Physical-Chemical Properties and Antioxidant Activity of Pectin-to-Glycerol Film Ratios with Quercetin Added for Wound Dressing Use

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Abstract

Natural-origin polymers in thin film form are promising candidates for dermal wound dressings due to their biocompatibility and ability to incorporate bioactive compounds. This study evaluated the physicochemical properties and antioxidant activity of pectin-to-glycerol films with added quercetin for potential wound dressing applications. Pectin was extracted from Citrus × aurantiifolia bagasse, yielding 15%, with a molar mass of 333,000 g/mol and a 54.1% degree of esterification, classifying it as high-methoxyl pectin. Films were produced via casting using a factorial experimental design. They exhibited homogeneous appearance, with some bubble formation at higher viscosities. Scanning electron microscopy confirmed good quercetin dispersion. Statistical analysis revealed quercetin content significantly influenced thickness, opacity, and water vapor permeability. Mechanical properties, modeled quadratically, showed that both glycerol and quercetin reduced film stiffness. Antioxidant activity exceeded 40%, attributed solely to quercetin. Thermal analysis indicated the presence of pure pectin fractions in high-pectin formulations, correlating with increased stiffness and permeability. Overall, the films demonstrated favorable characteristics for wound healing, with one formulation (F6) showing optimal mechanical, permeability, and antioxidant properties, making it a strong candidate for bioactive wound dressing applications.

Highlights

• Quercetin has good dispersion in pectin-to-glycerol film ratio

• Films showed good mechanical properties and high antioxidant activity

• Films with high pectin content have fractions of pure pectin

1. Introduction

Most of the current polymeric films have synthetic polymers as matrices due to their excellent physical, chemical, and mechanical properties. However, in general, synthetic polymers are detrimental to production because they are non-renewable, difficult to degrade, and harmful to the environment [1]. Films based on natural hydrocolloids have great potential to replace synthetic polymers in biomedical and pharmaceutical technology due to the advantages of their bioactivity, biocompatibility, and biodegradability. Some applications of these materials are in tissue engineering, controlled drug delivery systems, and capsules for oral ingestion [2]. They are predominantly derived from proteins and polysaccharides such as collagen, keratin, chitin or chitosan, starch, alginate, cellulose, albumin, agar, and pectin, among others [3].

Pectin stands out, which is an anionic polysaccharide usually obtained from plant cell walls [4]. It has mucoadhesive characteristics and biocompatibility, is almost degraded by bacteria in the colon and is not susceptible to gastric or intestinal enzymes [5, 6]. Chemically, pectin (polygalacturonic acid methyl ester) contains mainly α-D-galacturonic acid residues [4]. Pectin is composed of at least 17 different

types of monosaccharides with D-galacturonic acid as the main component, followed by D-galactose, and L-arabinose. There are two different classes of pectins based on their degree of methoxylation: high-methoxylation pectin (greater than 50%) and low-methoxylation pectin (less than 50%) [7].

Recent studies showed that pectin can be used to prepare controlled-release systems for drug delivery, for implantable cell transporters in tissue engineering, and prebiotics [8], and even promote the modification or different techniques of production of cross-linked films to obtain "intelligent" matrices capable of "detecting" external changes in temperature, humidity, ionic strength, or even pH (pH-responsive materials) [9]. The functionalization of polysaccharides with phenolic compounds can greatly improve their bioavailability and applications. Correlating these molecules can lead to materials that have interesting activities in the treatment of inflammatory or infectious conditions, and also optimize the physical and mechanical properties of films [10]. Among the compounds that can improve antioxidant properties, quercetin can be highlighted.

Quercetin, the most abundant flavonoid in edible plant foods [11], has been the subject of many studies as a dye and as a biologically active molecule because of its chemical structure and antioxidant, anti-inflammatory, antiviral, anticarcinogenic, and anti-diabetic properties. Flavonoids are chemical compounds that share a structure composed of three cyclic structures, two aromatic rings with hydroxyl groups attached. The antioxidant activity of a flavonoid is intrinsically associated with its chemical structure [12, 13]. However, despite presenting several biological properties that make them of great interest, their therapeutic use becomes difficult due to their low solubility. To overcome this problem, studies have been carried out to correlate phenolic compounds with polysaccharides [14] and successfully obtained cross-linked phenolic compounds in different types of polysaccharides, such as chitosan [15], dextran [16], and starch [17]. In this context, this study aimed to evaluate the chemical and physical properties and antioxidant activity of pectin-to-glycerol film ratios with quercetin added with potential use as dressings on dermal wounds.

2. Materials and Methods

2.1 Materials

All chemical reagents used were of analytical grade. Hydrochloric acid 37%, NEON; absolute ethanol 99%, scientific exodus; ethanol 95%, scientific exodus; glycerol 99.5%, BIOTEC; Quercetin > 95%, Sigma-Aldrich. The prepared solutions followed quality criteria such as calibrated glassware and equipment, and deionized water in a reverse osmosis purifier.

2.2 Extracting Pectin from Orange Bagasse

The waste of *Citrus* × *aurantiifolia* (Christm.) Swingle (synonym *Citrus* × *limettioides* Yu. Tanaka; *Citrus limettioides* Tanaka; common name in English: sweet lime or Indian sweet lime, and in Portuguese: *lima*) [18] were collected from a local orange juice industry in Toledo-PR, Brazil. The waste, orange bagasse composed of peel (flavedo) and pulp (albedo and endocarp) without seeds, was washed under running

water to remove gross impurities, washed with distilled water, cut into pieces of approximately 1 cm^2, and dried in air circulation oven at 40°C for 48 h. The dried bagasse was crushed and sieved to obtain particles < 60 mesh, similar to a fine powder with a brown color. The extraction was performed according to Canteri-Schemin et al. [19] with modifications. The crushed bagasse was dispersed in a hydrochloric acid solution at pH 2.5 in a 1:40 ratio (mass:volume). The extraction took place at reflux for 60 min. After extraction, the solution was filtered to remove solids and cooled to 25°C. Pectin was precipitated by adding 95% ethanol in a 1:1 ratio (volume:volume). After homogenization, the solution was kept static for 30 min at 30°C for complete pectin precipitation. The pectin was filtered through polyamide sieves and the retained material was washed four times with 99% ethanol. Finally, the material was dried in an air circulation oven at 40°C for 24 h.

2.3 Characterization of Pectin

2.3.1 Degree of esterification and viscosimetric properties

The degree of esterification (DE) was determined by the titrimetric method [20]. For this, Kf values (number of free carboxyl groups), Ke values (number of esterified carboxyl groups), and Kt values (total number of carboxyl groups) were obtained. The DE was obtained by the expression:

$$\mathrm{DE} = \frac{K_e}{K_f}\, x\, 100 = \left(\frac{1 - K_f}{K_t}\right) x\, 100 \qquad (1)$$

The kinematic and intrinsic viscosity and the viscosimetric molar mass were determined in a Cannon Fenske viscometer with capillary number 100. The viscometers were held in a constant temperature bath (25 ^{o}C). The specific viscosity was determined concerning the water viscosity and the reduced viscosity was determined concerning the pectin solution concentrations.

The Huggins intrinsic viscosity (η) is defined as the value of the viscosity reduced to infinite dilutions and was determined through the graph of the reduced viscosity as a function of the pectin solution concentration. The viscosimetric average molecular mass of the pectin samples was determined by applying the Mark Houwink-Sakurada equation, which relates [η] to the viscosimetric molecular mass (Mv).

$$[\eta] = K\,[M_V]^{\alpha} \qquad (2)$$

Where: K is a constant with a value of 0.30, and α is a constant with a value of 0.613.

2.4 Experiment Planning

The research was developed from a 2^3 factorial design to obtain an accurate evaluation of the effect of the variables: pectin content (P), quercetin content (Q), and glycerol content (G), all set in grams. The

results obtained concerning the properties of the films were evaluated using the software Statistica version 10. Table 1 shows the real variables and their coded values.

Table 1
Real variables and coded values for each level.

Variable	Level		
	-1	0	+ 1
Pectin content (g)	1.0000	1.2500	1.5000
Quercetin content (g)	0.0050	0.0075	0.0100
Glycerol content (g)	1.0000	1.2500	1.5000

2.4.1 Preparation of polymeric films

The films were produced from a random mixture of pectin, glycerol, and quercetin with defined levels by mass and their respective production conditions can be seen in Table 2 considering the coding in Table 1.

Table 2
Experimental conditions for the production of the films.

Film	Level		
	Pectin	Quercetin	Glycerol
F1	0	0	0
F2	-1	+ 1	+ 1
F3	-1	-1	+ 1
F4	0	0	0
F5	+ 1	+ 1	+ 1
F6	+ 1	+ 1	-1
F7	-1	+ 1	-1
F8	-1	-1	-1
F9	+ 1	-1	-1
F10	+ 1	-1	+ 1

The films were produced using the casting technique. In 40 mL water, the plasticizer (glycerol) was added and homogenized for 30 min in a magnetic stirrer at room temperature. Then the polymer (pectin) was added, remaining under constant agitation for 24 h at room temperature. Then quercetin was

solubilized in 10 mL distilled water. The pectin:glycerol solution was heated to 60°C and the quercetin solution was added dropwise. The resulting solution was stirred at 60°C for 4 h and followed by immediate pouring in equal volumes on two 8-cm diameter, 1.5-cm deep Teflon plates. The plates were dried in an air circulation oven at 25°C for 48 h.

2.5 Characterization of Polymeric Films

2.5.1 Visual and morphological properties of polymeric films

The films were visually evaluated to identify conditions of uniformity and appearance for use as dressings. The morphology of the films was examined using a scanning electron microscope (model Tescan® Vega 3). The films were coated with gold and fixed with carbon tape. Then, the samples were observed under an accelerating voltage of 10.00 kV and a maximum magnification of 2000x.

2.5.2 Thickness

The thickness of the films was determined according to ASTM D5729-97, using a micrometer with a precision of 0.001 mm. Thickness was determined at five different locations on each film (center and four corners) and the values expressed were the average of the five measurements.

2.5.3 Opacity

The films with thickness (T) were cut into rectangular pieces and positioned in the measurement compartment of the spectrophotometer. The absorbance (ABS_{600}) of the films was measured at a wavelength of 600 nm using a UV-Vis spectrophotometer (Hach DR2700). The opacity of the films was calculated from the expression:

$$Opacity = \frac{ABS_{600}}{T} \qquad (3)$$

2.5.4 Solubility

The films were cut into pieces of 1 cm^2 and dried in an oven at 60°C until constant mass, obtaining the initial mass of the film (Fi). Each film was then placed in a cell containing 20 mL distilled water and maintained for 24 h under agitation at 50 rpm at 25°C. The resulting solution was filtered through glass fiber filters and the filtrate was dried in an oven at 60°C until constant mass to determine the final mass of the film (Ff). Tests were performed in triplicate. The solubility of the film was calculated from the following expression:

$$Solubility\ (\%) = \frac{F_i - F_f}{F_i} * 100 \qquad (4)$$

2.5.5 Water vapor transmission rate and water vapor permeability

The water vapor transmission rate (WVTR) was determined according to ASTM E96/E96M-16 with adaptations. In the ASTM standard test plate, 5 mL phosphate-buffered saline (PBS) pH 5.7 was added and kept in a hermetically sealed pot containing silica gel and kept in an oven at 32 ^{o}C. The set containing the test plate, buffer solution, and membrane had its mass measured periodically for 24 h, obtaining a total of nine points. The WVTR was calculated by Eq. (5), where ΔW is the amount of water lost in the function of time, A is the membrane area (m^2), and (ΔW/Δt) is the angular coefficient obtained by linear regression. To determine the water vapor permeability (WVP), the WVTR value was multiplied by the thickness (mm).

$$WVT\ (g/h\ m^2) = \frac{\Delta W}{\Delta t\ A} \qquad (5)$$

$$WVP\ \left(mm\ \frac{g}{h}\ m^2\right) = WVTR\,Thickness \qquad (6)$$

2.5.6 Mechanical analysis

The mechanical properties of the films were determined using a portable universal testing machine (Biopdi-Brazil). The films were cut in standard size with dimensions of 50 mm × 10 mm. The thickness of the samples was determined with an analog thickness gauge (Mitutoyo, NO.7301). A 100 N load cell was used to perform the tensile test with a stretching speed of 5 mm min^{-1} at 30°C and at 30% relative humidity.

2.5.7 Thermogravimetric analyses

Thermogravimetric analyses were performed using a thermal analyzer (STA 8000 Perkin Elmer TM, USA). For each sample, 6 mg of produced films were placed into an open platinum crucible and heated from 30 to 300°C at a 20°C min^{-1} heating rate under an inert atmosphere (N_2, White Martins TM, Brazil, 99.99% grade) and dynamic atmosphere with a 20 cm^3 min^{-1} flow rate.

2.5.8 Antioxidant activity

The methodology was according to Blois [21] with modifications. The films were cut into 2 cm^2 pieces and solubilized in 30 mL of distilled water for 4 h at 25°C. Then, 3.9 mL of film extract solution was mixed with 0.1 mL of 1.0 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution. The mixture was vortexed and incubated in the dark at room temperature for 30 min, then the absorbances were measured at a wavelength of 517 nm using a spectrophotometer (HACH DR2700). The negative control consisted of 3.9 mL of methanol and 0.1 mL of DPPH solution. Antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity } (\%) = \frac{ABS_{contole} - ABS_{amostra}}{ABS_{controle}} * 100 \qquad (7)$$

$ABS_{control}$ is the absorbance of the negative control, and ABS_{sample} is the absorbance of the samples.

3. Results and Discussion

3.1 Extraction Yield

The extractions had an average yield of 15% and it was similar to those reported by Obele et al. [22] of 15.9% and Khan et al. [23] of 17% obtained in similar conditions but higher than those reported by Yang et al. [24] ranging from 8.4 to 9.8%.

3.2 Degree of Esterification and Viscosimetric Properties

The values obtained for Kf and Ke were, respectively, 7.4 ± 0.027% and 8.7 ± 0.062%. From these data, a degree of esterification of 54.1 ± 0.088% was obtained, classifying the extracted material as high degree of methoxylation [24, 25]. According to Hua et al. [26], the pH, temperature, and extraction time factors directly affect the degree of esterification and the size of the polymer chain, since the polysaccharide bonds can undergo hydrolysis. Both types of pectin, low and high esterification, can form thin films, however, films of low esterification pectin need a multivalent cation for the formation of a consistent and homogeneous film; in a comparison, high methoxylation does not require the addition of cations for film formation [27].

Viscosimetric parameters were determined to evaluate the rheological characteristics of pectin. The Huggins intrinsic viscosity, 78.64 mL g^{-1}, was defined as the viscosity reduced to infinite dilution [28]. Ideally, according to Shivangi et al. [29], a film-forming solution should have a moderate viscosity. The value found can cause defects in the film since high viscosity values can make it difficult to disperse the compounds in the solution, eliminate visible air bubbles, and cause discontinuities in solid films [29].

The viscosimetric average molecular mass found was approximately 333,000 g mol^{-1}, defined by applying the Mark Houwink-Sakurada equation, relating the intrinsic viscosity to the viscosimetric average molecular mass [30]. The variations in molar mass found in pectin may be related to the various forms of extraction, vegetable source, and raw material. According to Canteri et al. [19], the molar masses reported in the literature range from 20,000 to 360,000 g mol^{-1}, and commercial samples from 35,000 to 120,000 g mol^{-1}.

3.3 Visual and Morphological Aspects

The image of the films presents a brown coloration typical of pectin (Fig. 1). In general, the films present good homogeneity, with small bubbles in some cases (Fig. 1, F5, F6, F9, and F10). The presence of bubbles is related to the formation of solutions with high viscosity, which makes the elimination of bubbles difficult and reduces the uniformity of the films.

The morphology of the films was observed through images obtained by scanning electron microscopy (Fig. 2).

In most cases it is possible to evidence a smooth and uniform surface, with little or no evidence of particles, characterizing a good dispersion of quercetin in the film matrix. It can be observed that there are some differences in morphology when evaluating the films that have the highest contents, F5 (maximum contents) and F8 (minimum contents) (Fig. 2). The F8 film presents discontinuous zones with the presence of cracks (Fig. 2). In the F5 film, besides cracks, the presence of bubbles is observed, possibly due to the high viscosity of the solution.

3.4 Thickness, Opacity, Solubility, Water Vapor Transmission Rate, and Water Vapor Permeability

The results for thickness (T), opacity (Op), solubility (So), water vapor transmission rate (WVTR), and water vapor permeability (WVP) are shown in Table 3.

Table 3
Average values and standard deviation for thickness (mm), opacity (Abs nm), solubility (%), water vapor transmission rate (g/d m^2), and water vapor permeability (g mm/d m^2)

Films	Thickness (mm)	Opacity (Abs mm)	Solubility (%)	Water vapor transmission rate (g/d m^2)	Water vapor permeability (g mm/d m^2)
F1	0.225 ± 0.005	2.695 ± 0.201	91.514 ± 0.640	883.46	198.78
F2	0.180 ± 0.001	4.046 ± 0.219	89.418 ± 1.002	895.17	161.13
F3	0.172 ± 0.003	5.394 ± 0.211	94.711 ± 0.565	1079.01	185.59
F4	0.236 ± 0.003	2.979 ± 0.066	92.178 ± 0.953	1010.21	238.41
F5	0.335 ± 0.013	1.833 ± 0.096	93.624 ± 0.408	1021.91	342.34
F6	0.356 ± 0.004	1.848 ± 0.246	89.956 ± 0.325	998.32	355.40
F7	0.170 ± 0.015	4.248 ± 0.369	93.551 ± 0.454	1208.22	205.40
F8	0.190 ± 0.007	6.856 ± 0.274	93.350 ± 0.662	1006.96	191.32
F9	0.363 ± 0.004	2.889 ± 0.132	88.204 ± 0.314	1020.48	370.43
F10	0.356 ± 0.003	3.247 ± 0.120	91.887 ± 0.262	901.57	320.96

Thinner films provide better conditions of use for covering wounds since they are more moldable to irregular surfaces. Film thickness ranged from 0.363 ± 0.004 to 0.170 ± 0.015 mm for F9 and F7, respectively (Table 3). The linear model without interactions adequately represents the system (R^2 = 0.958). The pectin variable is the only one that is significant for film thickness. Thus, higher pectin contents promote greater film thickness.

Opacity is an important parameter in materials that will be applied in wound dressings because the ability of a product to be transparent allows visual inspection of wounds without removing the dressing [30, 31]. Therefore, films with low opacity are sought after. The films presented a variation of 6.856 ± 0.274 Abs mm to 1.833 ± 0.096 Abs mm for F8 and F5, respectively (Table 3). The most appropriate statistical model to represent the system is the quadratic model with variable interactions (R^2 = 0.983). Linear variables pectin and quercetin are significant. The response surface for opacity concerning quercetin content and pectin content variables can be seen in Fig. 3.

The equation that describes the response surface can be seen below:

$$Op = 47.47 - 50.96P + 15.33P^2 - 917.55Q + 300.20PQ + 5.01P + 221.75Q - 8.76 \quad (8)$$

High contents of pectin and quercetin result in an opaquer film. Opacity has a direct correlation with the degree of crystallinity of the material. Polymers with a high degree of crystallinity generally have a semitransparent or opaque character, unlike amorphous polymers or polymers with a low degree of crystallinity, which tend to be transparent. The presence of very small inclusions or crystallite additives makes the material semi-transparent since these inclusions act by scattering light [32].

For solubility data, values ranged from 88.2 ± 0.314% to 94.7 ± 0.565% for F9 and F3 films, respectively (Table 3). No statistical analysis model was applied to the data referring to solubility because in all tested models, no significance was observed related to any variable. The high solubility of the films is mainly due to the presence of the plasticizer, glycerol. The plasticizer decreases the cohesive forces of the polymer by pushing the polymer chains apart, has a hygroscopic character, and is highly soluble in water. Even though quercetin is poorly soluble in water, it is believed that its low content present in the film is associated with the high solubility of pectin and glycerol and the dispersion of quercetin in the polymeric matrix promote a high solubility, making the solubility independent of the composition of the film. High solubility values make it difficult to use the films in wounds with high volumes of exudate, so they are indicated for situations of low or medium exudate.

The WVTR and WVP values analysis is important in films applied in dressings. This parameter may indicate favorable conditions for the healing process (humidity and oxygen permeation). The WVTR describes the rate at which water vapor permeates through the polymeric film at a specific time, as described by McHugh et al. [33]. In an ideal polymer, the WVTR is independent of film thickness. However, for hydrophilic polymers, such as pectin, an increase in WVTR is observed as a function of thickness. Thus, for proper evaluation of the data, the WVP must be used, which is obtained through the product between the WVTR and the thickness of the polymeric film.

As shown in Table 3, the WVTR values vary from 883.460 g/d m^2 to 1208.22 g/d m^2 for films F1 and F7, respectively. For the WVTR results, no statistical model was applied, because in no case were significant variables observed. However, when evaluating the data referring to WVP, it was observed that the values range from 370.43 g mm/d m^2 to 161.13 g mm/d m^2 for films F9 and F2 respectively (Table 3). The linear model without interactions best represents the system (R^2 = 0.911). The pectin variable is the only significant one concerning water vapor permeability. The response surfaces for water vapor permeability with the other variables (quercetin and glycerol) can be seen in Fig. 4.

The equations describing the response surfaces can be seen below:

$$WVP = -74.73 + 322.85P - 56.27G - 1.513 \quad (9)$$

$$WVP = -74.73 + 322.85P - 201.78Q - 70.33 \quad (10)$$

The results regarding water vapor permeability are consistent with the results obtained for film thickness, where higher pectin contents result in greater film thickness. Thus, as described by McHugh and collaborators, hydrophilic polymer films, such as pectin, have a higher vapor permeability as a function of thickness. The results regarding the solubility of the film also corroborate the values of water vapor permeability, since films with greater solubility tend to have less resistance to diffusivity in water [34].

It should also be noted that even though the variables quercetin and glycerol are not significant, they have negative coefficients, that is, they reduce the permeability of water vapor. In the case of quercetin, it is believed that this effect is related to its low solubility, which decreases the hydrophilicity of the polymeric film. In the case of glycerol, this effect may be associated with its intercalation between the pectin polymer chains, occupying a space that would be related to water molecules.

3.5 Mechanical Properties

The most important mechanical properties for dermal wound dressings are tensile strength, breaking strain, and elastic modulus [35], these properties must be compatible with the damaged tissue, being important requirements of materials useful in tissue regeneration [36]. Data related to the mechanical properties of the films produced are presented in Table 4.

Table 4
Elastic modulus (MPa), tensile strength (MPa), and breaking strain (%) values of the films.

Films	Elastic modulus (MPa)	tensile strength (MPa)	breaking strain (%)
F1	3.21	16.88	668
F2	36.52	17.01	60
F3	1.71	6.89	521
F4	2.84	17.37	666
F5	2.22	10.98	563
F6	5.26	47.02	872
F7	15.61	24.59	121
F8	15.42	9.66	51
F9	60.52	34.51	63
F10	20.51	21.56	76

Although tensile strength and breaking strain are important parameters, they are very susceptible to imperfections in the polymeric film, which results in associated errors. However, the elastic modulus is a more adequate parameter to evaluate the characteristics of the film, since it is defined in the first stages

of material deformation. For application as dressings, it is expected that the polymeric film has a relatively small modulus of elasticity, which favors the moldability of the polymeric film on the skin.

The values for the elastic modulus of the films produced ranged from 60.52 MPa to 1.71 MPa for films F9 and F3, respectively (Table 4). The quadratic model with interactions between variables more adequately represents the system (R^2 = 0.999). The pectin content variable is significant in its quadratic form. In addition, all the main variables and their combinations are also significant and can be observed in the Pareto chart of the standardized effects (Fig. 5).

The response surfaces for the elastic modulus concerning the variables pectin content, quercetin content, and glycerol content can be seen in Fig. 6.

The equations describing the response surfaces can be seen below:

$$EM = 219.10 - 367.00P + 267.2P^2 + 7320Q - 21720PQ - 124.50P + 17900Q + 0{,}25 \quad (11)$$

$$EM = 219.10 - 367.00P + 267.20P^2 + 0.20G - 162.90P - 100.40PG + 107.40G + 54{,}90 \quad (12)$$

$$EM = 219.10 + 7320Q + 0.20G - 27150Q - 125.5G + 14320QG - 45 \quad (13)$$

Initially analyzing the variables without combination, it is observed that the pectin content is significant and positive both linearly and quadratically, which demonstrates that the elastic modulus increases with the pectin content. This effect was expected because high pectin contents represent fractions of the polymer without the action of plasticizers, which generates a material with greater cohesive forces and consequently more rigid.

Both variables glycerol and quercetin content are linearly significant and negative, demonstrating that the elastic modulus decreases with increasing content of both. This effect is expected for glycerol since it is a plasticizer. Plasticizers act by reducing the strong intermolecular interactions between polymer chains, decreasing their mechanical strength [37–39]. Regarding the quercetin content, it is believed that it acts similarly to glycerol since it is a molecule rich in hydroxyl groups that can assume positions and interactions in the polymeric matrix similar to those observed for glycerol. In this way, quercetin acts as a plasticizer in the polymeric film, decreasing the elastic modulus.

The interactions between the variables pectin content and quercetin content and the variables pectin content and glycerol content are significant and negative. Thus, the increase in quercetin and glycerol levels leads to a reduction in the elastic modulus, as expected. The interaction among the pectin and quercetin variables is the most significant variable of the system, it is believed that the volume of the quercetin molecule is greater when compared to glycerol, thus causing greater separation among the polymeric chains, reducing the rigidity of the material more intensely. As for the interaction between the glycerol content and the quercetin content, this is significant and positive, considering both negative linear variables, it can be observed that the minimum compositions of glycerol and quercetin will increase the elastic modulus because the low plasticizer content results in greater cohesion among the

pectin polymeric chains. However, maximum compositions of glycerol and quercetin will also increase the elastic modulus, it is believed that in these compositions, quercetin presents preferential interactions with glycerol, thus ceasing to act as a plasticizer.

3.6 Antioxidant Activity

To determine the antioxidant activity (AA) of the films, the DPPH radical elimination method was used. DPPH has a hydrogen-free radical and shows characteristic absorption at 517 nm. Upon encountering radical scavengers, the purple color of the DPPH solution quickly disappears [40]. The values referring to the percentages of antioxidant activity of the films obtained are described in Table 5.

Table 5
Average values and standard deviation of the antioxidant activity of the films.

Films	Antioxidant activity (%)
F1	41.78 ± 0.34
F2	44.44 ± 0.82
F3	40.32 ± 0.67
F4	42.17 ± 0.47
F5	45.37 ± 0.17
F6	43.84 ± 0.91
F7	44.44 ± 0.82
F8	40.43 ± 0.57
F9	40.49 ± 0.62
F10	40.84 ± 0.50

The values obtained from the films ranged from 40.3 ± 0.67% to 45.4 ± 0.17% for films F3 and F5, respectively (Table 5). The linear statistical model considering only the main effects represents the data more adequately (R^2 = 0.9585). The quercetin content variable is the only one that presents a significant difference. The response surfaces for antioxidant activity concerning the other variables can be seen in Fig. 5.

The equations that describe the response surfaces can be seen below.

$$AA = 34.73 + 0{,}45P + 800.5Q + 1.11 \quad (14)$$

$$AA = 34.73 + 800.5Q + 0.88G + 0.57 \quad (15)$$

The results observed about the antioxidant activity of the films are as expected since there are no reports in the literature on antioxidant activity for both glycerol and pectin, these act specifically on the structural properties of the films. However, several authors describe the antioxidant activity of quercetin and its application in wound healing systems [40–42]. The values found were higher than those described by Rubini et al. [43], in a similar study (approximately 35%).

3.7 Thermal Properties

Figure 6 represents the thermogravimetric profile and the differential thermogravimetric profile of pure pectin, of a mechanical mixture between pectin and glycerol in a 1:1 ratio (mass:mass), of the F8 film, which represents the lowest levels of all components and of the films F3, F7, and F9 which indicate an increase in pectin, quercetin, and glycerol levels, respectively (Fig. 3).

Pure pectin has greater thermal stability (T_{onset} = 228 ^{o}C) when compared to the produced films. The thermal event related to pectin mass loss is related to the depolymerization of pectin chains followed by polymer pyrolysis [44–46]. This event appears in the differential thermogravimetric profile as a defined and symmetrical peak at 239 ^{o}C, indicating the purity of the polymer.

The mechanical mixture between pectin and glycerol presents two thermal mass loss events the first is related to glycerol with maximum degradation observed by the peak at 175 ^{o}C, and the second mass loss event is related to pectin degradation this is presented as a peak wider with a maximum of 230 ^{o}C, a temperature lower than that observed for pure pectin, an effect that is common in mixtures [44–46].

The other films have lower thermal stability than pure pectin with T_{onset} ranging from 165 ^{o}C to 176 ^{o}C. These values are expected since glycerol acts as a plasticizer, reducing the cohesion forces of the material and consequently decreasing its thermal stability.

Analyzing the differential thermogravimetric profile, it is observed that films F3, F7, and F8 present a single mass loss event characterized by a large and asymmetrical peak with a maximum at 205, 209, and 214 ^{o}C, respectively (Fig. 3). The peak asymmetry is more pronounced at lower temperatures, indicating that in these compositions, glycerol decreases the cohesive forces of pectin, favoring the thermal degradation of the material. Furthermore, it can be assumed that in these cases more homogeneous materials were obtained since they degrade in a single step. It is believed that the content of glycerol bigger or equal to that of pectin in the formed films indicates a complete intercalation of glycerol between pectin polymeric chains.

Regarding the F8 film, the differential thermogravimetric profile shows two thermal events of mass loss, the first with a peak at approximately 200 ^{o}C and related to the degradation of the material intercalated with glycerol (Fig. 3). The second peak at approximately 230 ^{o}C is believed to be related to a fraction of pure pectin present in the film since the temperature at which the thermal event occurs is similar to pectin degradation when in a mechanical mixture, in addition, the film F8 has a pectin content greater than the glycerol content (Fig. 3).

The presence of a pure pectin fraction in the F8 film corroborates data related to elastic modulus and water vapor permeability (Fig. 3). In both cases, the statistical analyzes demonstrated that the pectin content is more effective in terms of increasing both properties showing the highest values for the related datasets. In the case of the elastic modulus, it is believed that the pure fraction of pectin in the film does not have its cohesive forces reduced by the action of the plasticizer, which increases the rigidity of the material, concerning the permeability of water vapor, a pure fraction of pectin represents a greater hydrophilic polymer content without intercalation of plasticizer, thus being more available for water vapor permeation.

3.8 Considerations on the Application of Films as Dermal Dressings

Normal wound healing physiology depends on low levels of reactive oxygen species and oxidative stress [47]. Overexposure to oxidative stress leads to wound healing problems [48]. Antioxidant molecules are substances that inhibit the oxidation process, offering resistance to oxidative stress, assisting in the process of scavenging free radicals, and inhibiting lipid peroxidation processes [49]. Thus, higher values of antioxidant activity are more appropriate for application in dressings.

Regarding the data obtained for antioxidant activity, all films produced showed high values, greater than 40%, with the variation between the highest value obtained (Table 5, F5, 45.4%) and the lowest value obtained (Table 5, F3, 40.3%) of 12%, thus it is believed that concerning antioxidant activity all films produced have potential application as dressings.

The mechanical properties and water vapor permeability are of great importance in evaluating the use of these films as useful materials in wound dressings. The films produced in the present work must present values comparable to that of human skin, which presents an elastic modulus between 2.5 and 16 MPa [50], as well as the rupture deformation values, which must present values higher than those of human skin, which is around 70% [35]. Considering the elastic modulus and rupture deformation data, it is observed that films F1, F4, F6, and F7 are within the specifications related to human skin (Table 4).

Higher values of water vapor permeability represent higher levels of moisture at the wound location, favoring the healing process. Among the films selected concerning the mechanical properties, it is observed that the film F6 (Table 3) presents greater water vapor permeability, so the film F6 is, among the films produced in this study, the best candidate for application as a dressing for dermal wounds.

4. Conclusion

Polymeric films of pectin and glycerol were obtained with the addition of quercetin. Pectin was extracted from the orange bagasse of *Citrus* × *aurantiifolia* with a high extraction yield of 15%. In addition, pectin showed a high degree of methoxylation, 54.1 ± 0.088%, and a viscosimetric molecular mass of approximately 333,000 g mol^{-1}.

The films were obtained by the casting technique using a factorial design of random mixing among the variables pectin, glycerol, and quercetin. The films obtained showed good homogeneity and brown color with relative opacity. Morphological analyzes carried out using scanning electron microscopy showed a homogeneous dispersion of quercetin throughout the film.

Thickness and opacity were represented by linear and quadratic models respectively. In both cases, the increase in pectin content is more significant and increases the observed responses. As for solubility, no representative statistical adjustment was observed due to the high solubility of the film components. The water vapor permeability was adjusted by a linear model, with the pectin content being the only significant variable.

The analysis of the mechanical properties of the films was carried out from the modulus of elasticity and in this case the quadratic model with interactions adequately represented the system with all the variables and their respective significant interactions. The interaction between pectin and quercetin contents was the most intense variable, demonstrating that high quercetin contents have a great effect on decreasing the stiffness of the material.

The values related to the antioxidant activity of the films ranged from 40.3 ± 0.67% to 45.4 ± 0.17%. The statistical model that best represented the data was the linear model with only the pectin content as a significant variable and the values obtained are higher than those found in the literature in similar studies.

Thermal properties of the films under conditions where the pectin content is greater than the glycerol and quercetin contents resulted in the formation of a fraction of pure pectin, which can be correlated with the high values of vapor permeability of water and modulus of elasticity observed in some films.

Therefore, it was possible to identify that among the films produced, the F6 film is the one that has the potential to be used as wound dressings.

Declarations

Author Contribution

Jean Colombari Neto: Conceptualization, Formal analysis, investigation, writing - original draft.Fernando Reinoldo Scremin: Conceptualization, Formal analysis; investigation; data curation; writing – original draft, writing - review & editing; visualization.Josiane Caetano: Formal analysis, ConceptualizationDouglas Cardoso Dragunski: Conceptualization, investigation, supervision, and writing - review & editing.

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Figures

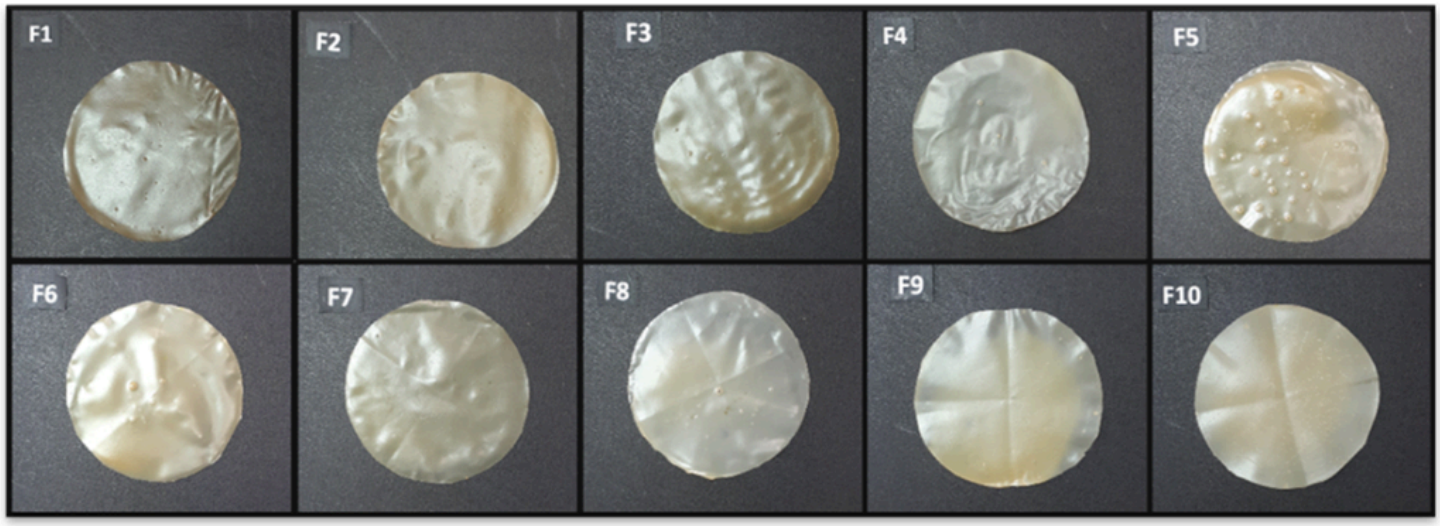

Figure 1

Image of the films obtained in each composition condition.

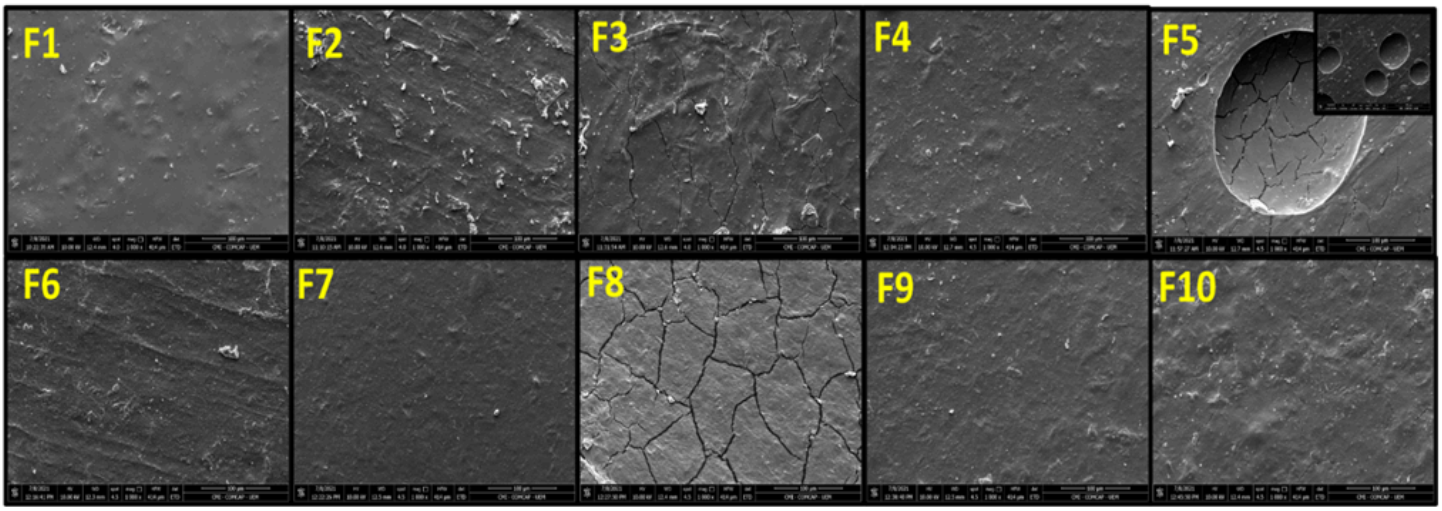

Figure 2

Scanning electron micrographs of the film surface. Acceleration of 10.00 kV and magnification of 1000x.

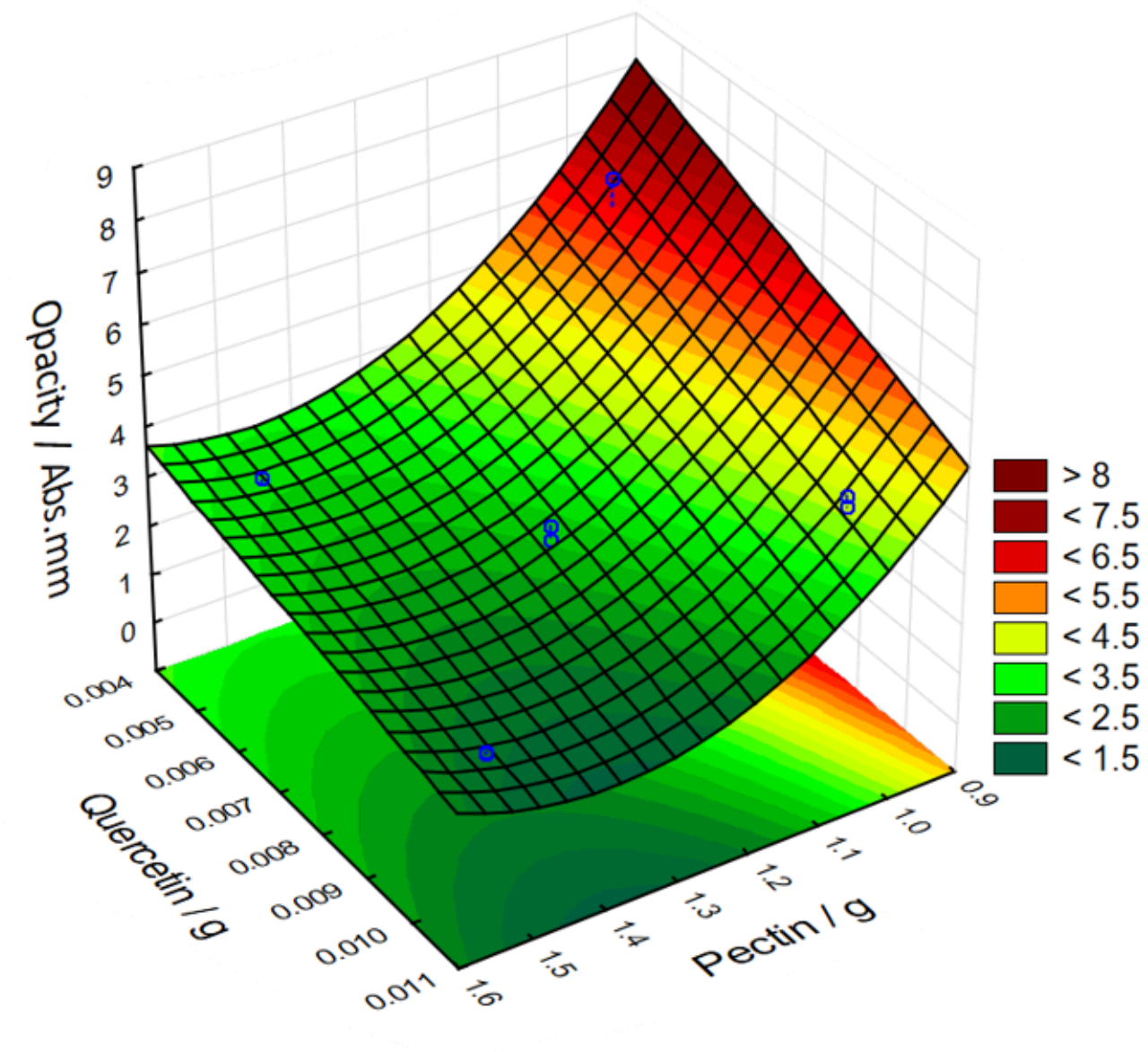

Figure 3

Response surface for opacity concerning the variables quercetin content and pectin content.

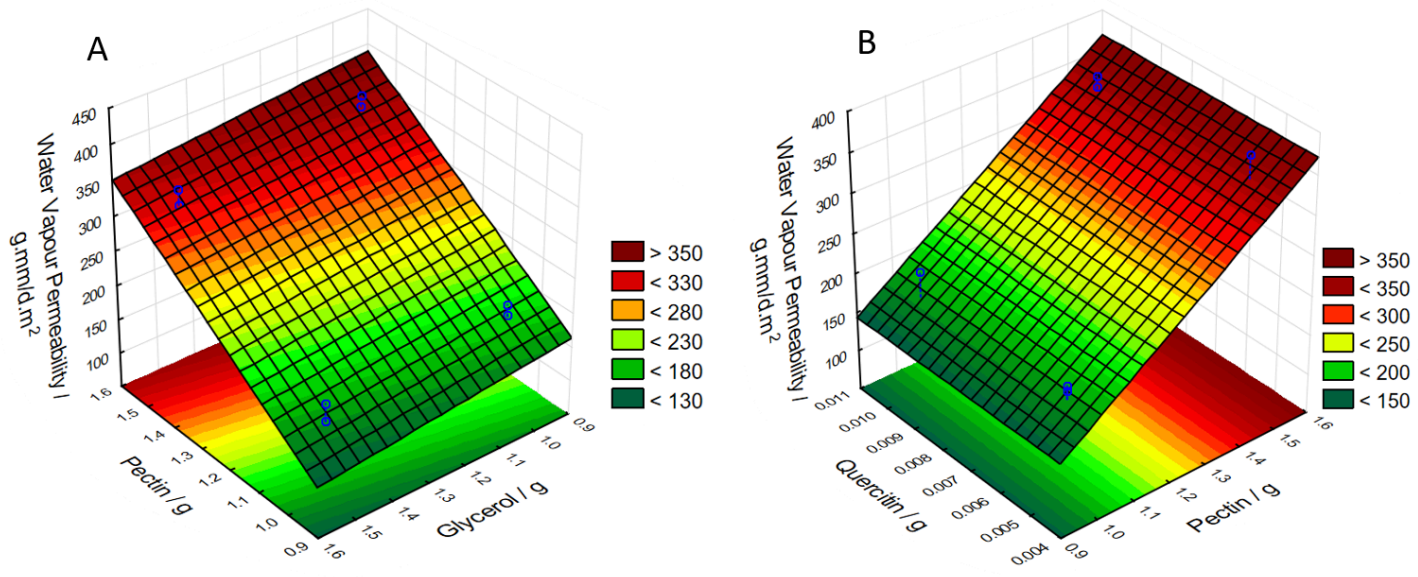

Figure 4

Response surface for water vapor permeability concerning pectin content and glycerol content variables (A) and concerning quercetin content and pectin content variables (B).

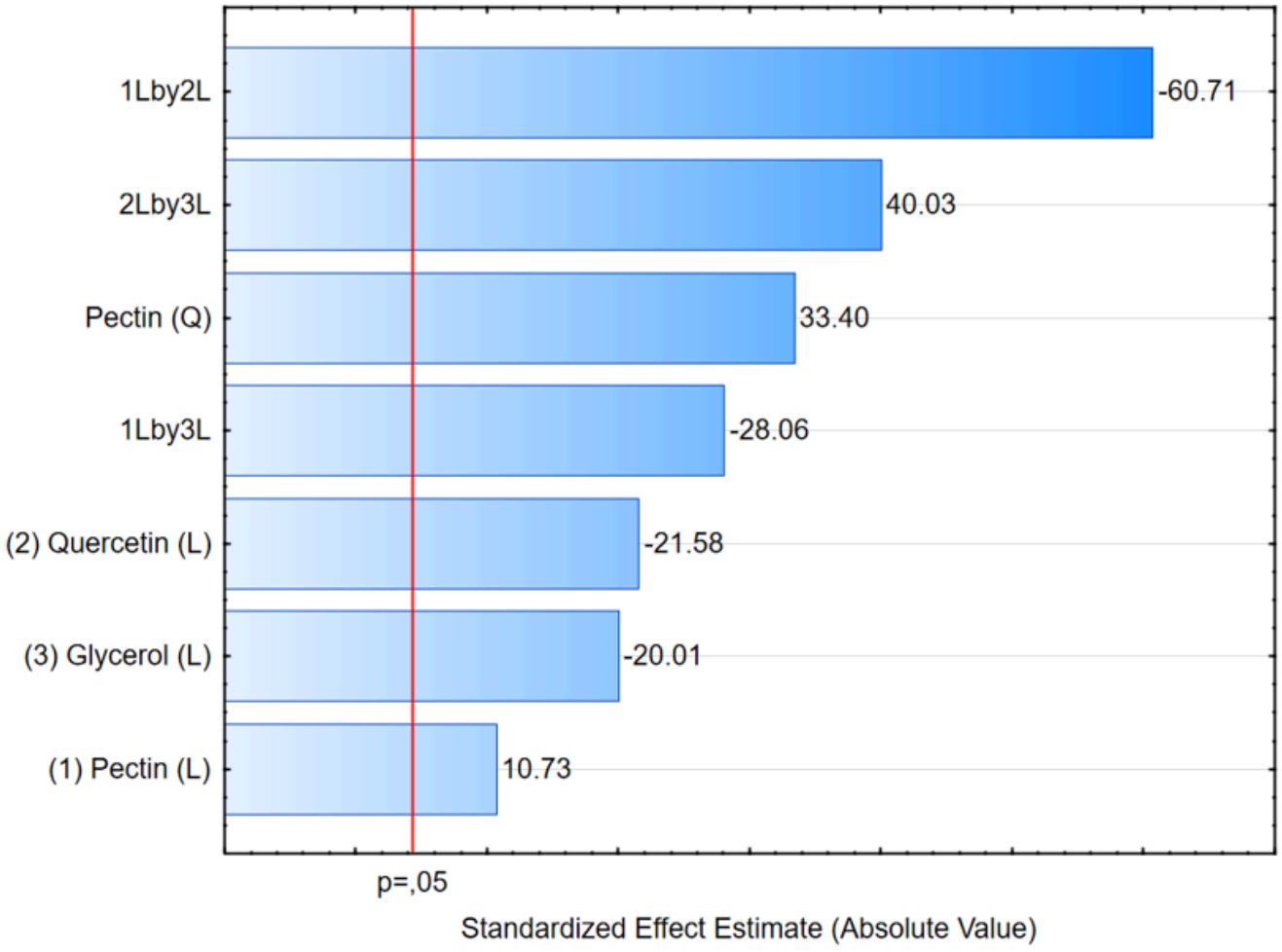

Figure 5

Pareto diagram of the standardized effects concerning the elastic modulus considering the variables pectin content, glycerol content, quercetin content, and their combinations.

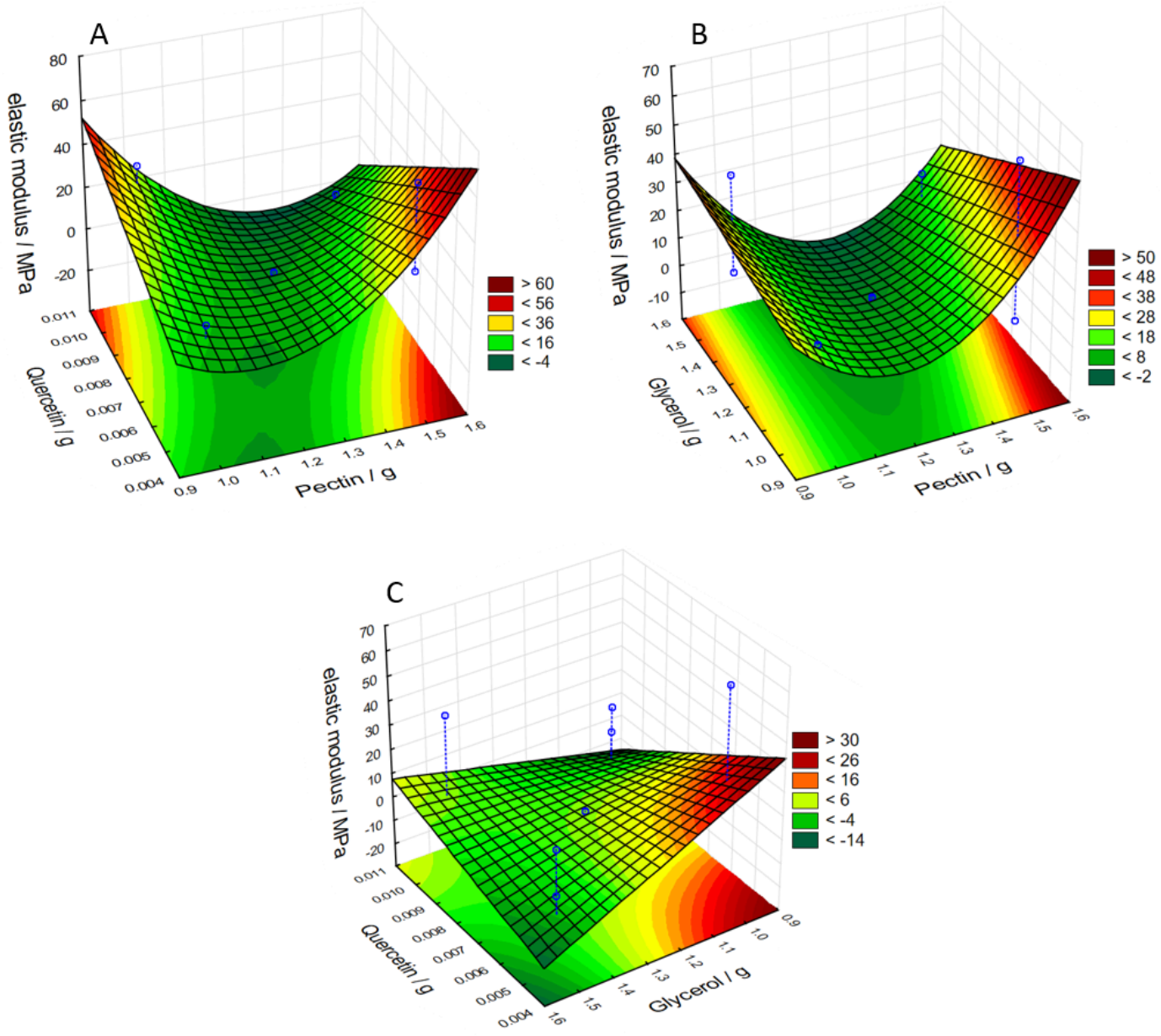

Figure 6

Figure 4: Response surface for elastic modulus concerning quercetin content and pectin content variables (A), glycerol content and pectin content variables (B), and concerning quercetin content and glycerol content variables (C).

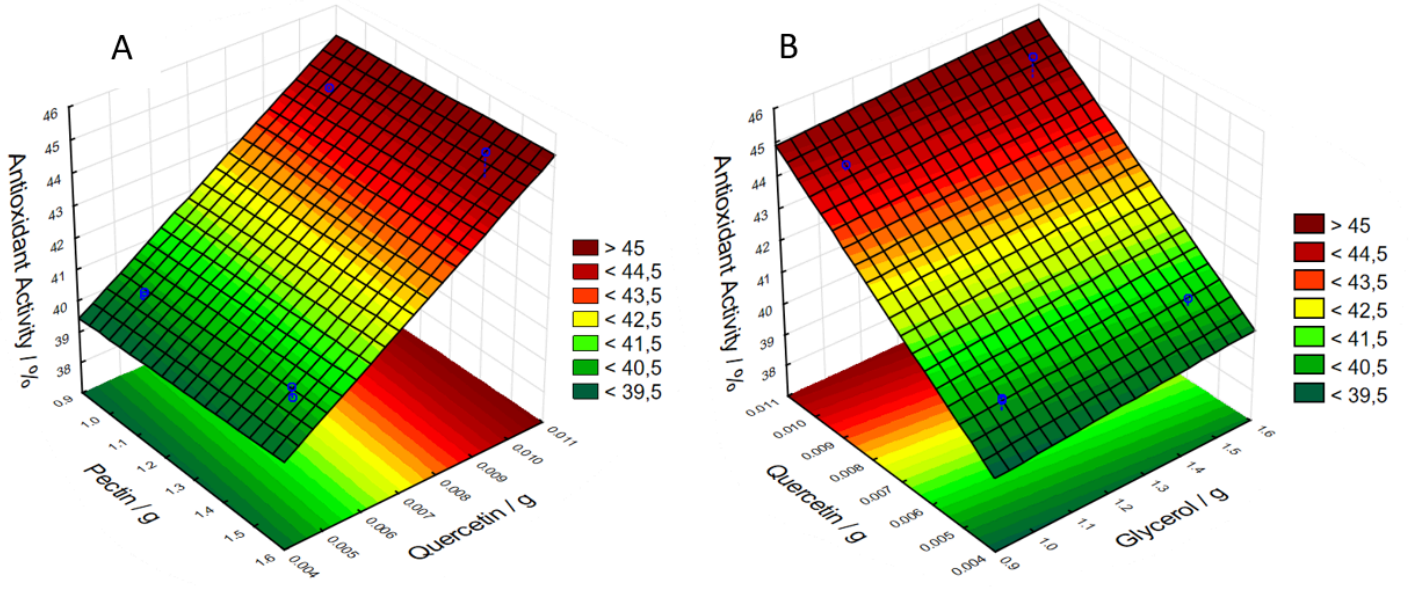

Figure 7

Figure 5: Response surface for antioxidant activity concerning the variables pectin content and quercetin content (A) and concerning the variables quercetin content and glycerol content (B).

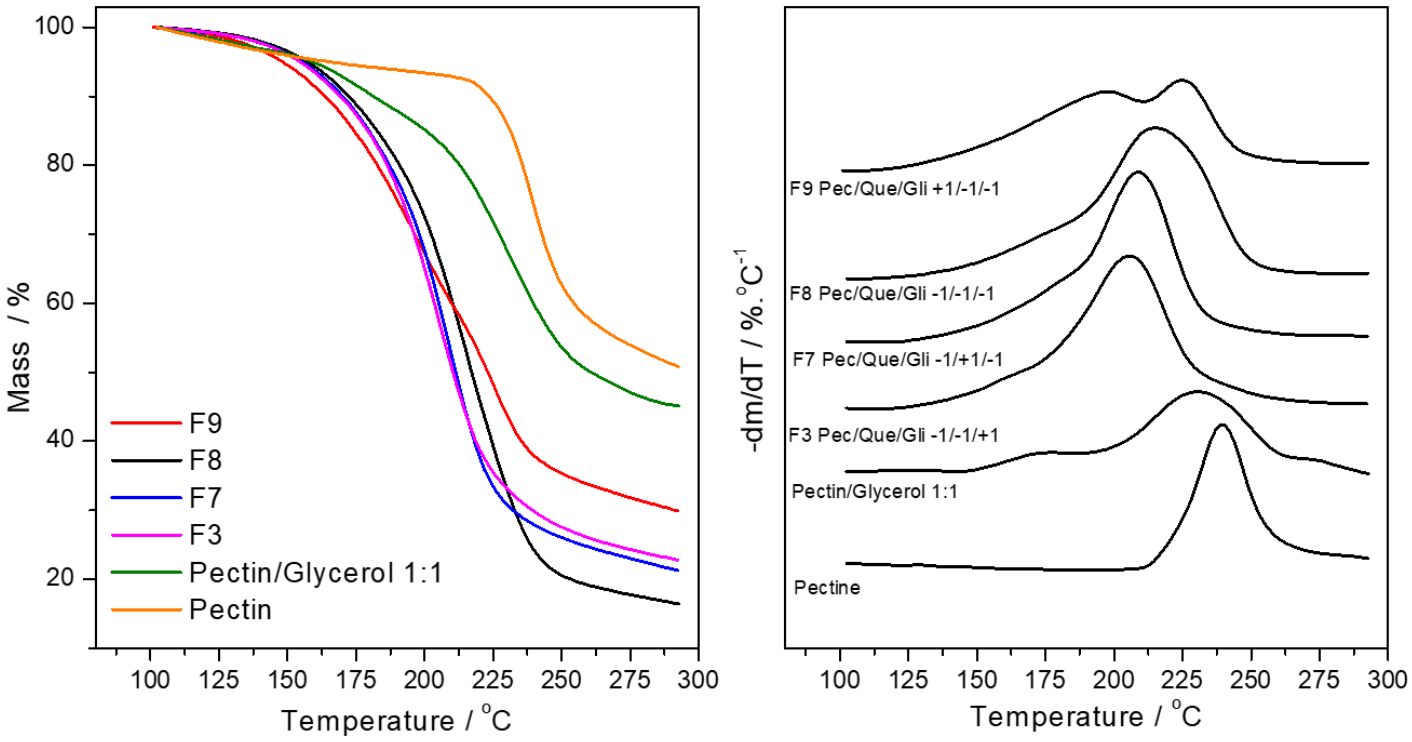

Figure 8

Figure 6: Thermogravimetric profile on the left and differential thermogravimetric profile on the right of films F3, F7, F8, and F9, a mechanical mixture between glycerol and pectin and pure pectin.