.L.-P.; supervision, N.P.D.-Z., C.V.-S. and L.M.S.-O.; project administration, N.P.D.-Z., C.V.-S. and C.G.M.-H.; funding acquisition, D.B.C.-P. and L.M.S.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors thank to the Tecnológico Nacional de México (TecNM) for the institutional project 18406.23-P and the Instituto Tecnológico de Ciudad Madero for its institutional support through CDMA-PYR-2026-24845. During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GPT-5.6, with the image-generation tool) to generate illustrative graphical elements included in the graphical abstract. Specifically, the AI-generated elements correspond to the visual representation of the storage conditions at 6 °C and 25 °C for up to 36 h, as well as the illustrations included in the "Data Science & Machine Learning" section. The latter includes a general illustrative Machine Learning graphic and individual graphical representations of digital image analysis based on RGB channels, Spearman correlation analysis, feature relevance analysis (Information Gain, Gain Ratio, and ANOVA), and K-means clustering of colorimetric variables and pH. The prompts used were: Prompt 1: "Create a clean scientific infographic illustrating the storage conditions of the samples at 6 °C and 25 °C for up to 36 h. Include appropriate visual symbols representing refrigeration and room-temperature storage, using a professional style suitable for a scientific graphical abstract." Prompt 2: "Create a scientific infographic illustrating a Data Science and Machine Learning workflow for the analysis of intelligent films. Include a general illustrative Machine Learning graphic, together with separate visual representations of digital image analysis based on RGB channels, Spearman correlation analysis, feature relevance analysis using Information Gain, Gain Ratio, and ANOVA, and K-means clustering of colorimetric variables and pH. Use a clean, professional scientific style suitable for a graphical abstract." The generated graphical elements were subsequently integrated, arranged, and edited by the authors as part of the graphical abstract. The AI-generated elements were used solely as illustrative visual components and did not generate or modify the experimental data, statistical analyses, or scientific results reported in the manuscript. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

RGB	Red, Green and Blue
ANOVA	Analysis of Variance
pH	Potential of Hydrogen

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